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MELANOMAS. INTERFERON THERAPY

Immunotherapy has been used for the treatment of human cancers since Coley's report in 1893[1]. A number of immuno-stimulants have been applied in cancer treatment[2,3]. Interferon (IFN) is the first human cytokine to be widely studied

Our first results with peritumoral and intratumoral application of human natural leukocytic interferon (HNLI) in melanoma patients were published in 1981[4], and the last in 1989[5].

Markovic et al [6] evaluated the effect of HNLI after surgical procedure in 11 patients with skin melanoma and malignant neoplasm of the breast for the values of C_{19} - steroids and particularly of dehydroepiandrosterone The application of HNLI in a dose of $2x10^5$ IU resulted in normalization of C_{19} - steroid metabolism

The experience of Knežević et al.[7] suggests that HNLI increases the invasiveness and lytic activity of macrophages. The activated macrophages are significantly larger. Another characteristic of HNLI application is the proliferation of lymphoid tissue. HNLI stimulates the infiltration of tumor stroma with the masses of lymphocytes and macrophages, they pass into the streaks of tumor cells and destroy them A comparison of the changes in parenchyma and tumor stroma before and after HNLI therapy has shown that it takes 2-4 weeks to make the defence stronger A significant role of HNLI is the activation of regional lymph nodes even if it has not been applied directly to them It seems that HNLI strongly increases the active defence of lymph nodes against the metastasis. In many nodes, a hyaline mass surrounding damaged cell debris, and necrotic tumor cells were found They observed it in head and neck tumors, gynecologic carcinoma and breast cancer. In all these cases, HNLI was applied locally, peritumorally and intratumorally, and not to regional lymph nodes.

A literature survey demonstrated a low but significant response rate in patients with disseminated menalomas after systematic use of r IFN alpha. In a clinical trial Robinson et al.[16] achieved complete remission in four out of 40 patients (10%) with cutaneous primaries, whereas six (15%) melanoma patients had partial remissions, with an overall response rate of 25%. In two patients, complete remission persisted 15 and 32

months from the onset of treatment, respectively. Interferon was given in a dose of 10 million IU/M2 subcutaneously three times *per* week for one year Response rates of 10 and 20 % have been reported by most investigators using different interferons and different dosing schedules[17-25]. A small proportion of patients with disseminated malignant melanomas may achieve a complete response and some of these may have a prolonged remission (5 out of 50 patients)[26-27]. Von Wussow et al. [28] studied the effect of intralesional injection of HNLI and r.IFN alpha 2b in 51 patients. There was complete or partial regression of injected nodules and 21% of objective systematic responses (i e regression of noninjected metastasis outside the lymphatic drainage region of the injected tumor).

The aformentioned studies and results have justified further research of IFN in the treatment of melanoma

PATIENTS AND METHODS

PATIENTS

Twenty-seven patients (14 before and 13 after surgery) were treated with r.IFN alpha 2c Four patients were treated with HNLI. Six patients were male and 21 female, aged 28-78 years The size of the melanomas at the longer diameter were from 5 to 56 mm, and at the shorter diameter from 5 to 50 mm The sites of the tumor were leg 14, back 7, arm 3, sub-and retromandibular 2, face 2, axilla 1, anocutaneous border 1 and hard palate 1 Clinical stage I (melanoma confined to the skin) was recorded in 22 patients, clinical stage II (regional lymph node involvement) in two patients and clinical stage III (disseminated disease) in three patients Clinical stage I group consisted of T_1 (melanoma confined to the epidermis, radial, superficial growth phase) in one patient, T_2 (vertical growth phase, nodular melanoma) in 17 patients and T_3 (satellite appearance) in four patients

INTERFERON

Recombinant HuIFN alpha 2c was applied locally peri- and intratumorally. The duration of treatment was from one day through four weeks In two patients, r.IFN alpha 2c was administered intralymphatically The number of injections was 1-40 Interferon was not administered on Saturdays and Sundays A single dose was 6 mcg (corresponding to 1.36×10^6 IU standard Gxa01-901-535), and the total dose was 120 mcg in two cases after and in five cases before surgery A single dose was 150 mcg (corresponding to 3.4×10^6 IU standard Gxa 01-901-535), and the total dose was 300 mcg in nine cases before surgery and in seven cases after surgery. After surgery, one patient (No 15, Table 3) received 60 mcg in 10 injections, and one (No 17, Table 3) 45 mcg in seven injections One patient (No 16, Table 3) received 75 mcg and 30 mcg additionally in two intralymphatic applications, and one (No 25, Table 3) received 15 mcg intralymphatically

HNLI was applied locally. One patient received 25 injections and a total dose of $1.2x10^6$, one patient 18 injections and a total dose of $0.5x10^6$. One patient received a daily dose of $2x10^6$ for 60 days, and one - the same daily dose for 60 days in addition to the same dose intramuscularly every other day

CLINICAL EVALUATION OF THE RESULTS

Clinical results were evaluated on the basis of the tumor size before and after interferon therapy.

PATHOHISTOLOGIC EVALUATION

The pathohistologic confirmation of the clinical diagnosis was done on the bioptic material Excised tumor represented the biotoptic material

IMMUNOHISTOLOGIC EVALUATION

The immunohistologic evalution of the results primarily took into consideration lymphoid cells and macrophages in stroma

0 = no response, nonreactive stroma

+ = poor response, scattered lymphoid cells

++ = moderate response, numerous lymphoid cells, narrow infiltration areas, few macrophages

+++ = good response, massive infiltration of lymphoid cells, numerous macrophages

++++ = very good response, massive infiltration and formation of lymphoid nodules in stroma, numerous macrophages

RESULTS

Generally speaking, according to the clinical evaluation, the tumor size was reduced by more than 50% in four patients, by more than 25% in eight patients and by less than 25% in two patients after interferon treatment Before surgery, the appearance of the tumor was changed, the surface peeled off and the edges were becoming clearly bordered in all patients.

Data on seven living out of the fourteen patients treated with interferon before surgery, are presented in Table 1 Three patients (Nos 3,4), Clark level II-III, Breslow

level 1 0 to >2 0 mm, clinical stage I, T_1 - $2N_0M_0$ classification, are still alive five or more years after the treatment Patient No. 1, with a melanoma sized 50-55 mm and satellites on the planta of the right foot, Clark level V, Breslow level >2.5 mm, clinical stage II, $T_3N_1M_0$ classification, refused amputation of the right leg above the knee and accepted the interferon treatment. After the treatmant, the tumor was so strongly delineated from the healthy tissue that the surgeon pecled the tumor off from the planta of the right foot The amputation of the right leg above knee was thus avoided due to the interferon treatment. Three out of seven patients (Nos 5,6,7) Clark level III-IV, Breslow level >2 5mm, clinical stage I, $T_2N_0M_0$ classification, are still alive four years after the treatment

Table 1

Before surgery

Living melanoma patients

PATIENT No	AGE	SEX	TUMOR SITE	CLARK	BRESLOW (mm)	CLINICAL STAGE	SURVIVAL (years)	IMMUNOLOGIC GRADE	DURATION OF TREATMENT (WEEKS)	r IFN alpha 2c TOTAL AMOUNT IN MGC	REMARKS
1	1907	M	Planta right leg	V	>2 5 IV	II T3N1M0	6	III	4	120	After 6 years of follow-up no contact with the patient due to the war
2	1942	I	Right lower arm	II	2 0 III	I T2N0M0	6	III	4	300	
3	1941	I	Left lower leg	III	2 0 III	I T2N0M0	6	I	4	300	
4	1931	I	Right lower leg	II	1 0 II	I T1N0M0	6	III	4	300	
5	1946	M	Left Upper arm	III	>2 5 IV	I T2N0M0	4	III	4	300	
6	1922	M	Back	III	>2 5 IV	I T3N1M0	4	I	4	300	
7	1928	M	Back	IV	>2 5 IV	I T2N1M0	4	III	4	300	

Data on seven deaths in fourteen patients, Clark level III-V, Breslow level 2.0 to > 2.5 mm, who were treated with interferon before surgery, are given in Table 2 Three clinical stage III, classification $T_3N_1M_1$ patients (Nos. 8,11,14), two clinical stage I, classification $T_3N_0M_0$ patients (Nos. 9,13) and two $T_2N_0M_0$ classification patients (Nos. 10, 12) lived for 1 - 3 years after the treatment.

Table 2

Before surgery

Deaths in melanoma patients

PATIENT No	AGE	SEX	TUMOR SITE	CLARK	BRESLOW (mm)	CLINICAL STAGE	SURVIVAL (years)	IMMUNOLOGIC GRADE	DURATION OF TREATMENT (WEEKS)	r IFN alpha 2c TOTAL AMOUNT IN MGC	REMARKS
8	1912	I	Right submandibular	IV	>2 5 IV	III T3N1M1	2	I	4	120	
9	1948	F	Right lower leg	III	>2 5 III	I T3N0M0	3	I	4	120	
10	1944	I	Left lower leg	IV	>2 5 II	I T2N0M0	3	III	4	300	
11	1927	I	Right retromandibular	III	>2 5 IV	III T3N1M1	2	I	4	120	
12	1939	I	Back	IV	>2 5 III	I T2N0M0	2	II	4	300	Bleeding
13	1922	I	Right lower leg	III-IV	2 0 II	I T3N0M0	2	III	4	300	
14	1944	I	Right upper leg	V	>2 5 IV	III T3N1M1	1	I	4	120	

Data on seven living out of the thirteen patients treated with interferon after surgery are given in Table 3 Thirteen melanoma cases were treated with interferon after surgery. Seven of them are still alive. six patients Clark level III-IV, Breslow level 2.0 mm to >2 5 mm, clinical stage classification T_2-$3N_0M_0$, and one patient clinical stage II classification $T_3N_1M_0$. Three patients (Nos 15,16,17) have survived for 6-7 years, and four cases (Nos. 18,19,20,21) for 3-4 years up to the present Patient No 16, Clark level IV, Breslow level >2 5 mm, clinical stage II, $T_3N_1M_0$ classification is still alive six years after the surgery. In addition to $24x10^6$ units locally, the patient also received $10x10^6$ units intralymphatically into the leg.

Data on six deaths in thirteen patients treated with interferon after surgery are given in Table 4 Those patients, Clark level III-V, Breslow level >2 5 mm, clinical stage I, clinical grade T_2-$3N_0M_0$, lived for 2-5 years after the treatment. In two cases, Nos. 22 and 25, the tumor was not completely removed Case No. 25 with a melanoma not completely removed on anocutaneous border received only one intralymphatic injection of $5x10^6$ units and lived for three years after the surgery.

Table 3

After surgery

Living melanoma patients

PATIENT No	AGE	SEX	TUMOR SITE	CLARK	BRESLOW (mm)	CLINICAL STAGE	SURVIVAL (years)	IMMUNOLOGIC GRADE	DURATION OF TREATMENT (WEEKS)	rIFN alpha 2c TOTAL AMOUNT IN MIOC	REMARKS
15	1951	F	Left axilla	III	>2.5 III	I T2N0M0	7	++++	2	60	
16	1956	M	Left upper leg	IV	>2.5 IV	II T3N1M0	6	+++	2	105	Intralymphatic
17	1938	F	Back	III	>2.5 III	I T2N0M0	6	++++	2	45	
18	1922	F	Right lower leg	V	>2.5 IV	I T2N0M0	4	+++	4	300	
19	1916	F	Right lower leg	IV	>2.5 III	I T3N0M0	4	+	4	300	
20	1930	F	Face	IV	>2.5 IV	I T2N0M0	3	+++	4	300	
21	1938	F	Back	III	2.0 III	I T2N0M0	3	++	4	300	

Four patients were treated with human natural leukocytic interferon (HNLI). In one melanoma patient, a fifteen-year-old girl with a lesion on the nose, whose diagnosis was confirmed by incision biopsy, complete remission was achieved after four weeks of treatment, including eighteen injections and a total of $500x10^5$ units. The girl is still alive 10 years after the treatment. In a patient aged 75 with a lesion on the hard palate and a poor prognosis, complete remission was achieved locally after four weeks of treatment with 25 injections and a total of $1.2x10^6$ units, but there were no effects on the lung and neck metastases. The patient died one year after the HNLI treatment. In two patients (aged 63 and 70) with recurrent melanomas within the surgical scar, in one in the form of satellites and in the other a tumor within the scar, both with a poor prognosis, the tumors were treated with a daily dose of $2x10^6$ HNLI for sixty days. Recurrent melanoma regressed completely. In the patient with satellite metastases, 75% of the nodules disappeared completely and the remaining were reduced in size. In both patients, both the original surgical specimens and recurrences were confirmed by microscopic examination. Both patients died within 2 years after the treatment.

Table 4

After surgery

Deaths in melanoma patients

PATIENT No	AGE	SEX	TUMOR SITE	CLARK	BRESLOW (mm)	CLINICAL STAGE	SURVIVAL (years)	IMMUNOLOGIC GRADE	DURATION OF TREATMENT (WEEKS)	r IFN alpha 2c TOTAL AMOUNT IN MGC	REMARKS
22	1951	F	Right upper leg	V	>2.5 IV	I T2N0M0	2	III	4	120	Tumor was not completly removed
23	1931	M	Neck	IV	>2.5 IV	I T2N0M0	2	III	4	120	
24	1953	F	Back	V	>2.5 IV	I T2N0M0	2	II	4	300	
25	1938	F	Anocutaneous border	IV	>2.5 IV	I T2N0M0	3	IIII	1 day	15	Intralymphatic Tumor was not completely removed
26	1928	F	Right upper leg	IV	>2.5 IV	I T2N0M0	3	II	4	300	
27	1959	F	Left lower leg	III	>2.5 III	I T2N0M0	5	III	4	300	

DISCUSSION

A variety of clinical staging classifications have been recommended for a melanoma. Perhaps the most simple and commonly used is clinical stage I melanoma confined to the skin (T_1 superficially spreading melanoma, radial melanoma, T_2 nodular melanoma, T_3 nodular melanoma with satellites), clinical stage II when there is regional lymph node involvement (N1), and clinical stage III when there is dissemination of the disease (M1).

The likely outcome in an individual is determined by the depth to which the tumor has spread into the skin, the site of the tumor, the sex and age of the person, and many of other variables.

In general, patients with lesions on the head and neck, trunk, hands and feet have a poorer prognosis than those with lesions on the arms and legs The clinical presence of ulceration and bleeding in melanoma is a sign of poor prognosis The presence of microscopic satellites also means a poor prognosis, as well as metastases to regional lymph nodes, but such patients still live longer than those with metastases beyond the regional lymph nodes Older age has been shown to be an unfavorable prognostic factor. Overall, women tend to have a better long-term prognosis than men, as well as patients under 50 years of age as compared to older age groups

While melanoma is confined to the epidermis, it is known as *in situ*, level I or intraepidermal melanoma which is noninvasive, has no ability to metastasize and if removed at this stage, a cure will result in almost 100% of the cases[15] If the growth pattern changes from the superficial spreading growth phase to the vertical growth phase (melanoma which has invaded from the epidermis into the dermis), the tumor has a potential to enter the blood and lymphatic vessels, and spread elsewhere in the body from the primary site

The prognosis of melanoma is inversely related to the depth of tumor invasion This concept, suggested by Mehnert and Heard[8] has been refined by Clark et al [9] who have defined five levels of microinvasion, and by Breslow[10] who has introduced the quantitative measurment of the vertical thickness of melanoma with the use of ocular micrometer Clark et al.[11] claim that primary melanomas evolve through two clearly defined phases the radial growth phase and the vertical growth phase Only the cells in the vertical growth phase exhibit all the properties of fully transformed cells A variety of new cell-surface molecules are present on vertical growth melanomas, including the gangliosides GD2 and GD3 All these factors are relevant to invasive and metastatic behavior and selective growth advantage

Breslow[12] has suggested that lesions with a thickness of less than 0 76 mm carry an excellent prognosis Slingluff et al [13] found that 4 8% developed recurrences, but those with a 3-mm thickness and patients with Clark IV or V lesions had a poor prognosis.

To evaluate the interaction of many prognostic factors, Mackie et al.[14] have developed a prognostic scoring system Eleven prognostic factors are assigned a point value. The higher the patients score, the worse the prognosis. Tumor ulceration and satellite presence are poor prognostic signs.

Is there any evidence that the use of interferon can raise the survival rate of patients or at least improve the quality of life of melanoma patients? The effects of IFN on clinical prognosis are not yet completely known Is it possible to delay recurrence in patients?

Partial regression, a decrease in the size and change of melanoma are seen in almost 100% of patients. Does this mean a better quality of life and a prolonged survival for melanoma patients?

Two patients belonged to clinical stage I, classification T_1 (radial melanoma with good prognosis), one of them received r IFN alpha 2c before surgery and is still alive six years after the surgery, and the other patient, who received HNLI, is still alive nine years after the surgery

Clinical stage I, classification T_2 (vertical growth phase, nodular melanoma), was diagnosed in seventeen patients, and seven of them received r IFN alpha 2c before surgery. Five out of these seven patients are still alive, two for six years and three for four years after the treatment, while two patients died, one of them two years and the other three years after the treatment It seems that in this group the survival rate is better

than could have been expected if only surgery had been performed. Ten patients received r.IFN alpha 2c after the surgery, and five patients are still alive, one of them for six and one for seven years, one for four years and two patients for three years after the treatment Five patients died, three of them lived for three years and two lived for two years after the treatment.

In this group in which r IFN alpha 2c was applied after surgery, the survival rate is lower in comparison with the group in which r IFN alpha 2c was applied before surgery.

Clinical stage I, classification T_3 (satellite melanoma) consisted of four cases with poor prognosis. Two patients who received therapy before the surgery, died two and three years after the treatmant, respectively One patient (Table 3, No 16) is still alive six years after the treatment and one died two years after the treatmant (Table 4, No 23).

Clinical stage II, classification T_3N_1 (with nodular involvement and poor prognosis), was found in two patients, who are still alive six years after the treatmant.

Clinical stage III, classification $T_3N_1M_1$ (melanoma with distant metastases and a very poor prognosis) consisted of three patients who received HNLI and two who received r IFN alpha 2c Three patients with metastatic melanoma received HNLI, two of them showed complete regression locally, one patient showed partial regression, but they all died from distant metastases within two years after the treatmant Two patients who received r IFN alpha 2c before surgery died within two years after the treatment.

What can be said about the efficacy of interferon in the treatment of melanoma based on our experience and our method and schedule of application?

1 Local application of interferon is efficient in melanoma clinical stage I classification $T_1N_0M_0$ with or without subsequent surgery, and is especially recommended on cosmetically difficult localizations

2. Interferon is useful as an adjuvant treatment in clinical stage I, classification T_2 and $T_3N_0M_0$ and clinical stage II, classification $T_3N_1M_0$ The recommended route of administration is local interferon treatment for four weeks (3-5 times per week) before and for twelve weeks (1-2 times per week) after surgery A significant role of local tumor application of interferon is the activation and hyperplasia of regional nodes. The local application of interferon hinders the growth of tumor cells and preventing dispersion of the neocytes, inhibits micrometastasis thereby reducing the number of tumor recurrences, increasing the chance of survival and improving the quality of life. According to our results, interferon application before surgery is more efficient than that after surgery.

3 Interferon therapy of metastatic melanoma is of a limited efficacy, as shown by our results. A response rate similar to that achieved by conventional chemotherapy has been accomplished in studies using higher interferon doses and much longer interferon treatment[26-28].

In patients with metastatic disease, it is important to find effective adjuvant and minimally toxic therapy to stimulate the resistance of the body. The identification of human melanoma cell surface antigens has led to the development of an array of mouse monoclonal antibodies against glycolipid surface antigens. Such antibodies, if specially prepared to overcome the immunogenicity of mouse monoclonal antibodies[29-31], might provide a useful and efficient combination with r.IFN alpha which could enhance the immune activity against distant metastatic melanoma cells.

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SUMMARY

Twenty-seven patients were treated with r IFN alpha 2c (fourteen before and thirteen after surgery) Four patients received human natural leukocytic interferon (HNLI) Interferon was administered locally.

Out of two patients belonging to clinical stage I, classification T_1 (radial melanoma with good prognosis), one received r.IFN alpha 2c before surgery and is still alive six years after the surgery, and the other received HNLI, and is still alive nine years after the surgery.

Clinical stage I, classification T_2 (vertical growth phase, nodular melanoma) included seventeen patients, seven of them received r IFN alpha 2c before surgery Out of these seven patients, five are still alive, two for six years and three for four years after the treatment, while two patients died, one of them two years and the other one three years after the treatmant It seems that in this group, the survival rate is better than could have been expected if only surgery had been performed Ten patients received r IFN alpha 2c after surgery, and five patients are still alive, one for six and one for seven years, one for four years and two patients for three years after the treatment Five patients died, three of them lived for three years and two lived for two years after the treatment

In this group, in which r IFN alpha 2c was applied after surgery, the survival rate is lower in comparison with the group in which r IFN alpha 2c was applied before surgery

Clinical stage I, classification T_3 (satellite melanoma) consisted for four cases with poor prognosis Two patients who received therapy before surgery, died two and three years after treatment, respectively One patient (Table 3, No 16) is still alive six years after the treatment and one died two years after the treatment (Table 4. No 23)

Clinical stage II, classification T_3N_1 (with nodular involvement and poor prognosis), included two cases still alive six years after the treatment.

Clinical stage III, classification $T_3N_1M_1$ (melanoma with distant metastases and very poor prognosis) consisted of three patients who received HNLI and two who received r IFN alpha 2c Three patients with metastatic melanoma received HNLI, two

of them showed complete regression locally, one patient showed partial regression, but they all died from distant metastases within two years after the treatment Two patients who received r IFN alpha 2c before surgery died within two years after the treatment

What can be said about the efficacy of interferon in the treatment of melanoma based on our experience and method and schedule of application?

1 Local application of interferon is efficient in melanoma clinical stage I classification $T_1N_0M_0$ with or without subsequent surgery, and is especially recommended on cosmetically difficult localizations

2. Interferon is useful as an adjuvant treatment in clinical stage I, classification T_2 and $T_3N_0M_0$ and clinical stage II. classification $T_3N_1M_0$ The recommended route of administration is local interferon treatment for four weeks (3-5 times per week) before and for twelve weeks (1-2 times per week) after surgery A significant role of local tumor application of interferon is the activation and hyperplasia of regional nodes. The local application of interferon hinders the growth of tumor cells and preventing dispersion of the neocytes. inhibits micrometastasis thereby reducing the number of tumor recurrences, increasing the chance of survival and contributing to a better quality of life. According to our results, interferon application before surgery is more efficient than that after surgery.

3 Interferon therapy of metastatic melanoma is of a limited efficacy, as shown by our results A response rate similar to that achieved by conventional chemotherapy has been achieved in studies using higher interferon doses and much longer interferon treatment

In patients with metastatic disease, it is important to find effective adjuvant and minimally toxic therapy to stimulate the resistance of the body. The identification of human melanoma cell surface antigens has led to the development of an array of mouse monoclonal antibodies directed against glycolipid surface antigens Such antibodies, if specially prepared to overcome the immunogenicity of mouse monoclonal antibodies, might provide a useful and efficient combination with r.IFN alpha 2c which could enhance the immune activity against distant metastatic melanoma cells.

MELANOMI TERAPIJA INTERFERONOM

S A Ž E T A K

Dvadesetsedmoro bolesnika liječeno je lokalno s rekombinantnim interferonom, a četvoro bolesnika s prirodnim humanim leukocitnim interferonom. Od dvadesetsedmoro bolesnika liječenih s rekombinantnim interferonom, četrnaestoro je primalo rekombinantni interferon prije, a trinaestoro nakon kirurškoga zahvata

O učinkovitosti primjene interferona u liječenju melanoma na osnovi našega nacrta, načina primjene i iskustva možemo reći kako je lokalna primjena interferona

učinkovita kod melanoma kliničkoga stupnja I klasifikacije $T_1N_0M_0$, što je zanimljivo kod tumora lokalizacija kojih nije pogodna s kozmetskog stajališta Interferon kao pomoćna terapija melanoma koristan je u kliničkom stupnju I klasifikacije T_2 i $T_3N_0M_0$, zatim u kliničkom stupnju II klasifikacije $T_3N_0M_0$ i u kliničkom stupnju II klasifikacije $T_3N_1M_0$ Preporuča se lokalna primjena interferona tijekom 4 tjedna, 3-5 puta u tjednu prije kirurškoga zahvata Značajna uloga lokalne primjene interferona je aktiviranje i hiperplazija regionalnih limfnih čvorova Lokalna primjena interferona u i u blizini tumora pomaže, uz ostale aktivnosti, blokiranje tumorskih stanica, sprječava disperziju malignih stanica, inhibira mikrometastaze, smanjuje mogućnost ponovne pojave tumora, poboljšava izglede bolesnika za preživljavanje Primjena interferona prije kirurške intervencije je učinkovitija, prema našim rezultatima, od primjene interferona nakon kirurške intervencije, ali najbolji put je primjena i prije i nakon kirurške intervencije

Liječenje interferonom metastatskog melanoma, tj. kliničkoga stupnja III klasifikacije $T_3N_1M_1$, bilo je ograničene učinkovitosti u našim ispitivanjima. Učinkovitije rezultate liječenja metastatskog melanoma, slične rezultatima postignutima s uobičajenom kemoterapijom, opisali su drugi autori koji su rabili mnogo veće doze rekombinantnog interferona i puno dužu primjenu interferona Za bolesnike oboljele od metastatskog melanoma potrebno je naći učinkovitu pomoćnu terapiju u svrhu pojačanja otpornosti organizma, koja je minimalno toksična Identifikacija površinskih antigena dovela je do razvitka niza mišjih monoklonskih protutijela protiv glikolipidnih površinskih antigena stanice humanoga melanoma Takva monoklonska protutijela, priređena tako da premoste imunogenost mišjih monoklonskih protutijela, bilo bi svrhovito vezati s rekombinantnim interferonom u svrhu pojačanja imunološke aktivnosti protiv metastatskih melanomskih stanica

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LIP CANCER. INTERFERON THERAPY

Our first results on the application of human natural leukocyte interferon (HNLI) in patients with basal cell carcinoma were published in 1975, and they marked the beginning of the local treatment with HNLI[1]. Results of the following study in seventeen patients (basal and squamous cell carcinoma) treated with HNLI and recombinant IFN alpha 2c were published in 1981[2], and the latest publication describing 161 patients appeared in 1991[3]. The efficacy of recombinant IFN alpha has also been confirmed by other authors[4-7]. The local production of interferons has an important role in the immune system. The local antitumor effect of interferon is essential[2,8,11]. The local treatment with interferon stimulates the immune response at the site of the tumor, and regional lymph node enlargement was observed upon the local application of interferon, even when it was not directly applied to them. In our present study, HNLI and recombinant HuIFN alpha 2c were used in therapy for lip cancers.

PATIENTS AND METHODS

PATIENTS

Twenty-one patients with squamous cell carcinoma were treated with HNLI. There were 11 men and ten women, age range 34-78 years. The sites of tumor were lower lip in 18 patients and upper lip in three patients. The tumor size before treatment was longer diameter 10-20 mm and shorter 5-15 mm. Six patients with squamous cell carcinoma were treated with r IFN alpha 2c. Five of them were men and one a woman, aged 54-81. The tumor size before treatment was longer diameter 5-15 mm and shorter diameter 2-15 mm. The site of tumor in these six patients was at the lower lip. Three patients with basal cell carcinoma were treated with HNLI. In all of them, the site of tumor was at the upper lip, dimensions 5-15 mm.

INTERFERON

The duration of treatment with semipurified concentrated HNLI was 3-8 weeks A single dose consisted of 300,000 to 1 2 million units The number of applications ranged from 15 to 35, and the total dose was between 7.5 and 21 6 million units Interferon was administered in 1 ml of liquid, using a hypodermic needle (one injection but changing direction), taking care to inject the entire amount intralesionally No other medication was administered during the course of the interferon treatment HNLI was produced at the Institute of Immunology in Zagreb, Croatia. The duration of treatment with r IFN alpha 2c was 4 weeks Three patients received 20 intratumoral injections of 2 million units each, in the total amount of 40 million units, and three patients received 20 intratumoral injections of 5 million units, in the total amount of 100 million units r IFN alpha 2c was applied daily, except for Saturdays and Sundays No other medication was administered during the treatment. r IFN alpha 2c was produced by Boehringer Ingelheim, and was donated for our study

HISTOPATHOLOGIC EVALUATION OF THE RESULTS

Biopsy material was taken from the tumor edge and deep enough to include all the layers affected by tumorous growth The material was taken on examinations and immediately after the treatment, within 72 hours In the patients in whom the tumor had disappeared, the biopsy material was taken from the site where the tumor had been located In the patients in whom the tumor persisted after the treatment with interferon, it was surgically removed and the excised tumor represented the biopsy material The biopsy material was preserved in 10% formalin, embedded in paraffin, prepared with a Reichert microtome (5 mm) and dyed with hematoxilin-eosin To ascertain that the excisional biopsy specimens were free of microscopic foci of squamous or basal cell carcinoma, sections were examined by two histopathologists

CLINICAL EVALUATION OF THE RESULTS

\+ = the tumor size has regressed by less than 25%,

++ = the tumor size has regressed by 25% -75%,

+++ = the tumor size has regressed by more than 75% or a barely visible nonepithelialized lesion is present,

++++ = the tumor has disappeared, fully epithelialized

IMMUNOHISTOLOGIC EVALUATION OF THE RESULTS

+ = scattered lymphoid cells;

++ = numerous lymphoid cells, narrow infiltration areas, few macrophages,

+++ = massive infiltration of lymphoid cells, numerous macrophages,

++++ = massive infiltration of lymphoid cells, numerous macrophages, tumorous cells have disappeared completely

RESULTS

The results of HNLI therapy in 21 patients with squamous cell carcinoma are presented in Table 1. According to the histopathologic and clinical findings, 10 out of 21 squamous cell lip carcinomas were cured, and tumor cells were not found in the biopsy material taken after the interferon treatment In two patients, the tumor size was reduced by 75%-90%, in five patients by 25%-75%, and in four patients by less than 25%

The results of r IFN alpha 2c therapy in six patients with squamous cell carcinoma are presented in Table 2 According to histopathologic and clinical findings, one out of six patients with squamous cell lip carcinoma was cured and tumor cells were not found in the biopsy material squamous taken after the r IFN alpha 2c treatment In two patients, the tumor size was reduced by 75%-90%, in one patient by 50% and in two patients by less than 25%.

The response rate in 21 patients with lower lip squamous cell carcinoma treated by HNLI (Table 1), measured by size reduction of more than 25%, was 81%, and the complete response rate, i e the percentage of patients cured according to histopathologic and clinical findings, was 48% The response rate in six patients with lower lip squamous cell carcimoma treated with r IFN alpha 2c (Table 2), measured by the size reduction of more than 25%, was 67%, and the complete response rate 17% Histologically, neither HNLI nor r IFN alpha 2c induced any specific response which would not be present in normal interaction between the tumor cells and immune system Prior to the interferon treatment, as a rule, there were few cellular elements in the tumor stroma After the interferon application, the stroma was, as a rule, widely filled with masses of lymphocytes and macrophages which entered the rays of the tumor cells and destroyed them

Table 1

HNLI. Squamous carcinoma of lower and upper lip

PATIENT									
No	Age	Sex	Tumor site	Pathohistol findings after treatment	Duration of treat (weeks)	No of applic	Single dose (x106)	Total units (x106)	Clinical evaluatio
1	53	M	Lower lip	Sinc im	4	20	1	20	
2	49	M	Lower lip	Sinc im	3	15	0 8	12	
3	34	F	Lower lip	Sinc im	4	16	0 5	8	
4	64	F	Lower lip	Sinc im	4	16	0 5	8	
5	58	M	Lower lip	Sinc im	4	18	1 2	21 6	
6	74	M	Lower lip	Squamous cell ca	5	24	0 5	12	
7	78	M	Lower lip	Squamous cell ca	5	24	0 8	19 2	
8	70	F	Lower lip	Squamous cell ca	3	15	1 2	18	
9	66	F	Lower lip	Squamous cell ca	4	20	0 5	10	
10	50	F	Lower lip	Squamous cell ca	5	20	0 4	8	
11	50	M	Lower lip	Squamous cell ca	3	15	0 8	12	
12	64	F	Lower lip	Sinc im	4	16	0 5	8	
13	49	M	Lower lip	Sinc im	3	15	0 8	12	
14	66	F	Lower lip	Squamous cell ca	4	20	0 5	10	
15	42	M	Lower lip	Sinc im	7	35	0 3	10 5	
16	50	M	Lower lip	Sinc im	8	35	0 3	10 5	
17	70	M	Lower lip	Sinc im	8	25	0 3	7 5	
18	71	M	Lower lip	Squamous cell ca	8	30	0 3	9	
19	63	F	Lower lip	Squamous cell ca	4	20	1	20	
20	63	F	Lower lip	Squamous cell ca	4	20	1	20	
21	63	F	Lower lip	Squamous cell ca	4	20	1	20	

Table 2

R.IFN alpha 2c. Squamous cell carcinoma of the lower lip

PATIENT									
No	Age	Sex	Tumor site	Pathohistol findings after treatment	Duration of treat (weeks)	No of applic	Single dose (x106	Total units (x106	Clinical evaluation
1	55	M	Lower lip	Squamous cell ca	4	20	2	40	+
2	56	M	Lower lip	Squamous cell ca	4	20	2	40	+++
3	81	F	Lower lip	Squamous cell ca	4	20	2	40	+
4	64	M	Lower lip	Sine tm	4	20	5	100	++++
5	54	M	Lower lip	Squamous cell ca	4	20	5	100	++
6	38	M	Lower lip	Squamous cell ca	4	20	5	100	+++

Table 3

HNLI. Basal cell carcinoma of the upper lip

PATIENT									
No	Age	Sex	Tumor site	Pathohistol findings after treatment	Duration of treat (weeks)	No of applic	Single dose (x106	Total units (x106	Clinical evaluation
1	68	M	Upper lip	Sine tm	4	20	1	20	++++
2	56	M	Upper lip	Sine tm	4	20	1	20	++++
3	56	M	Upper lip	Sine tm	4	20	1	20	++++

The results of HNLI therapy in three patients with basal cell carcinoma of the upper lip are presented in Table 3 All the three patients were cured, as according to the histophatologic and clinical findings there were no cancer cells in the biopsy material taken after the HNLI treatment

A comparison of the clinical and immunohistologic evaluation showed that a massive infiltration of lymphoid cells with numerous macrophages was always followed by the disappearence of tumorous cells and by clinical cure of the lesions The results of the clinical evaluation and immunohistologic evaluation were in agreement in all the patients

DISCUSSION

Interferons are cytokines induced in cells by a variety of stimulants The effect of the interferon action is that cancer cells multiply more slowly and become less invasive, and that the immune system acts more effectively, which leads to the necrosis of tumor cells Interferon might have a maturational effect on tumor cells[9 12] Are there any signs that HNLI, which is a mixture of many subtypes, differs in terms of clinical efficacy? Although we would not expect r IFN alpha 2c to be more efficacious than HNLI, it seems that in the case of lip cancers, HNLI is more effective than r IFN alpha 2c, because the complete response was 48% as compared to 17% in favor of HNLI, and the response rate was 81% as compared to 67%, again in favor of HNLI Not only was the complete response rate higher, but the necessary doses of HNLI to achieve the complete response were of a lower concentration

We could not find any correlation with the size of the lesion and treatment outcome In most cases of lower lip carcinoma, the duration of successful treatment was four weeks Immunohistologic findings were the same in the cases of squamous and basal cell carcinomas, and in the cases of carcinomas of the breast and cervix[2 9 10] The degree of infiltration of tumor cell stroma with lymphoid cells and macrophages depends on the tumor cells invasiveness The less invasive the cancer cells, the stronger the infiltration of the stroma by lymphoid cell and macrophages, and *vice versa*

The prognosis for lip carcinoma with minor lesions is excellent and cosmetically good; but major lip carcinomas are always a big surgical problem and require surgical reconstruction of the lip Interferon can therefore be considered another important therapy for lip carcinoma, either with or without surgery

The recommended route of administration is intralesional, while the schedule of treatment should be 3-5 times *per* week for four weeks The recommended single dose of recombinant Hu IFN alpha 2c is $2x10^6$ I U, and of HNLI $0.5x10^6$ I U

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SUMMARY

The response rate in 21 patients with lower lip squamous cell carcinoma treated with HNLI, measured by the size reduction of more than 25% , was 81%, and the complete response rate, i e the percentage of patients cured according to histopathologic and clinical findings, 48% The response rate in six patients with lip

squamous cell carcinoma treated with r IFN alpha 2c, measured by the size reduction of more than 25%, was 67%, and the complete response rate 17% Three patients with basal cell carcinoma of the upper lip were treated with HNLI All three patients were cured, as according to histopathologic and clinical findings, there were no cancer cells in the biopsy material taken after the HNLI treatment Interferon can therefore be considered another important therapy for lip carcinoma, either with or without surgery

RAK USNICE TERAPIJA INTERFERONOM

S A Ž E T A K

Dvadesetjedan bolesnik sa skvamoznim karcinomom donje usnice liječen je s humanim prirodnim leukocitnim interferonom. U 17 bolesnika ili 81% slučajeva dimenzije tumora su smanjene više od 25%, a potpuno izliječenje, prema histopatološkom i kliničkom nalazu, postignuto je u 10 bolesnika ili 48% slučajeva

U šestoro bolesnika sa skvamoznim karcinomom donje usnice liječenih s r.IFN alpha 2c postignuto je potpuno izliječenje prema histopatološkom i kliničkom nalazu u jednoga bolesnika ili 17%, a u četvoro bolesnika ili 67% opseg tumora smanjen je za više od 25%.

Troje bolesnika s bazalnim karcinomom gornje usnice liječeno je s humanim prirodnim leukocitnim interferonom Svo troje bolesnika izliječeno je prema histopatološkom i kliničkom nalazu, a u bioptičkom materijalu nisu nađene tumorske stanice. Interferon se može smatrati još jednom važnom terapijom karcinoma usnice

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BREITENVARIATIONEN DES OS ETHMOIDALE UND SPHENOIDALE IN BEZUG AUF DAS GESCHLECHT UND ALTER

EINFUHRUNG

Bei der Endoskopie der Nase und der Nasennebenhöhlen, den endoskopischen Eingriffen, der transorbitalen Dekompression des Canalis opticus und der Fissura orbitalis superior kommt es gelegentlich zu ernsten, oft irrepablen Komplikationen[2 5 7,8 9 10]. Das ethomoidale Labyrinth und der Sinus sphenoidalis wurden zwar von vielen Autoren rontgenologisch und an Ct Aufnahmen studiert[1 3 4 6 11], jedoch ohne auf die erwahnten Komplikationen hinzuweisen.

MATERIAL UND METHODEN

Um die Verhaltnisse des Sinus ethmoidalis und sphenoidalis bei den oben erwahnten Eingriffen näher beschreiben zu können, wurden die Sinuse an 184 axialen CT Aufnahmen (102 mannliche und 82 weibliche) vom 1. bis zum 80. Lebensjahr untersucht Die Messungen wurden an drei Stellen der CT Aufnahmen in der Höhe des Canalis opticus durchgeführt am vordesten Teil und an der breitesten Stelle des Os ethmoidale, und am Os sphenoidale

BEFUNDE

Auf Grund der Messungen wurden 6 mögliche Variationen gefunden Typ I · Der mittlere Durchmesser ist am längsten so dass die gesamte Struktur eine Fassform aufweist Dieser Typ kommt am haufigsten vor (66.30%). Typ II . der vordere Durchmesser ist am kurzesten (26.10% der Falle) Typ III Der Sinus weist eine Trichterform auf, da der hintere Durchmesser am kürzesten und der vordere am langsten ist (3.27%) Beim Typ IV sind alle drei Durchmesser fast gleicher Lange (2,17%). Beim Typ V ist die Lange der zwei vorderen Durchmesser identisch, wahrend der hintere Durchmesser kurzer ist (1.08%) Der Typ VI weist eine Sanduhrform auf, da der mittlere Diameter am kurzesten ist. (1,08%, s. Abb 1,6)

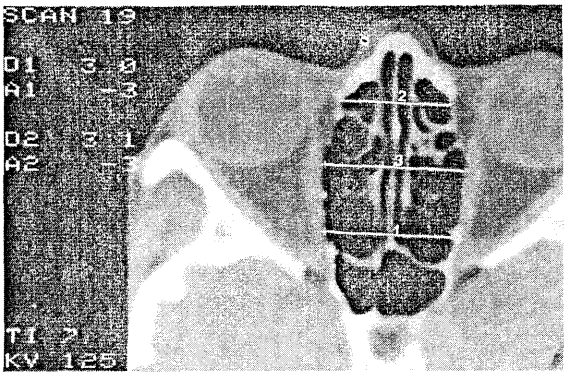


ABB.1. Typ I. Der mittlere Diameter (die breiteste Stelle des Os ethmoidale) (3) ist am längsten - Fassform. Der vordere Diameter (2) und der hintere Diameter (1) sind kürzer. Da an diesen Skenogramen auch andere Messungen durchgeführt worden sind, ist der hintere Diameter etwas mehr vorne statt im Bereich des Os sphenoidale eingezeichnet.

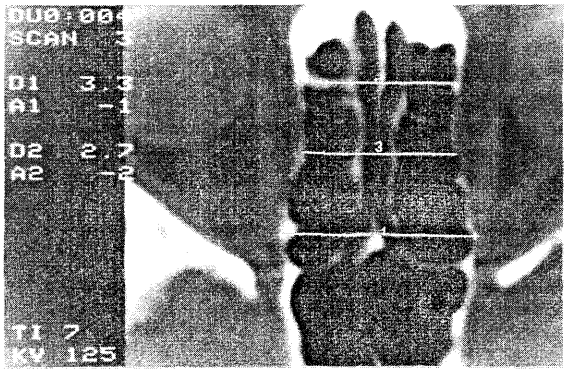
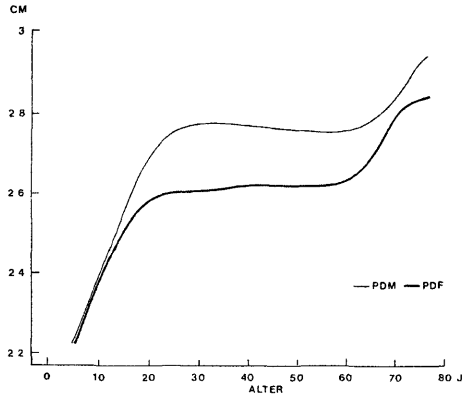
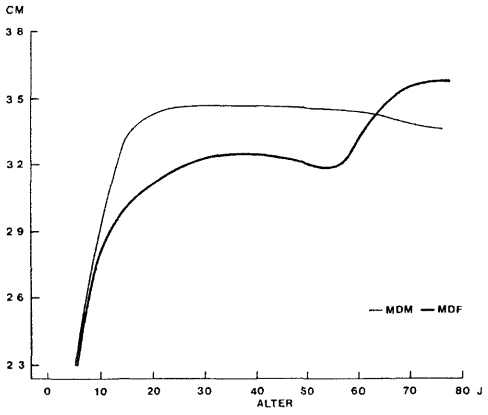


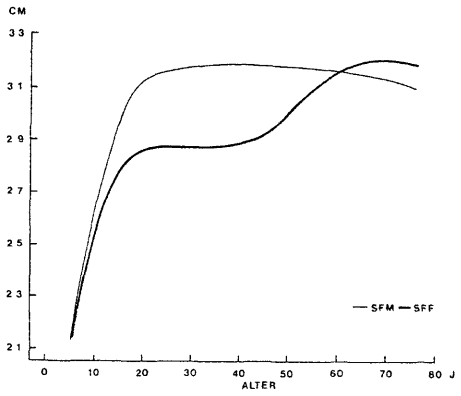
ABB.2. Typ VI. Der mittlere Diameter (die engste Stelle des Os Ethmoidale) (3) ist am kürzesten - Sanduhrform, während der vordere (2) und hintere Diameter (1) länger sind. Da an diesen Skenogramen auch andere Messungen durchgeführt worden sind, ist der hintere Diameter im hintersten Teil des Os ethmoidale statt im Bereich des Os sphenoidale eingezeichnet.



D 1 Diagram der Breitenvariationen des vorderen Diameters im männlichen (dünne Linie) PDM und weiblichen Geschlecht (dicke Linie) PDF



D 2 Diagram der Breitenvariationen des mittleren Diameters im männlichen (dünne Linie) MDM und weiblichen Geschlecht (dicke Linie) MDF



D 3 Diagram der Breitenvariationen des hinteren Diameters im männlichen (dünne Linie) SFM und weiblichen Geschlecht (dicke Linie) SFF

Beim Vergleich aller drei Diameter der beiden Geschlechtern ist ein deutlicher Anstieg der Werte erkennbar Der Anstieg ist steil bei beiden Geschlechtern vom 15 oder 20 bis zum 25 Lebensjahr (Diagramme 1-3)

Beim männlichen Geschlecht steigen die Werte von 2,2 cm bis auf 2,8 cm für den vorderen, von 2,3 cm auf 3,5 cm für den mittleren und von 2,1 cm auf 3,2 cm für den hinteren Diameter

Beim weiblichen Geschlecht steigen die Werte von etwa 2,2 cm auf 2,6 für den vorderen, von 2,3 cm auf 3,2 cm für den mittleren und von 2,1 cm auf 2,9 cm für den hinteren Diameter.

Beim männlichen Geschlecht bleiben die Werte nach dem 25. Lebensjahr auf der erreichten Höhe, d. h für den vorderen Diameter auf etwa 2,8 cm, für den mittleren auf 3,5 cm und für den hinteren auf 3,2 cm Der grösste Anstieg der Werte ist bei dem mittleren und hinteren Diameter ersichtlich

Beim weiblichen Geschlecht steigen die Werte nach dem 15 bzw 25. Lebensjahr von 2,6 cm auf 2,9 cm für den vorderen Diameter, von 3,2 cm auf 3,6 cm für den mittleren und von 2,9 cm auf 3,2 cm für den hinteren Diameter Besonders steil steigen die Werte beim weiblichen Geschlecht zwischen dem 45 und 60 Lebensjahr an.

DISKUSSION

Nach dem ersten Anstieg der drei Diameterwerte, d. h bis zum 25 Lebensjahr, anderen sich am wenigsten Diameter beim männlichen Geschlecht. Beim weiblichen Geschlecht andert sich am wenigsten der vordere Diameter Die beiden anderen zeigen einen bedeutenden Anstieg der Werte.

Angeblich ist eine stärker ausgeprägte Osteoporose für einen steilen Anstieg der drei Diameterwerte beim weiblichen Geschlecht nach dem 45 bzw dem 60 Lebensjahr verantwortlich. Dem Verhalten der drei Diameter gemass, sind für die Operationen bei beiden Geschlechtern Typen III (Trichterform), IV. (alle Diameter gleich lang) und VI. (Sanduhrform) ausserst gefährlich. Bei beiden Geschlechtern weist eine ersichtlich maximale Steigerung der Werte des mittleren Diameters (die breiteste Stelle des Os ethmoidale) auf eine haufigst vorkommende Fassform (Typ I.) der Strukturen auf.

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ZUSSAMMENFASSUNG

Auf Grund der axialen CT Aufnahmen des ethmoidalen und sphenoidalen Sinus sind bei beiden Geschlechtern 6 Typen zu unterscheiden Am häufigsten war der fassformige Typ I (66,30%) Ein kurzerer vorderen Durchmesser wurde beim Typ II erkennbar Die beiden anderen waren fast gleichermassen lang (26,10%) Bei Typ III war der hintere Diameter am kurzesten, so dass die Strukturen eine Trichterform vortauschten (3.27%) Die Lange aller drei Diameter war beim Typ IV identisch (2,17%) Die beiden vorderen Durchmesser beim Typ V waren gleicher Lange, wahrend der hintere am kurzesten war (1,08%) Der Typ VI wies eine Sanduhrform auf, da der mittlere Diameter am kurzesten war (1,08%). Maximal gefahrlich bei chirurgischen Eingriffen scheinen Typen III , IV und VI zu sein

Im Bezug auf die Lange der Diameter ist bei beiden Geschlechtern ein steiler Ansteig der Werte zwischen dem 15. und 25 Lebensjahr zu beobachten. Nach diesem Zeitpunkt verhalten sich die Werte beim mannlichen Geschlecht mehr oder weniger auf derselben Hohe, d h. zwischen 2,8 und 3,5 cm Beim weiblichen Geschlecht ist ein zweiter steiler Anstieg der Werte zwischen dem 45, und 60. Lebensjahr von 2,6 cm auf 3,6 cm erkennbar, was mit der bei Frauen haufig vorkommenden Osteoporose in Zusammenhang gebracht werden kann

SEX-AND AGE-RELATED VARIATIONS IN THE ETHMOIDAL AND SPHENOIDAL SINUS WIDTH

SUMMARY

Axial CT images of the ethmoidal and sphenoidal sinuses taken at the level of the optic canal revealed six types of nasal cavity shapes in a group of 102 male and 82 female subjects The barrel-shaped type I, in which the medial diameter is the widest, was most frequently observed (66 30%) In type II, where the anterior diameter is the shortest and the other two diameters are approximately identical, was found in 26 10% of the study subjects. Type III, characterized by the shortest posterior diameter, resulting in a funnel-shaped nasal cavity, was present in 3 27% whereas type IV with all the diameters approximately identical was found in 2 17% of the subjects. Type V has a reverse type II shape, i e the two anterior diameters are identical, and the posterior diameter is shorter (1 08%) In type VI, the medial diameter is the shortest, so that at the level of the optic canal the nasal cavity is shaped like a sand-glass (1 08%) Accordingly, types III, IV and VI are considered most inappropriate for surgical intervention.

Comparison of the curves obtained for all nasal cavity diameters in both sexes revealed a marked increase in the values between the age of 15 and 25 Thereafter, in men the values of all the three diameters remained at the same level, i.e ranged between 2 8-3 5 cm In females, the values significantly increased between the age of 40 and 60, ranging from 2 6 to 3 6 cm, which could probably be ascribed to osteoporotic alterations which are more pronounced in women

VARIJACIJE ŠIRINE ETMOIDNOGA I SFENOIDNOGA SINUSA S OBZIROM NA SPOL I DOB

S A Ž E T A K

Na aksijalnim CT snimkama etmoidnoga i sfenoidnoga sinusa, učinjenima u visini optičkoga kanala, ustanovili smo u oba spola (102 muškarca i 82 žene) šest vrsta oblika nosne šupljine Najčešći nalaz je tip I (66,30%) bačvasta oblika, gdje je srednji promjer najveći Kod nalaza tipa II najkraći je prednji promjer, a ostala dva promjera su približno jednaka (26,10% nalaza) Tip III ima najkraći stražnji promjer (3,27% nalaza), tako da nosna šupljina u takovim nalazima ima oblik lijevka. Kod nalaza tipa IV su svi promjeri otprilike jednaki (2,17% nalaza). Tip V ima obrnuti oblik tipa II, tj. oba su prednja promjera jednaka, dok je stražnji promjer najkraći (1,08% nalaza) Kod tipa VI najkraći je srednji promjer (1,08% nalaza), tako da nosna šupljina na razini optičkoga kanala ima oblik pješčanoga sata Za kirurške su intervencije najnezgodniji te stoga i najznačajniji tipovi III, IV i VI

Uspoređujući krivulje svih tipova promjera u oba spola, nalazimo velik porast vrijednosti od 15 do 25 godine. Nakon toga vremena, u muškaraca vrijednosti za sva tri promjera ostaju na istoj razini, tj. kreću se od 2,8 do 3,5 cm. U žena dolazi do značajna porasta tih vrijednosti u razdoblju između 40. i 60 godine života. Vrijednosti se u tom razdoblju kreću od 2,6 do 3,6 cm. Razlog takva porasta treba vjerojatno tražiti u osteoporotskim promjenama koje su izraženije u žena

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ON ENVIRONMENTAL/OCCUPATIONAL CANCER EPIDEMIOLOGY

INTRODUCTION

The great majority of cancers occur in epithelial tissue. This reflects the interaction between host factors (such as genetic endowment, health and nutritional status) and the external environment Genetic factors alone are believed to be responsible for only a small fraction of cancers[(1)].

In an approximation made by Doll and Peto[(2)], occupational exposure is involved in about 5% of cancer cases, dietary habit in 35%, cigarette smoking in 30%, and other unknown factors in the rest of about 30% of cancer cases

Based on IARC data[(3)] 55 environmental agents (chemicals) are known to be carcinogenic in humans, 45 agents show sufficient evidence for being probable carcinogens and 191 are possibly carcinogenic for humans Considering the fact that 60,000 to 70,000 agents are in current use, only a rather small portion are likely to be carcinogenic

One of the important questions is whether there is a safe or "threshold" level of exposure to environmental carcinogens The impossibility of identifying that level for the diverse human population has led to the assumption that even low-level exposure may have significant impacts. Health impact depends, of course, also on duration of exposure and the potency of the compound.

The types of evidence considered in qualitative analysis of the weight-of-evidence for human carcinogenicity[(4)] are summarized in Table 1.

The advantage of epidemiological studies lies in the fact that the humans, with whom they are dealing, are ultimate indicators of disease This type of study makes possible the evaluation of sensitive populations

The disadvantages of the epidemiological approach are that the studies are generally retrospective (based on death certificates, recall biases etc.) They are insensitive, costly and time-consuming; in this type of study, one often faces lack of appropriate cohorts and difficulties dealing with combination exposures.

Table 1

Types of evidence considered in qualitative analysis of the weight-of-evidence for human carcinogenicity

Primary evidence
Human evidence
Occupational mortality study
Correlation between environmental exposures and cancer incidence in the general population
Case control studies (rarely)
Lifetime animal bioassays (more available than human evidence)
Secondary (supportive evidence)
Short-term tests for mutagenicity, chromosome damage, cell transformation
Cancer screening bioassays, such as initation-promotion tests on mouse skin and pulmonary tumors in a highly susceptible mouse strain
Chemical structure - activity correlations, occurrence of carcinogenic metabolites

OCCUPATIONAL MORTALITY STUDY AND STUDIES ON CORRELATION BETWEEN ENVIRONMENTAL EXPOSURE AND CANCER INCIDENCE IN THE GENERAL POPULATION

For this type of study, continuous information on cancer incidence in the general population is needed. These data are considered as expected (predicted) cancer rates. The rates observed in specific groups occupationally or environmentally exposed to certain incriminated agents are then compared with the expected rates.

In **Croatia**, the Cancer Registry has been in existence for more than 25 years. It provides ample evidence for establishing a correlation between environmental exposures and cancer incidence in the general population.

OCCUPATIONAL EXPOSURE TO VINYL CHLORIDE MONOMER AND ANGIOSARCOMA OF THE LIVER

After the first observation that the occurrence of angiosarcoma of the liver may be associated with exposure to vinyl chloride monomer-VCM[5], a retrospective study was carried out in a factory for the manufacture and polymerization of VCM[6]. The factory started to operate in 1949. By the beginning of the study, 62 exposed workers had alreday died Analysis of the causes of death based on pension insurance data and medical files kept in the factory health unit showed that two workers died from primary carcinoma of the liver, five from bronchogenic carcinoma and eight from other malignant tumors (neoplasmas of the larynx, ribs, breast, spermatic cord, glioma of the brain, melanosarcoma, leukemia, Hodgkin's disease) Two workers with the diagnosis of primary carcinoma of the liver were treated at the local hospital and postmortem biopsy was performed The biopsy material kept at the hospital was rechecked and showed that both workers had angiosarcomas.

The expected rate of angiosarcoma of the liver is very small - about one per 8-9 million people per year In the Cancer Registry of Croatia - since it was founded - not a single case of angiosarcoma has been registered Therefore, the two cases of angiosarcoma could be attributed to the effect of exposure to VCM Occupational histories of both diseased workers showed that they were engaged for 19-20 years in the manufacture of VCM and polymerization process, respectively, and exposed to rather high levels of VCM (40-160 ppm)

PRIMARY CARCINOMA OF THE LIVER AND LUNG/BRONCHUS CARCINOMA IN AN AREA WITH POLYVINYL CHLORIDE INDUSTRY

The finding of angiosarcomas in occupational exposure to VCM was the reason for examining the incidence of carcinoma in a city area in which a polyvinyl chloride (PVC) industry was located[7] The area also housed several other industries with possible carcinogenic exposures As there were literature data indicating that VCM exposure may increase the risk of lung carcinoma, two types of tumors were chosen for the study malignant tumors of the lung and bronchus (162 1 WHO International Classification Code) and cytologically confirmed primary malignant tumors of the liver (155 0 WHO International Classification Code)

At the time of the study, the area had a total population of 185 047, 90 499 men and 94.548 women The city itself comprised 126 000 inhabitants The rest of the population lived in a greater city area which included the northern industrial part of the town

The incidence of malignant bronchogenic (lung) tumors and primary malignant tumors of the liver was analyzed only for a period of four years From the Registry data, a list of the diseased was compiled and followed up Interviews with the patients still alive or in the families of those who had died was also conducted The interview included questions about occupation, place of work, changes of place of residence, smoking habits and some details about medical history and the course of the disease.

Over the 4-year observation period, there were 193 (158 men and 35 women) cases of malignant tumors of the lungs and bronchus, and eight (4 men and 4 women) microscopically verified primary malignant tumors of the liver

Table 2 shows the observed mean annual incidence rates per 100,000 of these tumors compared with the expected rates in Croatia.

Table 2

Mean annual incidence rates per 100,000 of lung/bronchus tumors and primary carcinoma of the liver in the area under study and in Croatia

Tumor site	Raw rates						Age 35 and older *					
	Studied area			Croatia			Studied area			Croatia		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Lung and bronchus	43 7	9 3	26 1	46 4	7 1	26 1	112 1	20 5	63 3	107 8	14 1	56 3
Liver	1 1	1 1	1 1	1 1	0 9	1	2 8	2 5	2 7	2 5	1 8	2 2

* In the area under study, only two cases of tumors of the lung and bronchus were found in persons under 35 years of age (women) During the same period, in Croatia only 47 cases (31 men and 16 women) of tumor of the lung and bronchus, and four cases (men) of liver tumor were found in persons under 35 years of age

Analysis of the incidence of malignant neoplasms according to place of work or residence did not show any dependence as regards the primary cytologically verified tumors of the liver During the observed period, no malignant tumors of the bronchus (lung) were recorded in the PVC industry A higher number of these tumors were recorded in the cement industry (11 men, all employed in manufacturing for 25-40 years, only one non-smoker, and one woman, cleaner in the cement mill for 25 years, non-smoker, mean annual incidence rate per 100,000 men aged 35 and older was 165.4), and in the asbestos cement manufacture (3 men employed for about 40 years, all smokers, mean annual incidence rate per 100,000 men aged 35 and older was 171 6).

Most tumors were recorded in men who smoked. In women, 50% of the tumors occurred in non-smokers

The distribution of tumors according to place of residence within the area in which the PVC industry was situated was approximately the same as that for the entire city. In close proximity of the asbestos cement plant, six cases (4 men and 2 women) of lung/bronchus tumors were recorded Calculated as a rate, it was five-fold for men and ten-fold for women than in the rest of the area

Results of the study did not indicate that the occurrence of the followed up tumors was associated with exposure to vinyl chloride However, in the case of

malignant tumors of the lung (bronchus), they established a possible casual relationship of tumor incidence and some other occupational or ambiental exposures

MALIGNANT TUMORS OF THE RESPIRATORY TRACT, PHARYNX AND PERITONEUM IN AN AREA WITH ASBESTOS PROCESSING PLANT

A study of malignant tumors was also performed in an area with an asbestos processing plant[8,9] Primary malignant tumors of the respiratory tract, pharynx and peritoneum were studied in autochthonic inhabitants of the coastal community where a factory of asbestos textile and friction products has been in operation for more than 30 years.

The study covered a period of 19 years and the observed tumor rates were calculated per average number of inhabitants during that period using census data as a basis: 11,270 inhabitants - 5,560 men and 5,710 women. Data on tumors were collected from death certificates and were additionally checked using medical files from the local health service. The families of those who had developed cancer and died were interviewed in order to get additional information on their occupation, place of work, smoking habits, alcohol consumption and history concerning malignant tumors in the family. The tumor rates in the study area were compared with the expected rates based on the Cancer Registry of Croatia.

Over the observed 19-year period, there were 51 cases of malignant tumors - 40 in men and 11 in women. The distribution of tumors according to the site was as follows lung/bronchus cancer 36 (29 men and 7 women), mesothelioma 5 (2 men and 3 women), pharyngeal cancer 5 (men), laryngeal cancer 4 (3 men and one women), and peritoneal cancer 1 (man).

The mean annual incidence rates (per 100,000) in the area under study as compared with the expected rates for Croatia were lower for all followed up tumors except for mesothelioma which was three-fold that in Croatia. It should be pointed out that two out of five cases of mesothelioma were registered in workers from the asbestos processing plant, one person with mesothelioma was a farmer and two were housewives.

It is interesting that distribution of the observed tumors among/within different villages/towns varied· in some of them, the observed rate was higher compared with the expected rate, as seen in Table 3, suggesting a conclusion that air currents may be responsible for an uneven ambiental asbestos exposure.

Table 3

Malignant tumors of the lung/bronchus, pleura, larynx, pharynx and peritoneum in the area under study, according to place of residence

Settlement	No of inhabitants	Distance from asbestos factory (km)	No of tumors
Bačina	82	1 8	3 (lung/bronchus)
Brist	342	11 8	1 (lung/bronchus)
Gradec	1062	9 7	5 (lung/bronchus)
Ploče	5834	1	8 (lung/bronchus 4, pleura 3, peritoneum 1)
Komin	1428	7 9	13 (lung/bronchus 5, pleura 1, larynx 3, pharynx 4)
Plina	1386		
Plina, east	510	5 5	8 (lung/bronchus 7, pharynx 1)
Plina, west	876	5 7	
Podaca	183	13 6	1 (lung/bronchus)
Rogatin	742	3 5	5 (lung/bronchus 4, larynx 1)
Stoševica	976	7 7	7 (lung/bronchus 6, pleura 1)

A detailed analysis which took into account characteristics of the relief of the area, predominant winds and factors which may determine wind direction, showed that in a small town situated in the westerly wind direction, the rate of pharynx carcinoma was significantly higher than expected In another village, in the direction of southerly wind, lung carcinoma was significantly more frequent than expected. A significantly higher rate of pleural mesothelioma was observed in the town in which the factory was located as well as in the village lying in the direction of the southerly wind. Only one case of mesothelioma was registered in a village situated in the westerly wind direction (the same one with a higher rate of pharynx carcinoma)

Some other data obtained in the study support the assumption that air currents may be responsible for an uneven ambiental asbestos exposure and consequently for an

uneven distribution of tumor incidence among various villages/towns in the area Although the rate of tumors was much higher in smokers than in non-smokers, 1/3 of lung/bronchus tumors were found in non-smokers. Tumor cases were mostly registered in subjects who used to work in the open air farmers made up 39.2% in the total number of tumors, and 17 6% related to housewives, who in the area under study were also engaged in agricultural work

CONTROL CASE STUDIES

GASTRIC AND COLON-RECTAL CARCINOMA IN TWO AREAS OF CROATIA WITH DIFFERENT RISK FOR GASTRIC CANCER

Cancer Registry data for Croatia show a great difference in the incidence of gastric carcinoma between Varaždin-Čakovec and Dalmatia regions Literature analysis shows negative correlations between gastric and colon cancer incidence rates[10] The two regions are known for a substantial difference in nutritional habits of their populations.

The aim of the study was to analyze the reasons for the different incidence rates of gastric carcinoma in the two regions as well as of distinct risk factors for gastric, colon and rectal carcinoma[11].

During the 10-year study, 1,573 people were interviewed, out of whom 313 gastric cancer patients (247 in the high and 66 in the low risk area), 136 patients with colon cancer (39 in the high and 97 in the low risk area), 256 patients with rectal cancer (85 in the high and 171 in the low risk area) and the 868 controls (490 in high and 378 in the low risk area) Subjects from the same risk areas matched by sex and age (± 3 y) who did not have gastrointestinal tract carcinoma or any other acute gastrointestinal disease at the time of interviewing served as controls The questionnaire used consisted of 177 questions on age, region, family history of cancer, education, occupation, previous relevant diseases, nutritional habits in childhood and adulthood, smoking habits and alcohol consumption. Interviews were performed by trained interviewers according to the study protocol The data obtained were processed by computer Univariate analysis of the hierarchical log-linear module and canonic discriminatory analysis were applied to the specially reorganized data. Furthermore, nutritional scores were developed and univariate and multivariate analyses (including mean and median) were applied, followed by discriminatory analysis and predictions.

There was a substantial difference in nutritional habits between the high and low risk areas as well as between controls and all cancer patients in both risk areas. The subjects in the high risk area consumed more food both in childhood and adulthood, particularly some who were thus confirmed as risky. The subjects from the low risk area used to consume, more often than those in the high risk area, only several nutritional items which, with the exception of corn and other starches, are considered protective. These were fish, fruit, green leafy vegetables and whole wheat bread More subjects from the high risk area smoked and consumed alcohol than those from the low risk area

There was a strong childhood-adulthood correlation of nutritional habits in both risk areas and for all diagnostic groups of subjects.

There was a significant difference in nutritional habits between the controls and all three diagnostic groups in both risk areas. Based upon the results, the following food appeared to be risky for gastric carcinoma in childhood-pork, corn, pickled food, butter, and in adulthood corn, pickled food, eggs, dry cured meat and smoked food, animal fat, fried and barbecued (on charcoal) food, alcohol and smoking. Protective nutrition for gastric carcinoma included· in childhood regular consumption of green leafy vegetables, veal/lamb, general vitamins, and in adulthood green leafy vegetables, veal/lamb, milk, fish, vitamins and cooking as a mode of food preparation For some nutritional items, neither risky nor protective effect could be confirmed. Such were especially beef and pork in adulthood, as well as cheese consumption both in childhood and adulthood

For colon carcinoma protective nutrition consisted of. in childhood-cereals, bread and fish, in adulthood fish, fiber food, cottage cheese Risky nutrition included: in childhood corn, beef and pork, in adulthood whole milk, ice-cream, pork, dry-cured and smoked meat, animal fat and frying as a mode of food preparation

For rectal carcinoma protective nutrition was. in childhood - milk, especially vitamins, in adulthood fresh citrus fruit, other fresh fruit and vegetables, cereals, cooking as a mode of food preparation and low fat food Risky nutrition included in childhood corn and nuts, and in adulthood corn, eggs, nuts, sugar, pickels and hot, spicy food

The patients from the high risk area smoked more and drank more alcohol than those from the low risk area.

Based upon the results of the study, a nutritional profile of gastric, colon and rectal cancer patients is presented.

A person at an increased risk of developing gastric cancer is considered. aged 50 to 80 years, with a family history of gastric carcinoma or some other related disease, resident of a rural area and having a rather low standard of living, who in childhood used to eat corn, fatty food and spicy food and less food rich in vitamins, who consumed more pork meat and beef meat than poultry and fish, who more often eats fried and barbecued than cooked food, consumes pickled food and smoked food and eggs, does not often eat vegetables and fruit, does not drink too much milk, does not take vitamins, and smokes and drinks alcohol regularly

A person at an increased risk of developing colon cancer, and this to a certain extent relates to rectal cancer, is considered aged 50 to 70 years, with a history of carcinoma or positively correlated diseases in the family, who is overweight, not moving much (sedentary job), smokes, has a disease correlated with colon-rectal cancer (such as increased arterial blood preasure, intestinal polyposis), consumes more fat and "red" meat (pork and beef meat) than poultry and fish, more often eats dry-cured, barbecued, fried and processed food (canned food, cured and smoked food) than cooked

and roasted, as well as spicy food, consumes fruit and vegetables only occasionally, smokes, drinks alcohol in large amounts, and does not take enough vitamins A, C, and F

CONCLUSIONS

The aim of this paper was to elucidate, with the help of examples, the potential role of epidemiology in the research of cancer prevalence As seen from the presented examples, epidemiological observations can be used to detect the risks that have been overlooked or only suggested tentatively by laboratory tests, to check the correctness of conclusions on the cause of hazard, and to estimate the level of exposure that produces the highest additional risk of the disease

One of the major difficulties is identifying a possible low relative risk for common cancer, such as lung cancer, compared to a higher risk for rare cancer, such as mesothelioma or angiosarcoma. The former will not be as easy to discern from the background of cases possibly due to many other causes, whereas the latter will be more noticeable because it may be somewhat bizarre[12] In terms of the attributable number of cases that may be associated with the environmental/occupational exposure, the former (low relative risk) is likely to be more important than the latter (high relative risk)[13].

An important aspect in environmental and occupational epidemiology of cancer is to try to get more information into the way the exposure hazards interact with other possible causes and with some other factors, such as the age of the individual when first exposed. The question is also whether relative risks should be expected to increase progressively with duration of exposure or increase to a stable plateau, whether they generally remain constant or fall after the exposure ceases[14].

The advantage of epidemiological methods that study the real-life human situation is offset by the unfortunate necessity of allowing a natural experiment to play out. Thus, epidemiology cannot provide an early warning system essential to cancer prevention However, the epidemiological studies have to be involved in the procedures for risk assessment, which is the basis for risk management

At present, a need is recognized to incorporate multiple process into risk assessment models[15] . This means a transition from empirical models that are basically curve-fitting to physiologically based mechanistic models. Improvement of understanding of the concepts needed to physiologically based models as well as data needed to drive them include, among others, evidence obtained by epidemiologic studies

A novel approach in which epidemiology can also be involved is in merging sophisticated and highly sensitive laboratory methods with analytical epidemiological methods Conceptually, the bridge between the two disciplines - laboratory studies of chemical carcinogenesis and epidemiology - is provided by biological markers that

reflect molecular or biochemical changes resulting from exposure to carcinogens[1]. As known, a number of biological markers can now be measured by laboratory assays on human body fluids, cells or tissues in order to provide quantitative measures of the dose or adverse biological effects of environmental carcinogens Under development are markers that may indicate susceptibility to cancer.

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SUMMARY

Experience in using epidemiological methods for investigating cancer incidence from the point of view of occupational and environmental exposures is reviewed.

Results of the retrospective studies on the occurrence of angiosarcoma of the liver in occupational exposure to vinyl chloride monomer, as well as on primary carcinoma of the liver and lung/bronchus carcinoma in an area with polyvinyl chloride industry are presented

Malignant tumors of the respiratory tract, pharynx and peritoneum were studied in an area with an asbestos processing plant

In addition to these types of studies on correlation between exposures and cancer incidence in selected occupational groups and general population, the results of a case control study in two areas of Croatia with different risks for gastric cancer are summarized

The role, advantages and limitations of the epidemiological approach are discussed.

EPIDEMIOLOGIJA RAKA I PROFESIONALNA I AMBIJENTALNA IZLOŽENOST

SAŽETAK

Uvodno se razmatraju procjene o uzrocima raka, posebice s obzirom na moguću ulogu profesionalne izloženosti karcinogenima. Iznose se podaci o broju kemijskih spojeva s utvrđenim te mogućim karcinogenim učinkom Raspravlja se ukratko o vrsti dokaza u kvalitativnoj analizi humane karcinogenosti Sumiraju se prednosti i nedostaci epidemiološkoga pristupa u proučavanju karcinogenih učinaka različitih ekspozicija.

Težište rada je na prikazu vlastitih iskustava u primjeni epidemioloških metoda u istraživanju incidencije pojedinih vrsta raka Izloženi su rezultati retrospektivne studije o pojavnosti angiosarkoma jetre u profesionalnoj izloženosti vinil kloridu monomeru, o učestalosti primarnih malignoma jetre i pluća na području s tvornicom za proizvodnju polivinilklorida, te o karcinomu pluća, mezoteliomu, karcinomu dušnika, ždrijela i potrbušnice u stanovnika područja s tvornicom za preradu azbesta Prikazani su i rezultati istraživanja tipa "case control" studije o raku želuca, debeloga i završnoga

crijeva na dva područja u Hrvatskoj s različitim rizikom u odnosu na incidenciju raka želuca. U ovom je istraživanju posebna pozornost bila usmjerena na ocjenu moguće uloge prehrane, odnosno prehrambenih navika, u pojavnosti tih oblika raka

Zaključno se govori o mjestu i dosegu epidemiologije u istraživanju raka i ocjeni rizika u profesionalnoj i ambijentnoj izloženosti karcinogenima.

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ELECTROCARDIOGRAM IN PATIENTS WITH *ANOREXIA NERVOSA*

INTRODUCTION

Anorexia nervosa is a disorder characterized by reduced food intake due to a fear of gaining excess body weight[1] It is most frequently encountered in healthy young women aged 15-34 years The patients either take no food or consume it in quantities lower than minimal daily requirements, with a relatively common use of diuretics, laxatives or induced vomiting Four to thirty percent of the patients may suffer a lethal outcome[2,3] Sudden death has been described due to malignant alterations of the ventricular rhythm[4,5] in patients with anorexia nervosa as well as in patients who have attempted to reduce body weight by use of liquid protein A prolonged Q-T interval was recorded several days prior to death in both anorexia nervosa patients and in those who had been consuming a liquid-protein diet[4,5] It has been established that a prolonged Q-T interval is associated with an increased arrhythmogenic risk of death as the result of malignant ventricular arrhythmias. The Q-T interval marks the electrical refractory period of the myocardium which, if prolonged, causes an uneven recovery of the excitable fibers of the myocardium. For that reason, some impulses may encounter unevenly recovered parts of the ventricular muscle and may lead to paroxysmal ventricular tachycardia and polymorphous ventricular tachycardia (torsade de pointes), which may lead to ventricular fibrillation[6] The aim of this study was to analyze the corrected Q-T interval measured in lead II in patients with anorexia nervosa, as compared to the findings in a control group, and to examine dispersion of repolarization by studing the Q-T interval in each of the 12 ECG leads, because an increased dispersion is a prediction of malignant ventricular arrhythmia. The aim of the study was also to compare the body mass index and Q-T interval in patients with anorexia nervosa, and to analyze the relative risk for a prolonged Q-T interval in both groups

PATIENTS AND METHODS

The study was performed on 30 patients with anorexia nervosa There were 26 female and four male patients aged 15-48, mean age 25 00±8 66 years

The diagnosis of anorexia nervosa was made according to the following criteria.

1 first appearance of the disease before the age of 25,

2 reduced body weight by at least 25% of the ideal body weight,

3 change of attitude toward food (starvation) and toward the environment (indifference),

4 elimination of an organic disease as a reason of weight loss,

5 elimination of other psychologic factors, and

6 at least two of the following criteria

a amenorrhea,

b sinus bradycardia,

c hyperactivity,

d bulimia, and

e vomiting

The severity of the disease was estimated by the body mass index[7],

$$\text{BMI} = \frac{\text{body mass/ kg}}{\text{body height/ m}^2}$$

(normal values 21 0-24 9)

A control group consisted of 30 patients, with the disease entities of oscillating arterial hypertension, ulcer disease, nonspecific cardiac oppressions, acute pyelonephritis or acute exacerbations of chronic pyelonephritis The group was selected at random from the patients treated at the cardiology clinic for several years preceding the study It included 19 women and 11 men aged 24-46 (mean 32 4±24 1 years) The Q-T interval was analyzed in the second standard lead distance from the beginning of Q interval to the end of T wave was recorded, or from the beginning of the R wave, if the Q wave was absent All Q-T intervals of that lead were analyzed, with the longest value taken for this analysis Q-T intervals were measured in seconds and corrected for heart beat frequency by the following equation[8 9]

$$\text{corrected Q} - \text{T interval} = \text{sec} \frac{\text{measured Q - T interval}_{\text{sec}}}{\sqrt{\text{R - R interval}_{\text{sec}}}}$$

The upper normal value of corrected Q-T interval is 0.422 and 0 423 sec for men and women, respectively In both groups, three consecutive cycles were measured in all of the 12 ECG leads and the level of difference between the minimum and maximum of the corrected Q-T interval was calculated On that basis, dispersion of the corrected Q-T interval was estemated in patients with anorexia nervosa and in control patients[10-13] Sodium and potassium serum concentrations were determined in all patients The relative risk for prolonged Q-T interval (RR) was calculated by the equation[10]

$$RR = \frac{a \times d}{b \times c}$$

The value is significant if higher than one In the equation, a denotes prolonged corrected Q-T interval in patients with anorexia nervosa, b is the normal value of corrected Q-T interval in patients with anorexia nervosa, c is prolonged corrected Q-T interval in the control group, and d is the normal value of corrected Q-T interval in the control group.

The correlation of corrected Q-T interval to body mass index was assessed by Spearman's method of range-correlation[14] and was also calculated employing Mann-Whitney's non-parametric test comparing two samples

RESULTS

The values of body mass index and corrected Q-T interval in the patients with anorexia nervosa are presented in Figure 1. Six out of 30 patients were found to have a prolonged Q-T interval with the values of 0 424 and 0 461 sec. Only one out of 30 patients in the control group had a prolonged Q-T interval of 0 424 sec, i e. 3% as compared to 20% in the group of patients with anorexia nervosa There was no statistically significant correlation between corrected Q-T interval and body mass index (r=+0 072, p=0 145) The analysis of the relative risk in patients with anorexia nervosa who had a prolonged Q-T interval and the patients without prolonged Q-T interval, as compared with the control group with prolonged corrected Q-T interval and the same group without it, produced a high value of 7 3

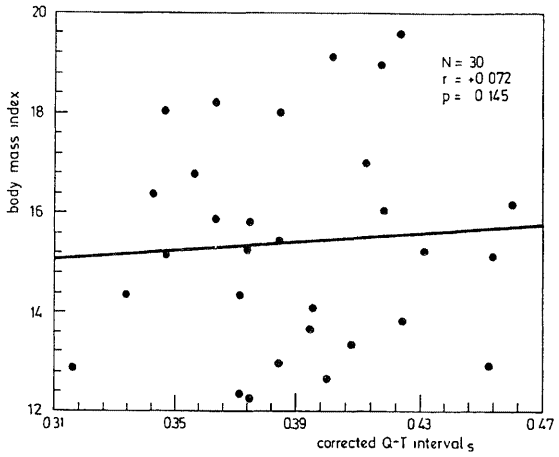

FIGURE 1 The relationship between corrected Q-T interval and body mass index in patients with anorexia nervosa

No patient in either group was found to have hypocalcemia or hypokalemia Dispersion of the corrected Q-T interval in msec in the patients with anorexia nervosa and in the control group, and the difference between the maximal and minimal Q-T interval are presented in Figure 2 The patients with anorexia nervosa were found to have a corrected Q-T interval of 26-116 msec (mean 59 63), as compared to 17-53 msec (mean 31 37) in the control group

Table 1
Dispersion of corrected Q-T interval: mean and standard deviation in patients with anorexia nervosa and control group

	N	Longest Q-T interval msec	Shortest Q-T interval msec	Mean	SD	Statistical singnificance z
Anorexia nervosa	30	116	26	59 63	24 12	1 374 *
Control group	30	53	17	31 37	9 97	

* Trialed probability of equaling or exceeding z=1 3740

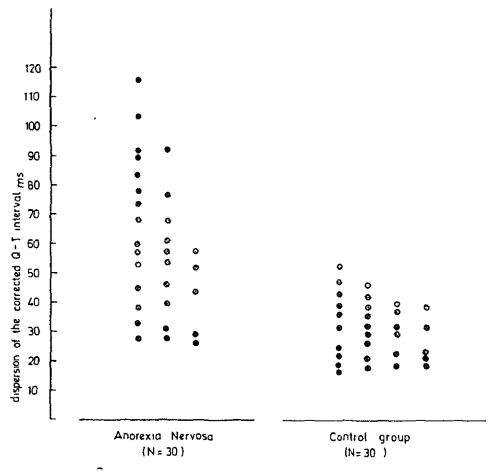

FIGURE 2 Dispersion of the corrected Q-T interval in ms in patients with anorexia nervosa and in the control group

DISCUSSION

There are many reasons for a prolonged Q-T interval. It may be congenital or acquired, as in patients with anorexia nervosa In our study, 20% of the 30 patients with anorexia nervosa were found to have a prolonged corrected Q-T interval The frequency of such a change described in the literature varies between 5 out of 9 to 0 out of 11, when only the Q-T interval alone was analyzed without correction for heart frequency[15,16]

In patients with anorexia nervosa, mortality is rather high, even up to 30%[2,3] When associated with the corrected prolonged Q-T interval, this condition leads to malignant arrhythmias Pathomorphology has demonstrated the heart weight to be reduced to half of the normal values in patients who died of anorexia nervosa associated with a prolonged corrected Q-T interval Histopathology has demonstrated an increased deposition of lipofuscin, conglomerates of the mononuclear inflammatory cells in the interstitial space, around the nerves and ganglionic cells and in the ventricular septum[17], whereas there were no changes in the sinus node, atrioventricular node or bundle of His Some of these changes may be the reason for impulse conduction abnormalities with consequent prolongation of the corrected Q-T interval, but the exact pathogenesis of the prolonged corrected Q-T interval still remains an open question[5] It has been postulated that such patients have pathologic changes in the

hypothalamus-pituitary axis, the result of which may be the prolonged corrected Q-T interval with malignant alterations of the heart rhythm[14]. It has been observed that patients with acute intracranial events may develop a prolonged Q-T interval with consequent malignant arrhythmia[18-20]. Malignant ventricular dysrhythmia may also be initiated by changes in the relationship between the left and right cervicothoracic ganglion or dysbalance of sympathetic innervation of the heart reduced activity of the left fibers (stellate ganglion - a direct pathway from brain to heart)[19,21-23] Changes between the sympathetic and parasympathetic tone may also influence prolongation of the corrected Q-T interval, which leads to the connection between the brain and heart by the autonomic nervous system as well[20] Other contributing factors may be changes in the concentration of electrolytes, such as hypocalcemia, hypokalemia. hypomagnesemia[24,25], sometimes seen in patients with anorexia nervosa Yet, neither our findings nor the reports of some other authors[4 5] have encountered an electrolyte dysbalance in the patients with anorexia nervosa

In this study, we attempted to evaluate the ratio between body mass index and corrected Q-T interval The index is affected by the height and mass of the body, the latter being of more importance in adults[26] We did not establish any connection between body mass index and prolonged Q-T interval.

Twenty percent of the patients with anorexia nervosa and only three percent of the control patients had a prolonged Q-T interval measured in lead II with a relative risk of 7 3 This lead us to the conclusion that the anorexia nervosa patients carried a much higher risk for a prolonged Q-T interval than the control group In our study, the prolonged Q-T interval measured in lead II was 0 424 and 0 461 sec, while data on three deceased patients in Boston were 0 46 - 0 61 sec[5] However, analysis of the corrected Q-T interval using three consecutive cycles in each of the 12 ECG leads revealed dispersion of the corrected Q-T interval of 116 msec in the patients with anorexia nervosa as compared to 53 msec in the control group We also observed a different cycle length in different leads and were not able to determine lead II as a reference one with a constant corrected Q-T interval length The corrected Q-T interval varied with all the 12 ECG leads both in the patients with anorexia nervosa and in the control group During the analysis of the corrected Q-T interval, it is necessary to measure this parameter in each of the 12 ECG leads and to use the longest value The Q-T interval dispersion was much longer in our patients with anorexia nervosa than in the control group, but less than in the patients with Romano-Ward or Jervell or Lange-Nielsen syndrome[10], in whom the authors found a mean dispersion of 178 msec with a maximum of 645 msec

At present, it is rather difficult to determine the threshold of corrected Q-T interval which might lead to malignant arrhythmias (it may develop irrespective of the corrected Q-T interval)[18-21] According to our results, the management of patients with anorexia nervosa should include careful analysis of electrocardiograms, and prospective investigations of this problem are obviously required We conclude that an abnormality in cardiac repolarization occurs in anorexia nervosa and may cause sudden death in this syndrome

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SUMMARY

Thirty patients with anorexia nervosa and thirty controls had an electrocardiogram with a corrected Q-T interval Prolonged Q-T interval was found in six out of 30 (20%) patients with anorexia nervosa and in one out of 30 (3%) patients from the control group No correlation was found between body mass and corrected Q-T interval measured in lead II in the patients with anorexia nervosa (r=+0.07) In the patients with anorexia nervosa, the relative risk of Q-T interval prolongation was high (7 3) as compared to the control group This conclued that the risk of Q-T interval prolongation in electrocardiogram was higher in the patients with anorexia nervosa than in the controls. It is well known that a prolonged corrected Q-T interval is associated with a high risk of sudden death due to malignant alterations of the ventricular rhythm During the analysis of the corrected Q-T interval in the patients from both groups, the corrected Q-T interval dispersion of up to 116 msec (mean value, 59 63±9 97) in the controls was observed, varying from lead to lead Therefore, it was not enough to measure the corrected Q-T interval from the second ECG lead only, but to analyze it in each of the 12 ECG leads A statistically significant difference was observed between the two samples (z=1 3740)

ELEKTROKARDIOGRAM U BOLESNIKA S ANOREKSIJOM NERVOZOM

S A Ž E T A K

U 30 bolesnika s anoreksijom nervozom i 30 bolesnika kontrolne skupine analiziran je elektrokardiogram s osobitom pozornosti na korigirani QT interval. Produžen korigirani QT interval ustanovljen je u šestoro bolesnika (20%) s anoreksijom nervozom i jedne osobe (3%) iz kontrolne skupine. Nije nađena povezanost između indeksa tjelesne mase i korigiranoga QT intervala, mjerenoga u II. odvodu u bolesnika s anoreksijom nervozom (r=+0.07). Relativna opasnost (tzv. relativni rizik) za produženi korigirani QT interval bio je visok u bolesnika s anoreksijom nervozom (7,3) u usporedbi s kontrolnom skupinom. Poznato je kako je produženi korigirani QT interval povezan s iznenadnom smrti zbog zloćudne promjene ritma klijetki. Tijekom analize korigiranoga QT intervala, u bolesnika s anoreksijom nervozom nađena je raspršenost korigiranoga QT intervala do 116 msec (srednja vrijednost 59,63±24.12), a u kontrolnoj skupini do 53 msec (srednja vrijednost 31,37±9.97). Te su vrijednosti bile varijabilne u pojedinim odvodima i analizirane u svih 12 odvoda. U procjeni korigiranoga QT intervala u bolesnika s anoreksijom nervozom nije dovoljno analizirati ga samo u II. odvodu, nego ga treba analizirati u svakome od 12 odvoda. Raspršenost korigiranoga QT intervala u tim dvjema skupinama bila je statistički značajna (z=1.3740), značajno viša u bolesnika s anoreksijom nervozom nego u kontrolnoj skupini.

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DOES A CORRELATION EXIST BETWEEN ELECTROCARDIOGRAM AND HIGH PLASMA DIGOXIN LEVELS IN THE ELDERLY?

INTRODUCTION

The digitalis effect is important in elderly patients in whom pronounced cardiac changes are present and renal reserve is reduced by 30% to 50%, while serum creatinine concentration may be within the normal limits[1-7] Assessment of the digitalis effect, includes analysis of the P-R interval, corrected Q-T interval, S-T segment depression, arrhythmias and conduction disturbances in electrocardiograms

The aim of this study was to investigate whether the clinical symptoms and electrocardiogram are specific for the digitalis effect in elderly patients

PATIENTS AND METHODS

From April 1978 to April 1990, a high plasma digoxin level was found in 147 patients aged 65-89 years Age groups are presented in Table 1

All patients were treated with a maintenance dose (Lanitop, Pliva -Bochringer) of medigoxin of 0.1 or less mg/day The criteria for the use of digoxin for more or less pronounced symptoms and signs of congestive heart failure verified by echocardiography before the treatment were met in all patients Most frequently, the etiology of the diseases included atherosclerotic myocardiopathy with atrial fibrillation, hypertensive heart disease, chronic pulmonary heart disease with atrial fibrillation and fast ventricular rhythm, less frequently acquired valvular disease, and rarely constructive pericarditis and cardiomyopathy, in one or two patients In all patients, plasma digoxin levels were determined by radioimmunoassay (Abbott)[8]. The sensitivity of the method is 0 1 µg/L Coefficients of variation were 10% Five hours after the last medigoxin dose, 2 ml of venous blood were drawn from the patients in the reclining position, into a test tube into which 0 2 ml of heparin had been previously added The object was to determine the digoxin concentration in a state of dynamic equilibrium, i e when the speed of entry into the body is the same as the speed of elimination. The following clinical signs of digitalis overdose were recorded anorexia, nausea, vomiting, and less specific signs like fatigue and headache Electrocardiograms

were recorded when blood was taken for digoxin analysis with patients in the reclining position, with a three-channel electrocardiograph at a tape rate of 25 mm/s (RIZ - Zagreb - Croatia) The following leads were recorded· I-III, aVR - aVF, V_1 - V_6 The arrhythmia changes were analyzed frequent premature beats, atrial tachycardia, junctional rhythm, atrioventricular block of the first and second degree (apart from Mobitz II) Mobitz I, block 2 1 or 3 1. The following parameters were carefully examined. atrioventricular conduction disturbances, i e. the prolonged P-R (P-Q) interval according to the heart frequency and time of the electrical systole of the ventricle. namely, the corrected Q-T interval The latter was determined according to Bazett's formula [9 10], where it is considered normal if ranging between 0 396 and 0 432 in women and between 0 386 and 0 422 s in men, whereas it is considered shortened if measuring 0 37 s or less (lead II)

$$\text{I corrected Q} - \text{T interval} = \frac{\text{measured Q} - \text{T interval(s)}}{\sqrt{\text{R} - \text{R interval(s)}}}$$

The corrected Q-T interval was also analyzed according to the formula of Stamforth [13]

$$\text{II corrected Q} - \text{T interval} = \frac{\text{measured Q} - \text{T interval(s)}}{\sqrt[3]{\text{R} - \text{R interval(s)}}}$$

The P-T-Q index was calculated according to Joubert's formula [12 13], a condition for this analysis was the existence of P wave, i e sinus rhythm

$$\text{P} - \text{T} - \text{Q index} = \text{P} - \text{R interval (s)} \times \frac{\text{negative T wave (mm)}}{\text{I corrected Q} - \text{T interval (s)}}$$

Statistical analysis was carried out by means of the correlation coefficient[14]

RESULTS

Out of 147 elderly patients with a high plasma digoxin level, the majority were aged between 71 and 75 years There were more women (62%) than men (38%), as shown in Table 1 Clinical symptoms of digitalis overdose were observed in 71 (48%) patients, and 92 patients had both clinical symptoms and ECG changes Arrhythmias were present in 40%, and conduction disturbances in 42% of the patients Data on the patients with both changes are presented in a group of predominant disturbances Only 18% of the patients were free of these changes, as presented in Table 2

Table 1

Number of patients with a high plasma digoxin level according to age and sex

Age	Sex		Total
	M	F	
65-70	12	21	33
71-75	22	31	53
76-80	7	17	24
81-85	11	17	28
>85	4	5	9
Total	56	91	147

Table 2.

Arrhythmia and conduction disturbances in electrocardiograms in patients with a high plasma dioxin level

Plasma digoxin level (nmol/L)	Arrhythmias	Conduction disturbances	Without these changes	Total
2.5 – 3.0	18	15	14	47
3.1 – 3.5	17	32	1	50
3.6 – 4.0	9	8	5	22
4.1 – 4.5	11	5	3	19
4.6 – 5.0	1	2	2	5
>5	3	0	1	4
Total	59 (40.1%)	62 (42.2%)	26 (17.7%)	147 (100.0%)

In 72 (49%) out of 147 patients, sinus rhythm was present in electrocardiograms In 22 (30%) out of 72 patients, a prolonged P-R interval is recorded. There was a correlation between a high plasma digoxin level and P-R interval ($p<0.05$). A shortened I corrected Q-T interval (0.37 s or shorter) was found in 32 (22%) patients There was no correlation between these parameters A total of 53 (36%) patients had a shortened II corrected Q-T interval No correlation was found between this parameter and elevated plasma digoxin concentrations Fifty-six out of 147 (38%) patients had a negative T-wave with sinus rhythm in their electrocardiograms The P-T-Q index was also calculated A correlation was found between these parameters ($p<0.01$)

Lowered values of serum potassium concentrations were found in seven (5%) patients, elevated values in 21 (14%) patients, in three patients they were 6.0, and in one patient they measured 6.6 mmol/L There was no correlation between the increased plasma digoxin levels and serum potassium concentrations Elevated values of serum creatinine were found in 76 (52%) patients, 20 of whom had a value from 375 to 750 μmol/L, and this value amounted to 750-1500 μmol/L in 16 patients No correlation was found between serum creatinine level and elevated plasma digoxin level. These data are presented in Table 3

Table 3

Correlation between a high plasma digoxin level, and electrocardiogram parameters, serum patassium and serum creatinine values

Parameters		Correlation coefficient	Statistical difference
High plasma digoxin level	P-R interval	+ 0.207	$p<0.05$
	I corected Q-T interval	+ 0.088	n.s
	II corrected Q-T interval	+ 0.096	n.s
	P-T-Q index	+ 0.394	$p<0.01$
	serum potassium level	+ 0.041	n.s
	serum creatinine level	+ 0.094	n.s

None of these patients received either spironolactone or quinidine during therapy Verapamil was administetred to 26 (17.7%) patients in a mean dose of 240 mg/day, and an ACE inhibitor in 16 (10.9%) patients We were unable to find any correlation between verapamil doses and plasma digoxin level

DISCUSSION

In this study, no correlation was found between the I corrected interval (when the square root from the heart frequency was calculated in the denominator), II corrected Q-T interval (when the cube root from the heart frequency was calculated in the dominator) and a high plasma digoxin level. A shortened corrected Q-T interval analyzed by the first method was present in 22%, and analyzed by the second method in 36% of the patients. Although the corrected Q-T interval analyzed by the second method was often somewhat shorter than it had been when analyzed by the first one, no statistically significant difference was found. There was a correlation between a high plasma digoxin level and the P-R interval, and between a high plasma digoxin level and the P-T-Q index. This is partially in agreement with our previous observation [1] on a slight correlation between a high plasma digoxin level and the P-R interval (= +0.32) and I corrected Q-T interval (r = + 0.27) in all age groups. Correlation coefficient is somewhat higher in eldery patients than in all other groups.

Some authors have obtained a higher correlation between plasma digoxin level (when the values were within the therapeutic range) and the P-T-Q index in healthy volunteers[12,13]. In our study, more than 82% of the elderly patients had arrhythmias or conduction disturbances in their electrocardiograms. The clinical condition of digitalis hypersaturation was present in half of these patients. Although more than a third of the elderly patients had chronic renal failure, we did not observe any correlation between a high plasma digoxin level and serum creatinine level or serum potassium level.

We conclude that there are no specific changes in electrocardiograms that could be ascribed to the digitalis effect. In the elderly patients, however, the prolonged P-R interval and the P-T-Q index changes are more often connected with a high plasma digoxin level than in younger ones.

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SUMMARY

In 147 elderly patients, a significantly elevated plasma digoxin level (2 5 nmol/L or more) correlated with the clinical symptoms and electrocardiogram The conduction disturbances and arrhythmias, as well as the electrocardiogram P-R interval, P-T-Q-index and corrected Q-T interval were analyzed. In 71 (48%) patients, clinical symptoms of digoxin overdose were observed Arrhythmias were present in 40% of the patients, conduction disturbances in 42%, while only 18% of the patients were free of these changes. A correlation was found between a high plasma digoxin level and the P-R interval (r = +0 207) as well as between a high plasma digoxin level and the P-T-Q index (r = + 0 394), whereas there was no significant correlation between the plasma digoxin level and corrected Q-T interval No correlation was found between the plasma digoxin level and serum potassiun concentration, or between the serum creatinine level and plasma digoxin level, although 52 (35%) patients and chronic renal failure

POSTOJI LI VEZA IZMEĐU PROMJENA U ELEKTROKARDIOGRAMU I VISOKE KONCENTRACIJE DIGOKSINA U PLAZMI U BOLESNIKA STARIJE DOBI?

SAŽETAK

U skupini od 147 bolesnika starije dobi s povišenom koncentracijom digoksina u plazmi, analizirane su istodobno promjene u elektrokardiogramu, te koncentracije kalija i kreatinina u serumu. U elektrokardiogramu analizirane su smetnje provođenja i smetnje stvaranja podražaja. P-R interval, P-T-Q indeks i korigirani Q-T interval. Aritmija je postojala u 40% bolesnika, smetnje provođenja podražaja u 42%, a 18% bolesnika nije imalo tih promjena. Potvrđena je veza između povišene koncentracije digoksina u plazmi (2,5 nmol/L ili više) i P-R intervala, a također i između povišene koncentracije digoksina u plazmi i P-T-Q indeksa. Nije potvrđena veza između povišene koncentracije digoksina u plazmi i korigiranog Q-T intervala. Također, nije potvrđena veza između povišene koncentracije digoksina u plazmi i koncentracije kalija u serumu, kao ni između koncentracije digoksina u plazmi i vrijednosti serumskog kreatinina, iako su mnogi bolesnici imali znakove kroničnog zatajivanja bubrega.

Tijekom analize učinka digoksina na miokard, ni u starijih bolesnika kao ni u mlađih, ne postoje specifične promjene u elektrokardiogramu koje upućuju na učinak digitalisa. No, u starijih bolesnika, ipak, produženi P-R interval, kao i P-T-Q indeks, češće su povezani s povišenom koncentracijom digoksina u plazmi nego u mlađih bolesnika.

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ADVANCES IN VETERINARY VIROLOGY

1. MOLECULAR BIOLOGY OF VIRUSES

The techniques of molecular biology represent remarkable new tools for examination of evolutionary relationships between viruses There has been a rapid accumulation of the knowledge about nucleotide sequences of viral genes and of the whole viral genomes comprising the hereditary material of viruses[1] Thus, the word "virus" may just designate a genetic element with an intracellular mode of parasitism and the ability to infect new cells in one way or another, and not necessarily infer an evolutionary relationship[2].

Although the knowledge concerning molecular biology of viruses has rapidly accumulated, little is known about molecular mechanisms of the interaction between cells and viruses in the early steps of viral infection However, cellular materials that serve as virus receptors have recently been identified for several viruses Cellular receptors for viruses have important roles in the early steps of viral infection, such as binding to the cell surface, penetration and uncoating of the virus Therefore, elucidation of precise structures and functions of the virus receptors is very important for the understanding of molecular mechanisms of interaction between cells and viruses in each step of the early infection cycle[3] A number of host cell membrane proteins that serve as virus-binding proteins or receptors have been identified and cloned within the last few years, providing an opportunity to investigate the virus-host interaction more directly[4] The receptor for the influenza virus is a simple sugar, sialic acid Thus, virus receptors are actually very important components in the host. This area of research ties structural and mechanistic aspects of virology and cell biology together, a fascinating area which has been developing rapidly[3]

2. PATHOGENESIS

Many investigations are focused on molecular mechanisms in attempts to understand viral pathogenicity Mims (1992) claims that there are five areas of research where interesting aspects of viral pathogenesis are being elucidated, namely viral modification of cell function, transgenic mice, influence of cell differentation on virus expression, cytokines in virus infections and the interaction of viruses with the immune system

It is well known that viruses can infect cells and interfere with their function in a way that is useful to the infecting virus Certain viruses, however, by interfering with cell function, can cause disease (LCMV in mice) Retroviruses provide numerous examples of effects on the immune cell function

Transgenic mice provide an excellent opportunity to analyze the expression of the viral genome in host cells They are important in helping to account for viral tropism, and recently are used to study the role of the prion protein gene in the genesis of spongiform encephalopathy

It is now well established that as cells differentiate they often become more susceptible to productive viral infection (papillomavirus skin lesion in cottontail rabbit) This can be especially important in cells of the imune system (visna virus replication proceeds when monocytes differentiate into macrophages)

Cytokines play a major role in viral pathogenesis, both as mediators of antiviral resistance and as agents of tissue damage in the infected host The interaction of viruses with the immune system is an area of immense research interest Viruses that persist in the body for life. have achieved the ultimate triumph over host resistance One of the most interesting phenomena in virus-induced immunosuppression is the production of immunosuppressive molecules of the infective virus For instance, feline leukemia virus produces p15e, a molecule that interferes with T-cell function, perhaps binding to these cells and reversibly reducing interferon and IL-2 output[5]

Three immunopathologic mechanisms may determine the pathogenesis of viral diseases in animals

1) A variety of viruses cause transient or prolonged immunosuppression by infecting lymphoreticular tissues and interacting with components of the immune system There are several examples of temporary or permanent virus-induced immunosuppression Immunodeficiency associated with feline leukemia virus infection involves various components of the immune system. Abnormalities in cellular and humoral immunity and the phagocytic and complement system have been recognized Each mechanism may operate independently or together with others The precise pathomechanisms of immunosuppression included by feline immunodeficiency virus have not yet been fully elucidated. The exact mechanism involved in bovine viral diarrhea virus induced immunosuppression is not sufficiently clear The same can be said about feline panleukopenia parvovirus, canine parvovirus type 2, canine distemper virus, and partially for infectious bursal disease virus

2) In a number of persistent infections, effective immune response may result in tissue damage An important mechanism is T-cell-mediated destruction of infected cells Another principal effector mechanism, mediated by T cells, is delayed-type hypersensitivity In several viral infections of animals, the cellular immune response has been shown to be the cause of tissue damage These immunopathologic mechanisms may include lentivirus infection, borna disease of horses and sheep. and canine distemper encephalitis, while maedi-visna is considered a prototypic lentivirus

3) In certain viral diseases, immune complexes are formed when antibodies are produced and react with viral antigen molecules that persist in the host or are released from infected cells into extracellular fluids Such diseases include aleutian disease of mink, feline leukemia, feline infectious peritonitis, canine type-1 adenovirus infection, bovine viral diarrhea and African swine fever However, further studies are necessary to clarify the precise composition of immune complexes circulating in the blood and immune complexes deposited in critical target tissues[6]

3 NEW VIRAL INFECTIONS AND DISEASES

A mysterious swine disease, a disease causing abortion and respiratory distress in pigs, first detected in 1987 in North America, since 1990 has emerged in Europe under various names, now mainly designed as porcine reproductive and respiratory syndrome The disease has spread rapidly through many European countries and causes severe losses in pig breeding farms Early in 1991, a causative agent was identified as a virus, now known as the Lelystead virus[7].

Since 1988, morbillivirus have been increasingly recognized and held responsible for mass mortality amongst harbor seals *(Phoca vitulina)* and other seal species Virus isolation and characterization proved that morbillivirus from seals in Northwest Europe were genetically distinct from other known members of this group, and now are known under the name of phocid distemper virus.

An epidemic of Baikal seals *(Phoca sibirica)* in 1987 was apparently caused by a morbillivirus closely related, or even identical, to canine distemper virus The existance of an epizootiological link between mass mortality in Lake Baikal seals a year before, and the phocid distemper virus infections in other pinniped species in Northwestern Europe remain questionable

Among aquatic mammals, morbilliviruses have also been isolated from porpoises and more recently (1990) from striped dolphins in the Mediterranean Sea, showing that the epizootiology of morbillvirus-infections in mammals involves not just pinnipeds but cetaceans as well.[8]

Studies on the etiologic agents of rabbit hemorrhagic disease (RHD) and European brown hare syndrome (EBHS) show that the viruses responsible for these infections can be placed in the *Caliciviridae* family EBHS has been diagnosed in all western European countries, and since 1989 RHD has been additionally reported in many European countries Epizootiological investigations have demonstrated the presence of both virus infections in Europe long before RHD emerged in China in 1984 Examination of sera revealed the existence of hares seropositive to EBHS as far back as 1971 Furthermore, antibodies were found in rabbit sera collected in former Czechoslovakia up to 12 years before the first reported outbreak of RHD in Europe[9]

A new viral disease, lethal to pigs, was observed in Mexico in 1980 The disease was caused by a recently characterized paramyxovirus designated LPMV The

disease is characterized by disorders of the central nervous system, corneal opacity and pneumonia. Comparative analysis demonstrated LPMV to be most closely related to the human mumps virus[10]

Infectious salmon anemia is a disease affecting Atlantic salmon. The etiologic agent is unknown, but transmission trials indicate a viral origin. Virus - like particles were observed by electron microscopy[11]

4 ANIMAL IMMUNODEFICIENCY VIRUSES

In 1987, N.C Pedersen (Davis, California, 1987) reported on the discovery of a new retrovirus isolated from diseased cats It is now termed feline immunodeficiency virus (FIV) Already from the first report it was evident that this virus should become of considerable biomedical importance, as it does not only cause an immunodeficiency syndrome in cats, but also shares many physical and biologic properties with human immunodeficiency virus (HIV) FIV occurs in natural populations of an easily accessible animal species and does not cross species barriers

Bovine immunodeficiency virus (BIV) has been isolated only once, i e in 1972 by Van der Maaten et al , and all attempts to obtain additional isolates have failed Nevertheless, seropositive cattle have now been found in the Netherlands BIV has been well characterized, its genome cloned, sequenced and partially expressed, but still its veterinary significance is unknown[12].

Simian acquired immunodeficiencies or lymphoproliferative diseases can be caused by infectious (exogenous) oncoviruses (C- or D-type) or by lentiviruses. A T-cell tropic HTLV-III-like retrovirus, now designed as simian immunodeficiency virus (SIV), was isolated from macaques with an immunodeficiency syndrome (SAIDS) in 1984/1985[13]

The equine infectious anemia virus (EIAV) has recently been shown to be serologically cross-reactive with HIV and FIV Moreover, it shares some common nucleotide sequences with HIV, BIV, caprine arthritis encephalitis virus and visna The retrovirus team (Leis et al , 1988) has positioned EIAV in immediate proximity to HIV-1[14]

5 SPONGIFORM ENCEPHALOPATHIES

Transmissible spongiform encephalopathy is an accurate description of a group of inevitably fatal diseases in several mammals Those said to be naturally affected include sheep (scrapie), cattle (bovine spongiform encephalopathy), mink, deer, cats (eland /Taurotragus oryx/, nyala /Tragelaphus angasi/, gemsbok /Oryx gazella/, cheetach) and man (kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträusler-Scheinker syndrome) Although these diseases are primarily seen as brain diseases, the infective agents have been found in many tissues. The only evidence of causative agent is that of

the finding of prion protein in brains of infected sheep and other mammals Prion protein is the major component of scrapie-associated fibrils The central role of prion protein in the spongiform encephalopathies is confirmed by recent evidence

No etiologic agent, such as a virus, has yet been unequivocally established as the cause of these diseases, with the possible exception of inherited Creutzfeldt-Jakob disease Modern molecular biology is in search for an identifiable gene product, the gene itself and possible control mechanism[15]

6 DIAGNOSTIC TECHNIQUES

Developments of crucial importance were the discovery of the helical structure of DNA, and the existance of RNA-directed DNA polymerase, now better known as "reverse transcriptase" in avian and murine leukemia viruses These achivements have all been recognized by the Nobel Prize Award Another milestone was the development of the hybridoma technique for production of monoclonal antibodies which were directed against a single antigenic determinant or epitope The hybridoma technique has dramatically improved and intensified biological research in many areas, and diagnostic techniques have been improved accordingly[7,6] Nucleic acid probe technology is being increasingly used in the research and diagnosis of viral diseases of veterinary importance The major applications include detection of viruses in clinical samples, especially those which are fastidious and difficult to cultivate, differentation of virulent from avirulent viruses and vaccine strains from wild type isolates, typing of viruses, mapping genes, detection of latently infected or carrier animals, study of the pathogenetic mechanisms and epidemiological studies

Among viruses of veterinary importance detected by nucleic acid probes are adenovirus, African swine fever virus, bluetongue virus, bovine viral diarrhea virus, enterovirus, infectious bursal disease virus, foot-and-mouth disease virus, porcine parvovirus, pseudorabies virus, rhinovirus, rotavirus and transmissible gastroenteritis virus.

Nucleic acid probes have been developed for the detection of parvovirus and bovine viral diarrhea virus as contaminantes in cell cultures

The differentation of virulent from avirulent viruses and vaccine strains from wild type isolates and typing of viruses is accomplished immunologically by monoclonal antibodies, and genetically by restriction endonuclease analysis, sequence analysis and nucleic acid probes The latter have been used to type the rotavirus groups, subgroups and serotypes.

Nucleic acid probes provide a powerful tool for evaluating homology between related DNAs. This can be accomplished by dot blot, Southern blot or Northern blot hybridization For example, homology was evaluated between porcine and canine parvovirus and between polyoma and papovavirus

Nucleic acid probes are an excellent tool for the determination of molecular mechanisms of pathogenesis Mechanisms of latency induction by herpesviruses have been studied by *in situ* hybridization with bovine herpesvirus-1 The mechanism of persistant infection by equine infectious anemia virus was determined using Southern blot hybridization Hybridization technology was also used to better understand the mechanisms of leukemogenesis of bovine leukemia virus These studies, combined with immunochemistry, can provide valuable information on the presence of viral nucleic acid and antigens in cells, and on the status of virus expression The development of probes specific for viruses, groups of viruses, or specific serotypes provides a valuable tool for epidemiological studies, especially useful for determining the epidemiology of viruses which are difficult to cultivate or type[17].

Very recently, diagnostic techniques have received another impetus with the development of the polymerase chain reaction Minimal quantities of DNA, in principle single DNA molecules, can now be amplified by repeated cycles of transcription until detectable amounts are obtained This technique does not only open new avenues for diagnostic application but will become very important in many areas of research Further, recombinant DNA techniques have become available. From our point of view, the development of diagnostic reagents with improved sensitivity, which can be used for rapid verification of disease problems are appealing Monoclonal antibodies will also become of importance for the identification of individual animals[16]

7 ANTIVIRAL THERAPY

The field of antivirals is still very new The reason is that viruses replicate inside cells utilizing, to a large degree, the biosynthetic machinery of the cell, so that most compounds which interfere with virus growth are also active against human and animal cells, and thus of no therapeutic value It is generally perceived that the development of effective antiviral compounds will be accelerated by investigating, at the molecular level, how viruses replicate One of the aims of such research is to identify processes which are essential for virus replication and different from those of the cell, so that the differences can be exploited to selectively target the virus[18]

A potentially general antiviral strategy involves inhibition of viral gene expression by use of synthetic oligonucleotide analogues that have sequences complementary to viral DNA or RNA sequences. Such oligonucleotides are expected to hybridize specifically to their viral target sequences interfering with the viral life cycle. Thus, the use of sequence-specific oligonucleotides to target unique viral gene sequences offers an approach to the development of highly specific antiviral therapeutics[19] Improved understanding of the molecular processes involved in the replication of viruses has encouraged the design and further development of antiviral drugs which specifically inhibit the function of an identified virus gene product[20]

There are, at present, no antiviral compounds licensed specifically for veterinary use, though there are numerous reports dating from the late 1960's on the experimental use against viruses of veterinary importance of antiviral compounds

developed or licenced for use in man The two main reasons for the lack of commercial availability of antiviral drugs specifically for veterinary use are their high cost of development coupled with their generally narrow spectrum of activity However, the intense research activity on the human front since the proven success of acyclovir (ACV, Zovirax) and zidovudine (AZT, Retrovir), and use of animal models of feline immunodeficiency virus and feline leukemia virus infection for screening drugs for AIDS, is likely to lead to the discovery of potential drugs, especially antiretrovirus and antiherpes compounds for veterinary use[21]

8 DEVELOPMENTS OF NEW VACCINES

One of the approaches to making vaccines is to genetically manipulate a virus so that it carries and expresses a foreign gene (or part of a gene) which codes for a protective antigen for another disease. Adeno-, polio- and herpesviruses have been engineered to act as vectors in this way, but vaccinia virus remains the main candidate for a recombinant virus vector for vaccine use. Vaccinia virus has a genome consisting of about 185,000 base pairs of DNA, and carries over 100 genes[22]

The first recombinant DNA vaccines have become available for veterinary application, e.g., rabies and Aujeszky's disease These new vaccines not only solve many problems related to the use of living attenuated or killed whole agent vaccines, but they also offer the possibility of vaccinating animals against a broader range of diseases. They are safe, stable and effective, and abundant quantities can be produced inexpensively[16] The vaccinia rabies recombinant virus was found stable, safe for target and non-target animal species, and protective for most of the rabies vectors It is used in limited field trials and in an extensive open field trial as oral vaccine for foxes Preliminary results have confirmed its basic properties and potential for rabies eradication[23] Consequently, both attenuated live virus vaccines and a recombinant vaccine are available for routine field vaccination of the fox population[24].

Recombinant-derived vaccines are being developed to protect against diseases such as vesicular stomatitis, bluetongue, porcine and canine parvoviruses, bovine papilloma, influenza, feline leukemia, rinderpest and Rift Valley fever[16] A new generation of vaccines based on (re)constructed viruses and immunogens are expected to be stable and inexpensive to produce Research is now ever more focused on constructed modified live-virus vaccines.

The term "intracellular immunization" has been suggested by Baltimore (Nature, 1988) to describe the process whereby a gene is expressed in cells to generate a protein which interferes with virus replication The protein could be either one which possesses an antiviral phenotype or alternatively a mutated viral protein which would block the function of the wild-type viral protein and so exert a dominant-negative phenotype A cell expressing such proteins could become immune to viral infection Intracellular immunization has now been achieved in both tissue culture and mice There are clear grounds for the optimistic view that in the long-term, genetic

engineering approaches to produce resistance to viral infections by inducing expression of proteins with antiviral activities might succeed[25].

A possible approach to control bovine lymphoproliferative disease caused by bovine leukemia virus (BLV) may be the development of an "antiviral information immunity" based on the effect of anti-sense RNA. A number of constructs have been obtained, under control of various promotors, carrying a DNA against gene X, the expression product of which is a transactivator, a viral transcription from the BLV LTR promotor[26]

Experimental vaccine trials with simian immunodeficiency virus and HIV may represent the important first step in developing a feasible vaccine using an acceptable animal model[27]

9 EUROPEAN SOCIETY FOR VETERINARY VIROLOGY

The European Society for Veterinary Virology was founded in 1988, with a widespread and enthusiastic support from veterinary virologists throughout Europe. The objectives of the Society are

- to progress further in veterinary virology,
- to provide an organization for individuals who devote a significant portion of their professional activities to research, teaching or practical application of veterinary virology,
- to encourage and promote improved methods of diagnosis, prevention and treatment of animal viral diseases.
- to cultivate further education in veterinary virology, and
- to promote information exchange in the field of veterinary virology

In pursuit of these objectives, the Society held its first scientific congress at the University of Liége in Belgium, April 5-7, 1989, with the main topic: "The contribution of molecular biology to veterinary virology". Three specialist sessions focused on the seal morbillivirus, bovine herpesvirus 1, and bovine diarrhea virus, while a small informal meeting reviewed the state of art in the field of rabbit hemorrhagic disease[28].

Over 200 scientists gathered again September 23-26, 1991, at the second International Congress in Uppsala, Sweden, on the topic "The pathogenesis of viral diseases. Molecular, virological and immunological aspects". During this meeting, a tribute was paid to one of the Society founder members, by introducing the first Zvonimir Dinter (a Croat born in Osijek) Memorial Lecture, given by Professor Cedric Mims[29]

The Third International Congress of Veterinary Virology was held in Interlaken, Switzerland, September 4-7, 1994. The main topic of the Congress was "Immunobiology of viral infections"

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SUMMARY

The main interests in current veterinary virology, as well as in virology in general, are research of virus structure and assembly, expression of viral genomes, molecular basis of disease, virus-host interactions, immunobiology of viral infections, and impact of molecular biology on diagnosis and control Many investigations have been quite correctly focused on molecular mechanisms, in an attempt to understand viral pathogenecity Improved understanding of the molecular processes involved in the replication of viruses has encouraged the design and further development of antiviral drugs which specifically inhibit the function of an identified virus gene product In the last few years, epizootiological investigations, especially those at the molecular level, have revealed some previously unknown viral infections or diseases in various animals

Social events, at least those concerning the European veterinary virologists, are becoming very dynamic nowadays

NAPREDAK VETERINARSKE VIRUSOLOGIJE

SAŽETAK

Glavna događanja u današnjoj veterinarskoj virusologiji, baš kao i općenito u virusologiji, istraživanja su o virusnoj strukturi i tvorbi virusa, ekspresiji virusnih genoma, molekularnoj osnovi bolesti, odnosu virusa i njegova domaćina, imunobiologiji virusnih infekcija i utjecaju molekularne biologije na dijagnostiku i prevenciju virusnih bolesti i infekcija Mnoga istraživanja su usmjerena na molekularne mehanizme u pokušaju da se shvati patogenost virusa Bolje poznavanje molekularnih zbivanja uključenih u virusnu replikaciju pruža nadu u daljnji razvoj antivirusnih lijekova koji specifično inhibiraju funkcije nekog identificiranog proizvoda virusnoga gena U zadnjih nekoliko godina su epizootiološka istraživanja, pogotovo ona na molekularnoj razini, otkrila neke virusne infekcije ili bolesti u različitih životinja koje prije nisu bile poznate.

Društvena događanja, barem ona što se odnose na europske veterinarske virusologe, postala su u novije vrijeme vrlo dinamična

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ESTIMATE OF AN AVERAGE OCCUPATIONAL DOSE FOR WORKERS IN A NUCLEAR MEDICINE DEPARTMENT

Dose limits for professional exposure recommended by the International Commission on Radiological Protection (ICRP) were in 1990 set to 20 mSv y^{-1}, to be averaged over a period of five years with no more than 50 mSv in a single year ICRP has also re-confirmed that the dose limit for the public should be 1 mSv y^{-1}, averaged over five years[1] This new recommendation for professional exposure is described as an evolution of the 1977 dose limit of 50 mSv y^{-1}[2] The limit was adjusted due to the accumulation of results from 11 years of intensive nuclear bombs survivors studies as well as the analysis of long-term effects of radiation used in medical treatments. New estimates for the level of risks from low dose irradiation of fatal cancers and genetic effects have been raised by about a factor of four[3,4]

The ICRP recommendations provide the framework for radiological protection Although they have no legal status, most national and international regulatory agencies base their own regulations and standards of good-practices on them Impending changes in the Croatian dose limitation system should certainly reflect new ICRP recommendations Therefore, the implication of lowered annual dose limits for professional exposure should be considered for a typical nuclear medicine department where they could have an impact on radiation protection, organization of work and medical practices.

Past personal dosimetry records for the workers at the Department of Oncology and Nuclear Medicine, "Sestre Milosrdnice" University Hospital from Zagreb were reviewed to assess the situation and trends

In individual dosimetry, the main aim of monitoring is control and book-keeping of the doses a person has received The measured absorbed dose is the fundamental quantity in radiation protection However, it is the weighed absorbed dose that is of interest in controlling radiation exposure. It is not easy to correlate a straightforward physical quantity such as the absorbed dose (measured value due to radiation field in single point) with the traditional concept of dose equivalent of newly introduced equivalent dose[1] as useful operational quantities describing biological effects of a particular type and energy of radiation However, since most of the radiation field in a nuclear medicine department is a relatively constant external diffuse gamma radiation, it could be assumed that a personal dosimeter measures a dose equivalent value at a point in the body near to the position where the dosimeter is worn If the

dosimeter is worn at a representative position, the measured absorbed dose value could be simply converted and taken to be the upper limit or an approximation of the effective dose received by the wearer[5]

DOSE DATA

The Department of Nuclear Medicine and Oncology, "Sestre Milosrdnice" University Hospital, performs all common nuclear medicine diagnostic and therapeutic procedures in accordance with standard rules or recomendations for *per* patient quantities of radioactive material The volume of work could be described through the daily used total activity of material, which varied during years, but on an average it could be estimated to be in the range of 14 8 - 22 2 GBq (400-600 mCi), mostly technetium-99m and iodine-131

The work is organized in such a way that various tasks related to preparation and administration of radioactive material (eluation from the radionuclide generators, dispensing and injection) are spatially separated according to the complexity and/or activity handled Standard procedures for safe handling of radioactive material and radiation protection measures are respected Additionally, the time spent in the zone with elevated level of ionizing radiation is limited to 4 hours daily

The Department for Radiation Protection, Institute for Medical Research and Occupational Medicine of the Zagreb University is responsible for personal film dosimetry of all the workers The sensitivity threshold of the standard film dosimeters used is 500 μGy

Film dosimetry data reviewed cover the period of 7 years (from 1986 to 1992) for 63 workers, most of whom worked at the Department all the time (apart from holidays) Each year was divided into 12 control intervals, roughly corresponding to the months of the year This study was based on approximately 3800 man-control interval values

For the purpose of this analysis, the workers were divided into three groups (1) eluation from generators and preparation of iodine therapies (total of 28 workers, mark **gen**), (2) injection of radiopharmaceuticals, positioning of patients and operations with measuring instruments (24, **dia**), (3) control, cleaning and administrative tasks (11; **adm**) The study period was enough to assume that the time spent at the workplace and/or number of handled patients were averaged and thus the same for all the workers within a group

RESULTS

Ten percent of all of the measured values of absorbed doses were above the film dosimeter threshold sensitivity It has been suggested that the distribution of occupational doses generally follows a long-normal form[2 3] If so, the missing part of

the distribution would have suggested that almost 40% of all the doses were not registered at all, although they could be relevant for the analysis of the average or collective doses (*Figure 1*) Those ≈ 1500 missing dose values represent roughly a 300 mSv addition to the collective dose, since occupational irradiation, in the case of nuclear medicine, is prevalently of a systematic nature and not the result of accidents or unusual conditions

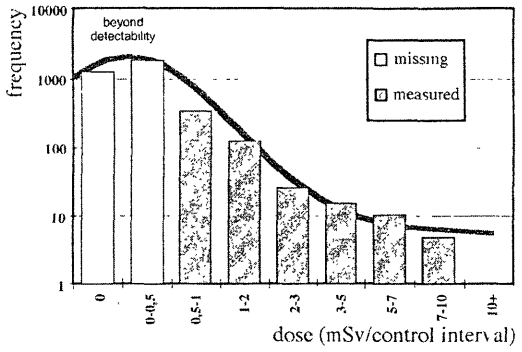

FIGURE 1 Distribution of measured dose values

This was also seen when the median dose for all received doses was determined for each individual (as the most representative for an individual over a prolonged period of time) and the corresponding distribution of calculated values plotted (Figure 2) The point representing 15 individuals with no measured dose was added to the distribution The shape of the curve suggests that higher doses *per* interval were sporadic and quite rare, and therefore they could not be attributed to a particular task or workplace If the values for missing doses were introduced in this type of data presentation, distribution would probably be of the same shape but shifted to the left, towards lower doses The maximum of such a curve, where all the received doses were taken into account, would be a better indicator for a typical dose *per* control interval for the population as a whole than simple data averaging

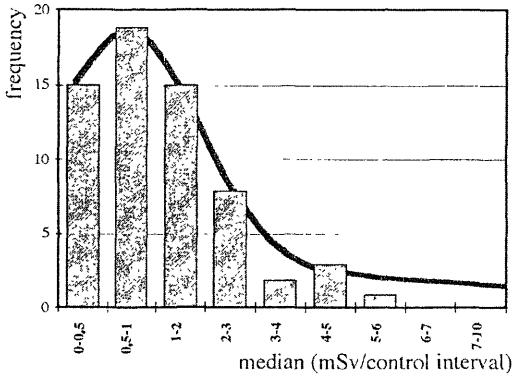

FIGURE 2 Distribution of individual median dose values

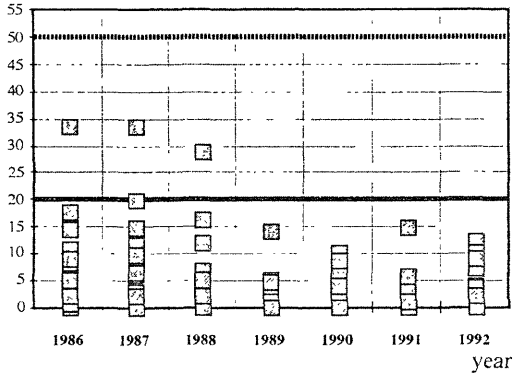

FIGURE 3 Annual doses for 11 workers and dose limits

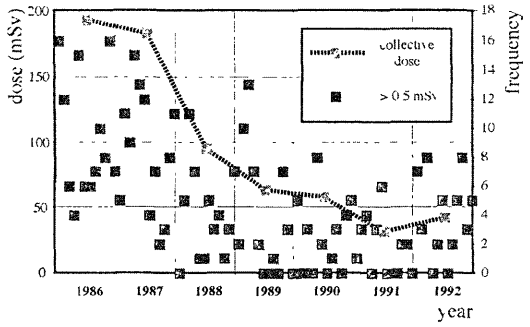

FIGURE 4 Annual collective dose and number of workers with more than 0 5 mSv per control interval

For 11 individuals from the right tail of the median distribution (most likely candidates to approach new dose limits), values of the measured annual doses over the years were plotted (Figure 3) Although an alarming possibility of approaching and even exceeding new dose limits in some of the workers seemed apparent, general behavior of the population (e g , through approximate collective dose) (Figure 4) clearly indicated that improvements in the radiation protection introduced at the department in 1988 and 1989 considerably reduced the overall irradiation This and a slight decrease in the number of patients passing through the department in recent years, have significantly lowered the probability of approaching the 20 mSv y^{-1} dose limit

Intergroup differences using group average as an indicator, are presented in Figure 5.

DISCUSSION

It is clear that most doses were delivered to **gen** and **dia** groups So, in the estimation of a representative approximation of the average occupational dose for the department, it appeared justified to exclude the **adm** group members. However, although having considerably lower doses than the **gen** and **dia** groups, the **adm** group members could not be treated as members of the general public because (a) on several occasions, and for several workers, doses above 0 5 mSv/control interval were observed, (b) some **adm** jobs at a nuclear medicine department could not be entirely separated from "hot" zones; and (c) theoretically, due to a very high sensitivity threshold, the members of this group could receive a notable dose of just under 6 mSv y^{-1} without detection!

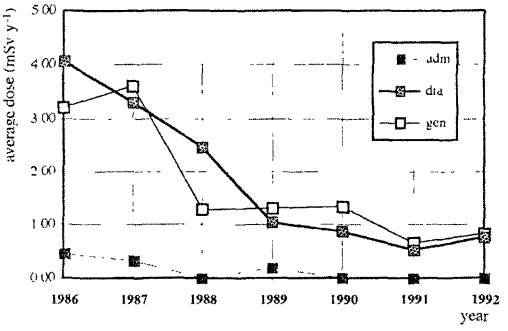

FIGURE 5 Annual average doses for three groups of workers (**adm** - control, cleaning and administration, **dia** - injecting and patient positioning, **gen** - elution from generators)

Also, it should be stressed that considerable intragroup differences were recorded (e g , in **dia** group, one individual average was 5-fold the group average due to systematically higher doses). Differences among workers were due to various reasons, mostly the type of workplace (type of duties and background radiation), but part of the differences were due to the individual approach to work and radiation protection (use of protective barriers, syringe shields and protective aprons) as well as individual capabilities (knowledge, experience and even handiness)

The average dose at the department decreased over the years from approximately 3 5 mSv y^{-1} to 0 8 mSv y^{-1} From these results, we can assume that 1 mSv is an acceptable estimate for the present average annual dose This value should be increased by at least 40% for missing doses due to high film dosimeter sensitivity Such a value must then be normalized to 8-hour work time (e g., multiplied by a conservatively chosen factor of 1.7) in order to compared it with other occupational doses for the same or similar type of work. The resulting value of the average annual dose ≈ 2.4 mSv is comparable with the 2 2 mSv estimate for staff in British nuclear medicine departments (6.7), but this value is, as expected, considerably higher than the average annual doses to ward nurses in radiotherapy departments (≈ 0 55 mSv), radiotherapists (≈ 0 4 mSv) or radiologists (≈0 2 mSv) [8 9]

Since the main phases in the use of radionuclides are elution from the generator, preparation, dispensing and injection of radioactive material, most of the occupational dose involves hands In that respect, as an orientation it was estimated that ≈ 20 mSv y^{-1} is received to the hands, if the total body dose is ≈ 2 mSv y^{-1} (6)

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SUMMARY

The doses of irradiation received by the workers at the Department of Nuclear Medicine and Oncology, "Sestre Milosrdnice" University Hospital, Zagreb, Croatia, were reviewed to assess the trends and impact of new annual dose limits of 20 mSv recommended by ICRP Film dosiometry data obtained monthly for the workers (N=63) at the department (for the period from 1986 to 1991) were analyzed A data set consisted of ≈ 3800 men/control interval values The workers were classified into three groups, according to the type and areas of work (1) preparation of radioactive material, (2) injection and patient positioning under diagnostic equipment; and (3) control, cleaning and administration. It is estimated that approximately 40% of doses are not registered at all due to a high threshold of film dosimetry Our estimate of the average annual dose was ≈ 2 4 mSv for two critical groups of workers Thermoluminiscent dosimetry is indicated as a better tool for monitoring at nucler medicine departments

PROCJENA PROSJEČNE PROFESIONALNE DOZE ZA DJELATNIKE U ODJELIMA NUKLEARNE MEDICINE

S A Ž E T A K

Pregledane su doze koje primaju zaposleni na Klinici za nuklearnu medicinu i onkologiju KB "Sestre Milosrdnice", kako bi se ocijenili kretanje i utjecaj novih godišnjih granica doze od 20 mSv koje preporuča ICRP Analizirani su podaci mjesečne filmske dozimetrije u razdoblju 1986-1991. g za 63 zaposlena Skup podataka sastojao se od vrijednosti za ≈ 3800 čovjek/kontrolni interval doza Zaposleni su razvrstani u tri skupine prema vrsti i području rada (1) priprava radioaktivnoga materijala, (2) injiciranje i postavljanje bolesnika pod dijagnostičkim instrumentima i (3) kontrola, čišćenje i administracija Procjenjuje se kako se približno 40% doza ne registrira zbog previsoka praga osjetljivosti filmske dozimetrije Naša je procjena kako prosječna godišnja doza iznosi ≈ 2 4 mSv za dvije kritične skupine zaposlenih Termoluminiscentna dozimetrija je znatno bolji vid praćenja za zaposlene u odjelima nuklearne medicine.

Adresa

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MODULATION OF NUMBER AND FUNCTIONS OF IMMUNOCYTES BY TAMOXIFEN IN BREAST CANCER PATIENTS

INTRODUCTION

Controversies concerning estrogenic and/or antiestrogenic effects of tamoxifen (TMX) need to be sorted in the view of its anticancer effects as well as its potential role in chemoprevention of breast cancer[1] According to results of monitoring healthy women in the prevention programs with TMX, it seems like a drug acting as a clear estrogen on serum lipids and fibrinogen, liver functions, endometrial proliferation and vaginal cytology[2]; yet it drastically interferes with estrogen promotion of breast cancer Since estrogens influence the immune system[3], and the immune reactions are known to contribute to host resistance to tumors[4], it is possible that the antitumor activity of TMX is not exclusively due to binding to estrogen receptors on tumor cells. It has been hypothesized that TMX acts as a biological response modifier, stimulating some immunologic functions like NK cell activity both *in vitro*[5] and *in vivo*[6], while suppressing some others, e.g , lymphocyte mitogenesis[7] and antibody formation[8] Monocyte, and particularly granulocyte phagocytic functions in tumor defence have so far been poorly discussed, especially in the context of using drugs as possible immunomodulatory agents Besides its involvement in intracellular killing of tumor cells, phagocytosis seems to play a role in other immunologic actions, thus becoming more interesting in studies of immune aspects of host tumor defence In this work, effects of TMX therapy on peripheral blood immunocytes number as well as NK activity and phagocytic functions of granulocytes and monocytes in breast patients are presented

PATIENTS AND METHODS

PATIENTS

Twenty-seven patients with localized invasive breast cancer, aged 45-78 years (mean age 61 yrs), clinical stage I-III, following radical mastectomy and postsurgical telecobalt therapy (TCT, total dose 40-55 Gy in 20 fractions) were included in the

study They were divided into two subgroups, one receiving endocrine therapy - TMX group - and the other receiving no further therapy - control group

TMX group thirteen pts, aged 47-78 yrs (mean 65 yrs), three clinical stage I (23%), seven clinical stage II (54%) and three clinical stage III (23%) They were receiving TMX (Tamoxifen-ratiopharm® 10, Ratiopharm, Germany) 20 mg daily following TCT. All of the excised tumors of were steroid receptor-positive, with estrogen receptor (ER) levels between 37 and 295 fmol/mg protein

Control group Fourteen pts aged 45-67 yrs (mean 59 yrs), three clinical stage I (22%), eight clinical stage II (56%) and three clinical stage III (22%) In five of them (40%), the excised tumors were steroid receptor positive, with ER levels 54-88 fmol/mg protein, while the remaining nine (60%) were ER negative All patients were tested twice for immunocompetence (1) 2-3 weeks after surgery but before the beginning of TCT, and (2) 7 months later

METHODS

Peripheral blood cell enumeration The number of granulocytes, monocytes and lymphocytes was determined from conventional blood smears Subpopulations of T lymphocytes and natural killer (NK) cells were determined by indirect fluorescent microscopy using monoclonal anti-CD4, -CD8 and -CD16 antibodies, respectively (Ortho Diagnostic Systems, USA) B lymphocytes were determined by indirect immunofluorescence using fluorescien isothiocyanate-labeled conventional anti-IgG+IgA+IgM antibodies (Institute of Immunology, Croatia), as previously described[9] At least 100 cells were recognized and expressed as proportions and absolute numbers ($x10^9$/L)

Phagocytosis Both ingestion and microbicidal capacities of granulocytes and monocytes were determined by combining techniques for determining ingestion[10] and intracellular killing[11] After erythrocyte sedimentation of heparinized peripheral blood taken by venipuncture, 0 5 ml of buffy coat was washed three times with Medium 199 (MEM, Institute of Immunology, Croatia) and the concentration of white cells adjusted to $1x10^6$/ml The suspension (0 25 ml) was placed into a small chamber of 1 5 cm in diameter, made on a microscopic slide and incubated for 30 min in tissue culture incubator at 37 °C and 5% CO_2 The supernatant was discarded and nonadhered cells gently washed out with MEM preheated at 37 °C Then, 0 25 ml of suspension containing $40x10^6$ /ml living yeast cells in MEM at 37 °C preheated at 37 °C were added to the chamber and placed in the tissue culture incubator After 30 min of incubation the chamber and its content were removed, the slide with adhered cells was rinsed in MEM at 37 °C, stained for 1 min with 0 05% (w/v) acridine orange in MEM (Sigma, USA) and washed again with MEM, covered by coverslip and examined under an Opton photomicroscope equipped with III RS epifluorescence condenser, at 800x magnification At least 100 granulocytes and monocytes were recognized separately, and the number of ingested yeast cells was counted and expressed as ingestion index, ii = number of ingested cells/100 granulocytes or monocytes The microbicidal capacity

was assessed by discriminating between dead(read fluorescence) and live (green fluorescence) ingested yeast cells, and expressed as a percentage of killing = number of ingested read yeasts/number of all ingested yeasts x 100. Yeast cells were prepared by keeping yeasts *(Saccharomyces cerevisiae)* in a 5-day culture in MEM at 37 °C and 5% CO_2, and then for another day with the addition of actinomycin D (Sigma, USA), 2 mg/ml of cell suspension, to stop cell proliferation. Cells were then washed three times with MEM and their concentration was adjusted to $40x10^6$/ml. Their viability was assessed by mixing equal volumes of cell suspension and 0.1% (w/v) acridin-orange solution and examination under fluorescence microscopy. At least 99% of the cells should be alive, i.e. should fluorescence green. If the percentage of dead cells exceeded 1%, a new cell suspension was prepared. The coefficients of variation of the test for granulocyte and monocyte ingestion were 16 and 26, and for granulocyte and monocyte microbicidal capacity 54 and 21, respectively.

NK cell activity. NK cell activity was determined by the cytotoxicity assay with ^{51}Cr-labeled K-562 target cells. In brief, peripheral blood lymphocytes (PBL) were separated by centrifugation on Ficoll- Hypaque gradient (Pharmacia, Sweden), washed three times in RPMI cell culture medium (Institute of Immunology, Croatia) containing 10% calf serum (Ruđer Bošković Institute, Croatia) and their concentration was adjusted to $5x10^6$/L. K-562 cells cultured and washed in the same medium were labeled with 3.7 MBq of sodium chromate in sodium chloride solution (Amersham, England), washed three times and their concentration was adjusted to $0.1x10^6$/L. Incubation mixtures of 0.1 mL were made containing equal volumes of effector (E) and target(T) cells, having E:T cell ratios of 12.5:1, 25:1 and 50:1. Control samples for spontaneous lysis contained an equal volume of medium, while control samples for total lysis contained an equal volume of 1% (v/v) of Triton x-100 (Sigma, USA) instead of effector cells. Following 18-h incubation at 37 °C and 5% CO_2, to each incubation mixture 1 ml of cold MEM was added and centrifuged. Supernatant aliquots of 0.8 ml were separated and counted in an LKB Clinigamma counter Model 1272.

The percentage of lysis was calculated as follows:

$$\frac{cpm_{sample} - cpm_{spontaneous\ release}}{cpm_{total\ release} - cpm_{spontaneous\ release}} \times 100 = \%\ of\ lysis$$

Statistics. Results are expressed as medians with corresponding interquartile ranges and analyzed by Mann-Whitney U test, using a Statistics program with graphics for Macintosh^{TM}, 1985, Data Metrics Inc.

Table 1

Absolute number ($x10^9$/L) of pheripheral blood cells before telecobalt therapy (TCT) and 7 months following TCT in two groups of patients, one receiving tamoxifen (TMX) and the other receiving no further therapy following TCT.

Cell type	Before TCT without TMX	After TCT without TMX	Before TCT with TMX	After TCT with TMX
Leukocytes	5 20a 5 10 – 6 73b	4 25 3 78 – 5 13	5 50 4 75 – 6 55	4 72 3 61 – 5 10
Granulocytes	3 39 3 13 – 3 55	2 88 2 61 – 3 79	2 97 2 61 – 4 03	3 14 2 56 – 3 55
Monocytes	0 20 0 16 – 0 30	0 24 0 11 – 0 30	0 23 0 14 – 0 34	0 20 0 14 – 0 22
Lymphocytes	1 43 1 16 – 1 85	0 80* 0 64 – 1 08	1 60 1 14 – 1 83	0 98* 0 74 – 1 26
CD4+ lymphocytes	0 80 0 63 – 0 96	0 33* 0 25 – 0 40	0 83 0 58 – 1 04	0 57** 0 41 – 0 85
CD8+ lymphocytes	0 44 0 35 – 0 53	0 21* 0 17 – 0 27	0 43 0 30 – 0 58	0 40** 0 33 – 0 46
B lymphocytes	0 09 0 07 – 0 13	0 06* 0 04 – 0 07	0 10 0 07 – 0 15	0 08 0 04 – 0 11
CD16+ lymphocytes	0 22 0 15 – 0 25	0 09* 0 07 – 0 16	0 14 0 09 – 0 27	0 12 0 10 – 0 20

[a] Median, [b] Interquartile range

* $p<0.05$ compared to the same group before TCT

** $p<0.05$ compared to the control group after TCT

RESULTS

The number of leukocytes, and proportion and absolute number of granulocytes, monocytes, lymphocytes, their subpopulations of inducer/helper (CD4+) and suppressor/cytotoxic (CD8+) cells, NK (CD16+) cells and monocytes, granulocyte and monocyte ingestion and intracellular killing capacities as well as NK cell activity, were determined before TCT and 7 months later in two groups of patients, one of them free of any further therapy (control) and the other receiving TMX. As shown in Table 1, the absolute number of lymphocytes ($p=0.002$) and both their subpopulations of CD4+ ($p<0.0009$) and CD8+ ($p=0.002$) cells, as well as B lymphocytes ($p=0.016$) and CD16+ cells ($p=0.015$) were decreased in the control group 7 months after TCT, as compared to their pre-TCT values, while in TMX patients only the absolute number of all

Table 2

The proportions of peripheral blood cells before telecobalt therapy (TCT) and 7 months following TCT in two groups of patients, one receiving tamoxifen (TMX) and the other receiving no further therapy following TCT

Cell type	Before TCT without TMX	After TCT without TMX	Before TCT with TMX	After TCT with TMX
Granulocytes	60a 57 – 65b	72* 67 – 74	65 51 – 72	66 60 – 70
Monocytes	4 3 – 10	6 3 – 7	5 2 – 6	4 3 – 8
Lymphocytes	26 23 – 31	18* 17 – 27	27 20 – 36	24** 18 – 29
CD4+ lymphocytes	50 47 – 54	49 44 – 54	52 49 – 57	49 44 – 51
CD8+ lymphocytes	28 24 – 30	30 28 – 34	32 28 – 35	35** 29 – 36
B lymphocytes	7 5 – 7	8 5 – 8	7 4 – 9	8 5 – 11
CD 16+ lymphocytes	14 10 – 18	13 9 – 19	12 9 – 19	13 10 – 17

[a] Median, [b] Interquartile range

* p<0 05 compared to the same group before TCT

**p<0 05 compared to the control group after TCT

lymphocytes was decreased in comparison to its pre-TCT value (p=0.015), while the numbers of CD4+, CD8+, B and NK cells were not statistically different from their pre-TCT values (Table 1) Moreover, the numbers of all lymphocytes (p=0 004), CD4+ (p=0 016) and CD8+ cells (p=0 048) in TMX patients after TCT were significantly increased in comparison to the corresponding values in control patients after TCT. although there was no difference in any of the parameters tested between these two groups before TCT (Table 1) Table 2 shows proportions of peripheral blood cells The proportion of all lymphocytes was decreased in control patients after TCT (p=0 017), causing a corresponding shift of granulocytes toward an increased value (p=0 003) However, the proportion of all lymphocytes in TMX patients following TCT was significantly increased in comparison to the corresponding value of control post-TCT group (p=0 036) The same was found for the proportion of CD8+ cells (p=0 044) There were no other differences either between the control and TMX group or between the pre- and post-TCT values in the two patient groups In functional tests, there was no difference in monocyte phagocytic functions (Figures 1 and 2) and granulocyte microbicidity (Figure 2). either between the two patient groups or between their pre- and post-TCT values The post-TCT value of granulocyte ingestion ability in TMX patients was decreased (p=0 003) in comparison to its pre-TCT value (Figure 2) The same tendency of decline in TMX patients was observed for NK cell activity, although the difference was not significant (Figure 3)

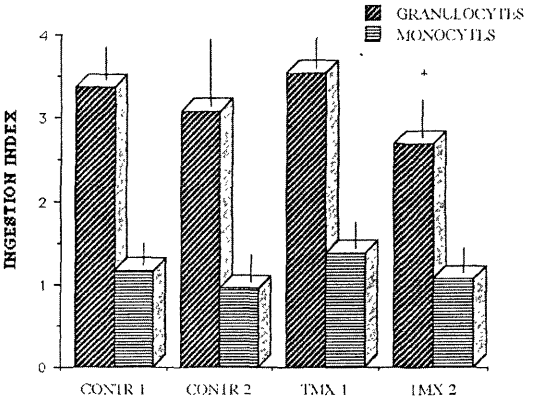

FIGURE 1 Granulocyte and monocyte ingestion abilities before (1) and 7 months following TCT (2) in patients free of further therapy (CONTR) and those receiving tamoxifen (TMX) Granulocyte ingestion was significantly decreased in patients following 7 months of TMX therapy in comparison to their values before therapy (* p=0 003)

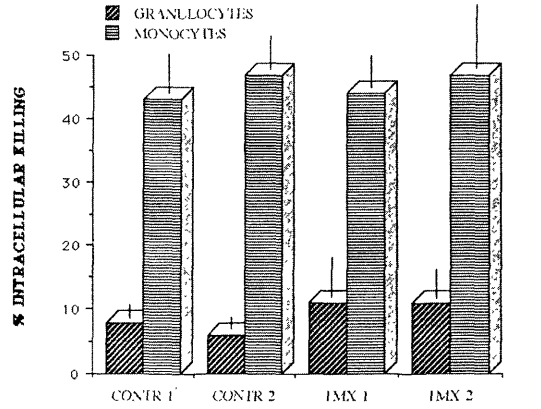

FIGURE 2 Granulocyte and monocyte intercellular killing before (1) and 7 months following TCT (2) in patients free of further therapy (CONTR) and in those receiving tamoxifen (TMX) There was no significant difference between any of the groups tested

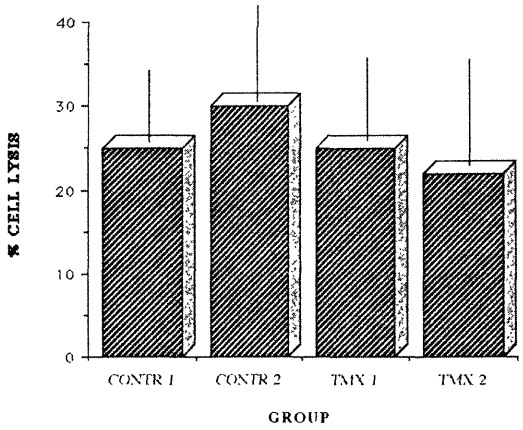

FIGURE 3 Natural killer activity (effector target cells ratio 50 1) before (1) and 7 months following TCT (2) in patients free of further therapy (CONTR) and in those receiving tamoxifen (TMX) Although the activity was somewhat lower in patients receiving TMX as compared to the values before starting TMX therapy as well as to the values in patients not receiving TMX, the differences were not statistically significant

DISCUSSION

We had two goals in our investigation to study the number of immunocytes and immune functions in breast cancer patients, and to evaluate the possible immunomodulatory effect of tamoxifen Results presented in this work indicate a more profound effect of TMX on the number of immune cells than on their functions. We did not observe significant differences between pre- and post-TCT values of NK activity either in control or in TMX patients In fact, the post-TCT NK activity in TMX patients was lower than the corresponding value in control patients (Figure 3). Although the difference was not statistically significant, it could indicate a possible adverse effect of TMX. This finding does not correspond with the results obtained by Berry and coworkers[6] who have reported *in vivo* potentiation of NK activity by TMX, but their patients were not irradiated prior to testing Concerning phagocytic functions, there was no difference in monocyte functions, but granulocyte ingestion was decreased following TCT in comparison with their pre-TCT value in TMX patients (Figure 1) This effect was not observed in control patients The results indicated the immunosuppressive activity, although slight, of TMX, which is in agreement with the results of Nagy and Berczi[8], who have reported that TMX is capable of suppressing both cell-mediated and humoral immunity in rats, and of Robinson et al [12], who have also reported a reduced

NK cell activity in TMX-treated breast cancer patients On the other hand, TMX more profoundly influenced the number of lymphocyte populations As shown in Table 1, the absolute number of all lymphocytes, their CD4+ and CD8+ subpopulations, B and CD16+ cells were significantly lowered following TCT in control patients This is in accordance with other authors who have reported that a decreased number of CD4 and CD8 peripheral blood cells can be observed immediately after irradiation[13] and persists for months[14] or even years[15] However, in TMX patients the numbers of these cells after TCT were not significantly decreased in comparison to their pre-TCT values. Moreover, the number of all lymphocytes as well as their subpopulations after TCT *were significantly higher in TMX patients than in control patients (Table 1)* Even the proportions of all and suppressor/cytotoxic lymphocytes following TCT were in TMX patients significantly higher than in controls (Table 2) Interestingly, the number of neutrophils and monocytes (Table 1) showed no significant changes following TCT in control patients nor was influenced by TMX therapy, showing their higher resistance to irradiation, but also to the potential effects of TMX It seems that TMX helps the recovery of lymphocyte populations decreased by radiotherapy, but does not influence the number of monocytes and granulocytes If the effect is related to estrogenic stimulation of cells carrying estrogen receptors, this would explain why lymphocytes responded to TMX, while granulocytes and monocytes did not In conclusion, TMX therapy caused depression of some immunocyte functions like granulocyte phagocytosis, did not significantly influence NK activity but increased the depressed number of lymphocyte populations caused by radiation therapy This beneficial influence of TMX on lymphocyte counts as well as slightly adverse effects on phagocytosis and probably on NK cell activity may reflect *in vivo* the immunosurveillance mechanisms and predict the disease behavior Therefore, the described immunomodulatory effects of TMX need to be kept in mind, particularly in its long-term use in both therapy and prevention programs for breast cancer

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SUMMARY

Tamoxifen (TMX) has been demonstrated to be an effective form of hormone therapy in estrogen receptor-positive breast cancer patients, in both adjuvant and metastatic settings Since estrogens influence the immune system, which supports host resistance of tumors, the effect of TMX on immunocompetence was investigated in a group of 27 patients with ductal invasive breast carcinoma, stage I-III, before and 7 months after postsurgical telecobalt radiotherapy (TCT) Patients were divided into two subgroups, one receiving tamoxifen (TMX group) and the other not receiving any further therapy (*control group*) *During the investigation, the number of leukocytes,* proportion and absolute number of granulocytes, lymphocytes, CD4+ cells, CD8+ cells, CD16+ cells, B-lymphocytes, monocytes, natural killer cell (NK) activity and granulocyte and monocyte phagocytic functions - ingestion and intracellular killing, were determined Post-TCT proportions of all and CD8+ lymphocytes as well as

absolute numbers of all and CD4+ and CD8+ lymphocytes in TMX patients were significantly increased in comparison to the same parameters in control patients, although there was no difference between the two subgroups before TCT. In the TMX group, only the absolute number of all lymphocytes remained decreased after TCT, while in the control group all the parameters observed were decreased after TCT in comparison to their pre-TCT values. On the other hand, the post-TCT value of granulocyte ingestion ability in TMX patients was decreased in comparison to its pre-TCT value. The same tendency of decline in TMX patients was observed for NK activity, but the difference was not significant. It seems that TMX has, at the same time, a stimulatory effect on the recovery of immunocyte populations decreased by radiotherapy, and an inhibitory influence on the immune functions. Thus, further investigation is warranted to determine the clinical relevance of long-term TMX treatment in the modulation of immune responses in breast cancer patients.

PROMJENA BROJA I DJELOVANJA IMUNOCITA TAMOKSIFENOM U BOLESNICA S KARCINOMOM DOJKE

SAŽETAK

Tamoksifen (TMX) se ustalio kao učinkoviti oblik hormonske terapije u bolesnica s karcinomom dojke s pozitivnim estrogenskim receptorima. Kako estrogeni utječu na imunološki sustav koji podržava obranu domaćina od tumora, ispitan je učinak TMX na imunokompetenciju u skupini od 27 bolesnica s invazivnim duktalnim karcinomom dojke, stadij I-III, prije i 7 mjeseci nakon postkirurške telekobaltne terapije (TCT). Bolesnice su bile podijeljene u dvije podskupine, jedna od njih je primala TMX (TMX skupina), a druga nije dalje primala terapiju (kontrolna skupina). Tijekom ispitivanja utvrđeni su sljedeći parametri: broj leukocita, postotak i apsolutni broj granulocita, limfocita, CD4+ stanica, CD8+ stanica, CD16+ stanica, B-limfocita, monocita, te djelovanje prirodno ubilačkih stanica (NK) i fagocitno djelovanje granulocita i monocita (ingestija i unutarstanično ubijanje). Postotci svih i CD8+ limfocita nakon TCT, kao i apsolutni broj svih CD4+ i CD8+ limfocita u TMX skupini bili su značajno povišeni u usporedbi s vrijednostima istih parametara u kontrolnoj skupini, iako ta razlika između podskupina nije postojala prije TCT. U TMX skupini jedino je apsolutni broj svih limfocita ostao snižen nakon TCT, dok su u kontrolnoj skupini svi praćeni parametri bili sniženi nakon TCT u usporedbi s njihovim vrijednostima prije TCT. S druge strane, vrijednosti sposobnosti granulocitne ingestije nakon TCT u TMX skupini bolesnica bile su snižene u usporedbi s vrijednostima prije provođenja TCT. Slična sklonost k padu vrijednosti uočena je i mjerenjem prirodno ubilačke aktivnosti (NK) u TMX skupini, iako razlika nije bila značajna. Sjeće se dojam kako TMX istodobno ima' poticajni učinak na oporavak populacija imunocita nakon radioterapije i kao negativan utjecaj na imunološka djelovanja. Zato su buduća

istraživanja okrenuta k utvrđivanju kliničkoga značenja promjena imunološkoga odgovora u bolesnica s karcinomom dojke dugotrajno liječenih tamoksifenom

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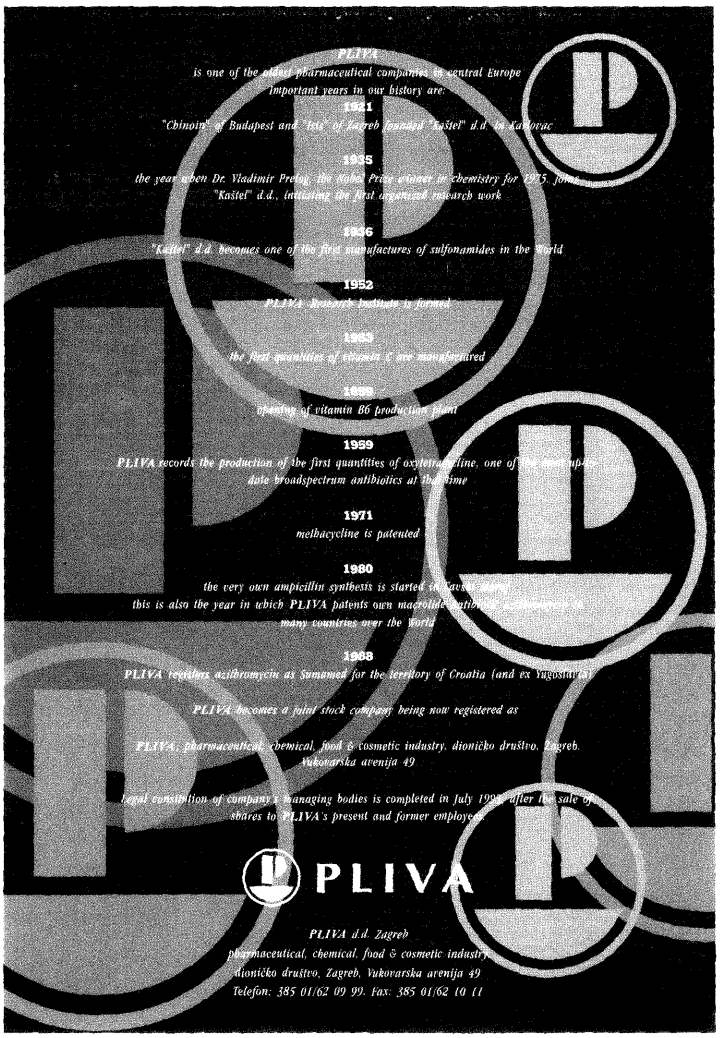
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