Drago Ikic[1], Josip Krušić[2], Davor Ivanković[3]

INTERFERON TREATMENT OF UTERINE CERVICAL CARCINOMA

THE RESULTS OF STUDIES CONDUCTED A LONG TIME AGO

Introduction

Interferon can be considered an important therapy for many gynecological diseases. It has been shown to be effective in the treatment of genital herpes [1,2,3,4]; condylomata acuminata [5,6]; uterine cervical precancerosis, interaepithelial neoplasia [7,8,9]. A treatment of the uterine carvical cancer was published by Lippman at al. [10,11]. According to Lippman at al. [10], the response rate is 58% [11 of 19] in patients with stage IIB or higher disease level and 66% [10 of 15] in patients with bulky disease (at least one dimension >10cm). Patients were treated with 13-cis Retinol Acid (cRA) lmg/kg orally per day and r.IF'N alpha $6x10^6$ IU sc per day for at least 2 months. The local adjuvant treatment of the cervical uterine carcinoma was published by Ikić, Krušić and coll [12,13 14,15]. After the application of human natural leucocytic interferon (HNLI) as local adjuvant treatment before surgery in patients with the uterine cervical as carcinoma the response was as follows: exellent in 6 patients, very good in 5, moderate in l, poor in 2 and no response in one [14]. The aim of our study is the follow-up of the uterine cervical carcinoma patients both HNLI treated and non treated up to 20 years ago, and to analyse the difference between these two groups. The IFN clinical studies are slow and it requires long assessment time. Therefore, it is valuable to analyse studies taken a long time ago which may lead to the procedure of using IFN as a part of the uterine cervical carcinoma treatment.

[1] Institute for Research and Standardization of Immunologic Substances, Croatian Academy of Sciences and Arts, Zagreb;
[2] Central Institute of Tumors and Allied Diseases, Zagreb;
[3] The Andrija Stampar School of Public Health, Medical Faculty, University of Zagreb.

Patients and methods

Patients. Fifty seven patients out of 72 volunteering patients with biopsy proven cervical uterine carcinoma took part in the final study. Twenty seven randomized patients were treated with HNLI and followed up, 30 randomized patients were included and followed up in the control group without being treated with HNLI. The staging of disease conformed with the 1987 Intemational Federation of Gynecology and Obstetrics (FIGO) staging system [16]. Surgical material removed from HNLI both treated and control patients was also examined. None of the patients had received any prior therapy. On completion of HNLI therapy the patients treated with HNLI, as well as patients in the control group, were treated with surgery. Table 1 shows the patients clinical stage, according to FIGO classification.

TABLE 1

PATIENTS CLINICAL STAGE. FIGO CLASSIFICATION

	IA	IB	IIA, IIB	TOTAL
HNLI TREATED	8	9	10	27
CONTROL GROUP	8	10	12	30

Interferon. HNLI was applied locally at $2x10^6$ units daily in pessaries. HM,I was given for 21 days before surgery. HNLI is semipurified concentrated (10^4 per 1 mg) protein,. human natural lekocytic interferon. The follow-up of some patients, both HNLI treated and not HNLI treated in the control group, has been organized for up to 20 years.

Statistics. The data were analyzed by using Kaplan-Meier method for survival analysis [17] and Cox's F-test for the comparison of the groups. The analysis was made by means of the statistical programme, Statistica, Stat Soft inc. Tulsa OK 741 10, USA.

Results

In the group of interferon treated patients 1 A, all the patients survived as well as patients in the control lA group. There were no differences between these two groups. There were 8 patients in the interferon treated group and 8 patients in the control group. The patients were followed up from 197 to 240 months in the HNLI treated group and 195-240 months in the control group.

Table 2 shows survivals of the 1B patients. In the group of interferon treated 1B patients 2 of 9 died, and in the control group 5 of 10 patients died. Regression lines for interferon treated and control 1B patients are shown in FIG 1. Survival analysis according to Cox's F-test shows that the difference in survival between the interferon treated 1B patients and the control group is significant on the level 9% $P<0,09$ which suggests there would have been an even greater difference, if we have had more patients.

TABLE 2

CARCINOMA, CERVICIS UTERI 1B

PATIENTS	SURVIVAL IN MONTHS	PATIENTS	SURVIVAL IN MONTHS
1	191	1	205
2	202	2	194
3	204	3	216
4	216	4	209
5	202	5	204
6	196	6	+60
7	216	7	+48
8	+60	8	+48
9	+18	9	+4
		10	+22
LIVING/DEAD = 7/2		LIVING/DEAD = 5/5	

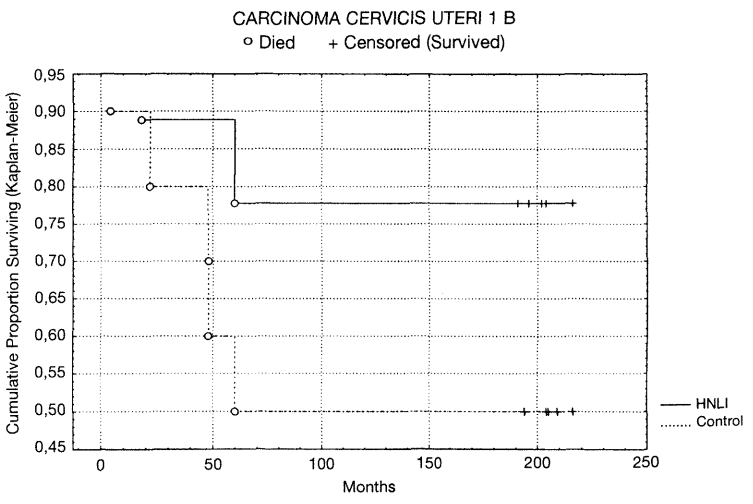

FIG 1

Table 3 shows survivals of the 2A and 2B patients. In the group of interferon treated 2A and B patients 5 of 10 died and in the control group 11 of I2 patients died. Regression lines for interferon treated and control 2A and B patients are shown in FIG 2. Survival analysis according to Cox's F-test shows that the difference in survival between the interferon treated 2A and B patients and control group is significant on the level 2% P<0, 02.

TABLE 3

CARCINOMA, CERVICIS UTERI 2A, 2B

HNLI		CONTROL	
PATIENTS	**SURVIVAL IN MONTHS**	**PATIENTS**	**SURVIVAL IN MONTHS**
1	216	1	214
2	192	2	+84
3	197	3	+48
4	214	4	+13
5	210	5	+13
6	+48	6	+13
7	+24	7	+24
8	+17	8	+32
9	+13	9	+36
10	+40	10	+36
		11	+30
		12	+34
LIVING/DEAD = 5/5		LIVING/DEAD = 1/11	

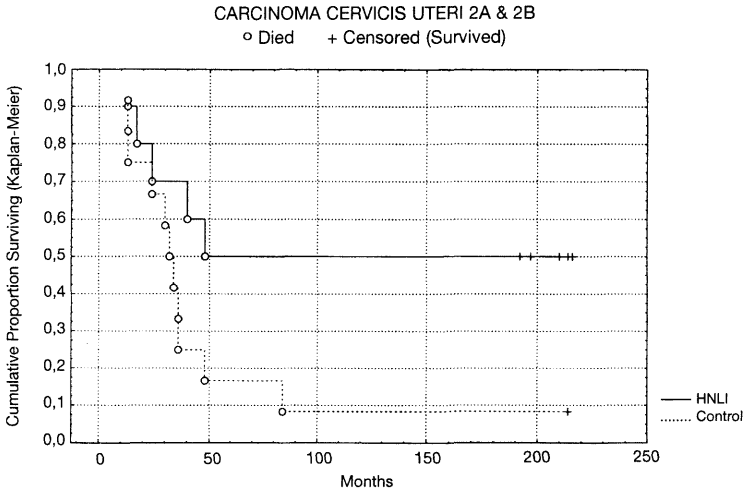

FIG 2

Discussion

The results of our long term follow-up show that it is possible to achieve the cure of the uterine cervical carcinoma patients 2A and 2B, treated locally with HNLI before surgery and that the cure persist. The difference in survival between the HNLI treated 2A and 2B patients and the control group is significant on the level of 2% P<0,02. The difference between groups 1B and control group was significant on the level 9% P<0,09. In our study, patients with squamous cell carcinoma of the cervix, were treated with HNLI $2x10^6$ units locally, daily for 21 days before surgery. In Lippman et al. study, r.IFN alpha 2a at the rate of $6x10^6$ units daily was applied subcutaneously for two months and 13 cRA 1 mg/kg daily, orally, after surgery [10,11]. It seems, then, that retinoids are potent regulators of epithelial differentiation within many neoplastic cell system [19]. In any case, in Lippman et al. study, the dosing of r.IFN alpha 2a after surgery was several times higher, the route of application different and the treatment duration longer than in our study where HNLI was applied locally before surgery [10,11]. Are there any signs that HNLI, which is mixture of many subtypes and r. IFN alpha 2a differ in clinical efficacy? That is an important question but we still lack data for clear-cut comparisons. The effects produced by HNLI are also comparable with those produced by with very pure interferon, although we have an impression that HNLI administered locally requires smaller doses than r.IFN alpha 2a [20,21]. Local interferon (IFN) application into tumor before surgery was introduced by Ikić et al. [12,14]. The local production of IFN has an important physiological role in the immune system. After the local application of HNLI to the tumor site, before surgery, the tumor cells were blocked and their dispersal during surgery was thus prevented, reducing the number of micrometastasis and increasing the chance of survival [18]. Therefore we advise for to be IFN administer locally in pessaries at least $2x10^6$ units daily before surgery for 3-4 weeks for the treatment of the patients IB, IIA, IIB or of higher grade and then to give them IFN after surgery, similar to Lippman et al. procedure.

REFERENCES

1. *Ikić D, Smerdel S, Šooš E, et al.*: Immunohistological protection and therapy of human genital herpes. Radovi Imun zavoda 1971;13:1 13-120.

2. *Ikić D, Smerdel S, Šooš E, et al.*: The therapy of some skin virus diseases of the genital region with human leukocytic interferon. In Gušić B, ed. Proceedings of the Symposium on Field Trials of Vaccines. Zagreb: Yugoslav Academy of Sciences and Arts 1973:193-198.

3. *Ikić D, Trajer D, Čupak K, et al.*: The clinical use of human leukocyte interferon in viral infections. Int J Clin Pharmacol 1981;11:498-506.

4. *Gross G, Roussaki A, Schopf E.*: Successful treatment of condylomata acuminata and bowenoid papulosis with subcutaneous injections of low-dose recombinant interferon.alpha. Arch Dermatol 1986;122:749-750.

5. *Ikić D, Bosnić N, Smerdel S, et al.*: A double-blind clinical trial with human leukocytic interferon in the therapy of condylomata acuminata. In Ikić D, ed. Proceedings of the Symposium on Clinical Use of Interferon. Zagreb: Yugoslav Academy of Sciences and Arts 1975;229-234.

6. *Scott GM, Csonka GW.*: Effects of injection of small doses of human fibroblast interferon into genital warts. Br J Venereal Dis 1979;55:442-445.

7. *Ikić D, Singer Z, Beck M, et al.*: Interferon Treatment of Uterine Cervical Precancerosis. J Cancer Rec Clin Oncol 1981;101:303-308.

8. *Spirtos N, Smith L, Teng N.*: Prospective Randomised Trial of Topical a-Interferon (a-Interferon gels) for the Treatment of Vulvar Intraepithelial Neoplasia. Gynaecol Oncol 1990;37:34-38.

9. *Singer Z, Šooš E, Feichter G.*: Treatment of cervical intraepithelial neoplasia associated with human papilloma virus by interferon vaginalettes. Radiol Oncol 1993;27:321-325.

10. *Lippman SM, Kavanagh JJ, Paredes-Espinoza M, et al.*: 13-cis retinoic acid plus interferon alpha 2a: Highly active systemic therapy for squamous cell carcinoma of the cervix. J Natl Cancer Inst 1992;84:241-245.

11. *Lippman SM, Kavanagh JJ, Paredes-Espinoza M, et al.*: 13- cis-Retinoic Acid plus Interferon-a 2a in Locally Advanced Squamous Cell Carcinoma of the Cervix. J Natl Cancer Inst 1993;85:499-500.

12. *Ikić D, Krušić J, Čupak K, et al.*: The use of human leukocytic interferon I patients with cervical cancer and basocellular cancer of the skin. In: Ikić D, ed. Proceedings of the Symposium on Clinical Use of Interferon. Zagreb: Yugoslav Academy of Sciences and Arts 1975:239-244.

13. *Krušić J, Ikić D, Knežević M, et al.*: Clinical and histological findings after local application of human leukocyte interferon in patients with cervical cancer. In: Ikić D, ed. Proceedings of the Symposium on Clinical Use of Interferon. Zagreb: Yugoslav Academy of Sciences and Arts 1977;167-177.

14. *Ikić D, Krušić J, Kirhmajer V, et al.*: Application of human leukocyte interferon in patients with carcinoma of the uterine cervix. Lancet 1981;1:1027-1030.

15. *Krušić J, Kirhmajer V, Knežević M, et al.*: Influence of human leukocyte interferon on squamous cell carcinoma of the uterine cervix: Clinical, histological and histochemical observations III Communication. J Cancer Res Clin Oncol 1981;101:309-315.

16. *Hoskins WJ, Perez C, Young RC.*: Gynecologic tumors. In Cancer: Principles and Practice of Oncology, 3rd ed. (DeVita VT Jr, Hellman S, Rosenberg SA, eds). Philadelphia: Lippincott, 1989:1099-1161.

17. *Kaplan E, Meier P.*: Nonparametric estimation from incomplete observations. J Am Statist Assoc 1958;53:457-481.

18. *Ikić D, Padovan I, Brodarec I, et al.* Application of human leukocyte interferon in patients with tumours of the head and neck. Lancet 1981;I:1025-1027.

19. *Lippman SM, Parkinson DR, Itri LM, et al.*: 13-cis retinoic acid and interferon alpha 2a: Effective combination therapy for advanced squamous cell carcinoma of the skin. J Natl Cancer Inst 1992;84:235-241.

20. *Ikić S, Padovan I, Pipić N, et al.*: Local interferon therapy for lip carcinoma. Eur Arch Otorhinolaryngol 1994;251 :293-296.

21. *Ikić D, Padovan I, Pipić N, et al.*: Basal Cell Carcinoma Treated with Interferon. Int J Dermatology 1991;30:734-738.

SAŽETAK

Liječenje cervikalnog karcinoma interferonom

Liječenje interferonom cervikalnog karcinoma uterusa pokazalo je pozitivne rezultate kod 50-60% pacijentica s 2A i 2B ili višeg stadija. Visoki mortalitet žena oboljelih od cervikalnog karcinoma uterusa pokazuje na potrebu potvrđivanja ovih rezultata. Zbog toga korisno je analizirati rezultate istraživanja izvršenih mnogo godina ranije koja mogu pridonijeti liječenju interferonom pacijentica oboljelih od cervikalnog karcinoma uterusa.

Dvadesetsedam randomiziranih pacijentica liječeno je s prirodnim humanim leukocitnim interferonom (HNLI) prije operativnog zahvata. HNLI primijenjen je lokalno, dnevno, $2x10^6$ jedinica kao prašak u pesaru u toku dvadesetjednog dana prije operacije. Trideset randomiziranih pacijentica u kontrolnoj skupini nisu liječene s HNLI. FIGO klasifikacija interferonom liječenih pacijentica bila je IA:8; IB:9; i B:10, ukupno 27. FIGO klasifikacija pacijentica u kontrolnoj skupini koje nisu liječene s HNLI bila je IA:9; IB:10; IIA, B:12; ukupno 30.

Rezultati promatranja pacijentica analizirani su nakon 20 godina pomoću Kaplan-Meirove metode i Cox-ovog F testa. U grupi IA sve su pacijentice preživjele, pa nije bilo razlike između HNLI liječenih pacijentica i onih u kontrolnoj skupini. U grupi IB umrle su 2 od 9 pacijentica liječenih s HNLI, u kontrolnoj skupini 5 od 10. Analiza prema Cox-ovom testu pokazala je da je razlika značajna na razini 9% P< 0,09. U grupi HNLI liječenih 2A i B pacijentica umrlo je 5 od 10, a u kontrolnoj skupini 11 od 12. Analiza prema Cox-ovom F testu pokazala je kako je razlika značajna na razini 2% P=<0,02.

Lokalna produkcija interferona igra važnu ulogu u našem imunološkom sustavu. Glavni razlog za primjenu HNLI prije kirurškog zahvata je induciranje reaktivnosti tumorske strome, regionalnih limfnih čvorova, te sprječavanje mikrometastaza prilikom kirurškog zahvata. Mišljenja smo da je potrebno prije operacije primijeniti interferon lokalno pomoću pesara u količini od najmanje $2x10^6$ jedinica u toku 3-4 tjedna za liječenje cervikalnog karcinoma uterusa kod pacijentica s 1B, 2A, 2B ili višim stupnjem, a zatim nastaviti poslije operacije 1-2 mjeseca.

SUMMARY

Interferon treatment of uterine cervical carcinoma

Background. The Interferon treatment of uterine cervical carcinoma has shown promising results, i. e. the response rate of 50%-60% in patients with stage IIA and IIIB or of higher grade. High mortality rate in women due to uterine cervical carcinoma indicates the need for the confirmation of these results. Therefore, it is valuable to analyse the results of studies conducted a long time ago which may contribute to establishing the procedure of using interferon as a part of the uterine cervical carcinoma treatment.

Methods. Twenty seven randomised patients were treated with human natural leucocytic interferon (HNLI) and followed up, while 30 randomized patients were followed up in the control group and were not treated with with HNLI. FIGO classification of IFN treated patients was IA:8, IB:9, IIA;IIB:lO; total 27. FIGO classification of control nontreated patients was IA:9, IB: 10 IIA and IIB:l2; total 30 patients. HNLI was applied locally at $2x10^6$ units daily in pessaries for 21 days before surgery. The data were analysed by means of Kaplan-Meier methods for survival analysis and by means of Cox's F-test for comparison between groups. We followed up the HNLI treated and nontreated patients up to 20 years.

Findings. In the group IA all the patients survived. There was not any difference between the IFN treated and the control group. In the group of IB patients 2 of 9 died and in the control group 5 of 10 patients died. Survival analysis according to Cox's F-test shows that the difference is significant at the level 9% $P<0.09$. In the group of IFN treated 2A and 2B patients 5 of 10 died, and in the control group 11 of 12 patients died Survival analysis according to Cox's F-test shows that the difference is significant at the level 2% $P<0.02$.

Interpretation. Local production of IFN has an important physiological role within the immune system. The main purpose of IFN local administration before surgery is to induce the reactivity of tumor stroma and regional lymph nodes and to inhibit micrometastasis. In our opinion it is important to apply IFN locally in pessaries at least $2x10^6$ units daily before surgery for 3-4 weaks for the treatment of uterine cervical carcinoma patients IB, IIA, IIB or of higher grade and then to give IFN after surgery.

Contributors: Drago Ikić was responsible for writing up the paper. Josip Krušić MD, ScD, departed from the life during preparation of the manuscript, was responsible for the design, execution and clinical work. Davor Ivanković was responsible for statistical analysis of data.

Acknowledgments

We thanks all the patients and medical staff who took part in this study.