Radiol Oncol 1993; 27: 280-5.

Combined treatment of murine SA-1 tumors by human leukocyte interferon alpha and electrotherapy

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Antitumor effectiveness of human leukocyte interferon alpha (IFN-α) was assessed in combination with electrotherapy. Subcutaneous fibrosarcoma SA-1 tumors were treated with IFN-α for five consecutive days. The results indicate that IFN-α given either locally (peritumorally) or systemically (intraperitoneally) as a single treatment has moderate antitumor effect. In order to potentiate its effectiveness, IFN-α was combined with electrotherapy. Low level direct current ranging from 0.2 to 1.2 mA for 60 minutes was delivered via Platinum/Iridium electrodes placed subcutaneously, outside the tumor. Combined treatment with electrotherapy and IFN-α given intraperitoneally, proved to have more than additive antitumor effect, assessed by tumor growth delay. Interaction between the two treatment modalities increased at higher current levels used for electrotherapy. The results indicate that IFN-α and electrotherapy interact in local tumor growth control. Therefore, electrotherapy can be used to locally potentiate systemic IFN-α treatment or vice versa, the latter agent can potentiate the effect of electrotherapy.

Key words: fibrosarcoma-therapy; interferon alpha; electric stimulation therapy; mice

Introduction

Interferons (IFN-s) are the members of a big family of regulatory cytokines.[1] These molecules control the growth and differentiation of many cells in the organism. By positive and negative feedback loops they interact with growth factors, oncogenes and other regulatory molecules.[2] Studies on IFN-s have yielded an expanding list of bioactivities; besides anti-viral and microbicidal action, antitumor effectiveness has drawn much of attention.[3, 4, 5] Interferons exert antiproliferative effect on a number of malignant cells, have transformation-suppressing effect and can regulate their differentiation.[2, 5, 6] Also, immunoregulatory effect of IFN-s is very important, which has put these agents in place of immunoadjuvant settings.[7] In clinical trials IFN-s have shown significant activity against a wide range of human cancers. Hematological disorders proved to be the most responsive to IFN-α treatment, contrary to solid tumors, where response rates seldom exceed 20–30 %.[8] From the vast experience it is evident that treatment in low tumor burden is more effective than in advanced, bulky disease. In this respect combination with other cytotoxic

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UDC: 616-006.327.04-085

treatments is possible, since they can reduce tumor burden and interact with IFN-s treatment.

One of the treatment modalities, which has recently proved effective in reducing tumor burden is electrotherapy.[9] It is effective as an anti-tumor agent which has been demonstrated on several tumor models as well as in clinic.[10-13] Application of electrotherapy is foreseen predominantly in combination with biological or cytotoxic treatments.[13-15] In our preliminary study combined treatment with human leukocyte IFN-α and electrotherapy demonstrated some positive interactions.[16] In the present study electrotherapy with electrodes placed outside the tumor, in order to avoid mechanical intrusion (field electrotherapy)[17] was combined with IFN-α treatment. Interaction of the two treatments was evaluated by tumor growth delay, according to the route of IFN-α treatment and direct current levels used for electrotherapy.

Materials and methods

Cell cultures

Fibrosarcoma SA-1 cell were grown in tissue culture flasks at 37° C in a humidified 5 % CO_2 atmosphere, using Eagle's MEM supplemented with 10 % fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml). To study the effect of IFN-α on cell growth, cells in petri dishes were treated continuously with 1, 5, 10 and 20×10^3 U IFN-α/ml. Three days after treatment viable cells in the cell cultures were counted in hemocytometer and the cell number/control (%) ratio was calculated. The statistical evaluation was done by means of Student-t test.

Animals

Female and male inbred A/J mice were purchased from the Institute Rudjer Bošković, Zagreb, Croatia. Animals were maintained in conventional animal colony at constant room temperature 24° C at natural day/night light cycle. Mice in good condition, without signs of fungal or other infection, eight to ten weeks old were included in the experiments. Experimental groups consisted of 8-10 animals.

Tumors

As a tumor model fibrosarcoma (SA-1) syngeneic to A/J mice was used. Single tumor cell suspension was obtained from an ascitic form of the tumor. Solid subcutaneous tumors, dorsolaterally in animals, were initiated by injection of 5×10^5 viable SA-1 cells. When the tumors reached 30–40 mm^3 in volume, animals were marked individually and on day 0 randomly divided into smaller groups, subjected to specific experimental protocol. On each consecutive day the tumor volume was calculated from the three mutually orthogonal diameters measured by vernier caliper gauge. Arithmetic mean (AM) and standard error of the mean (SE) were calculated for each day in all experimental groups. Tumor doubling time (DT) was determined for individual tumors and tumor growth delay calculated (GD) from mean DT of experimental groups.[14] The differences between the experimental groups were evaluated statistically by nonparametric Mann-Whitney Rank-Sum test, taking into account the Bonferoni adjustment when multiple comparisons were performed.

Electrotherapy

The direct current (DC) source for electrotherapy was designed and manufactured at the Faculty of Electrical and Computer Engineering, Ljubljana, Slovenia. Current and voltage were continuously monitored during electrotherapy with 0.2, 0.4, 0.6, 0.8 or 1.2 mA DC of one hour duration. Current was delivered through Pt/Ir (90/10 %) alloy needle electrodes (1.0 mm diameter, 22.0 mm long) with rounded tips and inserted subcutaneously 5–10 mm from the margin of the tumor on the two opposite sites.[17] The control group was treated in the same way as experimental groups, except that no current flowed.

Lymphokines and therapy protocol

Partially purified human leukocyte interferon alpha (IFN-α) was purchased from Immunological Institute, Zagreb, Croatia.[18] Animals were treated with 5×10^4 U IFN-α daily, for five consecutive days, starting one hour before electrotherapy. Therapy was performed either peritumorally with 0.1 ml IFN-α injected subcutaneously in the vicinity of the tumor, with precaution not to damage tumor capsule, or intraperitoneally with IFN-α dissolved in 0.5 ml phosphate buffer saline (PBS).

Results

Antitumor effectiveness of IFN-α was tested on fibrosarcoma SA-1 *in vitro* and *in vivo*. The effect of IFN-α *in vitro* on the growth of SA-1 cells is presented in Figure 1. Lower concentrations seemed to enhance tumor cell proliferation, but the effect was not statistically significant. At the highest concentration (2×10^4 U/ml) moderate antiproliferative effect was demonstrated which was statistically significant ($p < 0.05$).

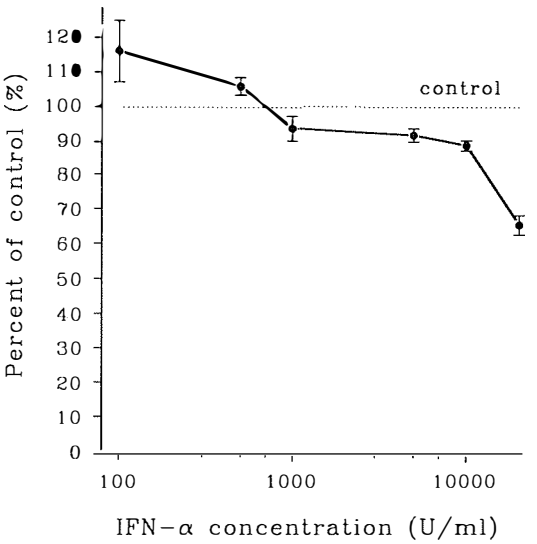

Figure 1. Effect of IFN-α on the growth of SA-1 cells *in vitro*. Cells were grown in different IFN-α concentrations for three days and thereafter their growth rate was determined.

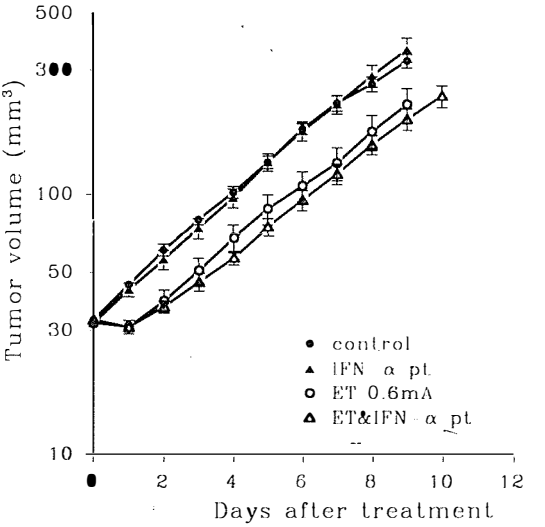

Figure 2. Antitumor effect of IFN-α and electrotherapy on subcutaneous SA-1 tumors. IFN-α (5×10^4 U) was injected peritumorally five consecutive days, starting on day 0. Electrotherapy was performed with 0.6 mA one hour after IFN-α treatment on day 0. Experimental groups comprised 8–10 animals.

Anti-tumor effect of IFN-α was tested also on SA-1 tumors *in vivo*. Solid subcutaneous SA-1 tumors were treated for five consecutive days with 5×10^4 U IFN-α daily. Different routes of IFN-α administration were tested; i.e. intraperitoneal and peritumoral application. Both, peritumoral (GD = 0.4 ± 0.2 days) and intraperitoneal (GD = 0.6 ± 0.3 days) treatment did not significantly delay tumor growth ($p > 0.05$) (Figure 2, 3). Also, there was no statistical difference between the effectiveness of IFN-α after peritumoral and intraperitoneal application ($p = 0.6$).

In order to test for interaction of IFN-α treatment with electrotherapy, both treatment modalities were combined. Electrotherapy (0.6 mA for 1 hour) as a single treatment statistically significantly delayed tumor growth ($P < 0.001$) (Figure 2, 3). In combined modality treatment electrotherapy was performed one hour after the first IFN-α application. The interaction was better when electrotherapy was combined with intraperitoneal IFN-α treatment than with peritumoral application (Figure 2, 3). Additive antitumor effect was obtained with peritumoral

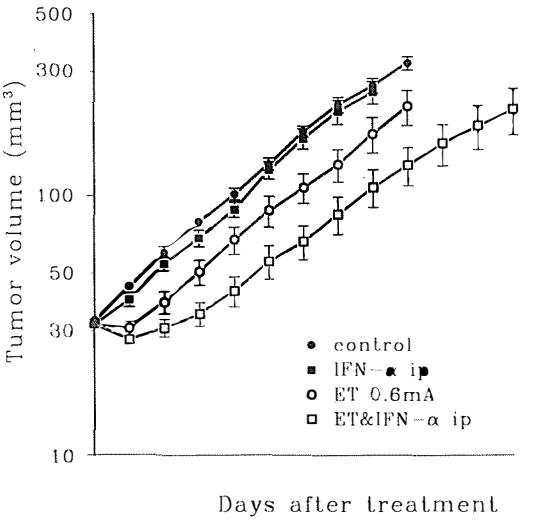

Figure 3. Antitumor effect of IFN-α and electrotherapy on subcutaneous SA-1 tumors. IFN-α (5×10^4 U) was injected intraperitoneally five consecutive days, starting on day 0. Electrotherapy was performed with 0.6 mA one hour after IFN-α treatment on day 0. Experimental groups comprised 8-10 animals.

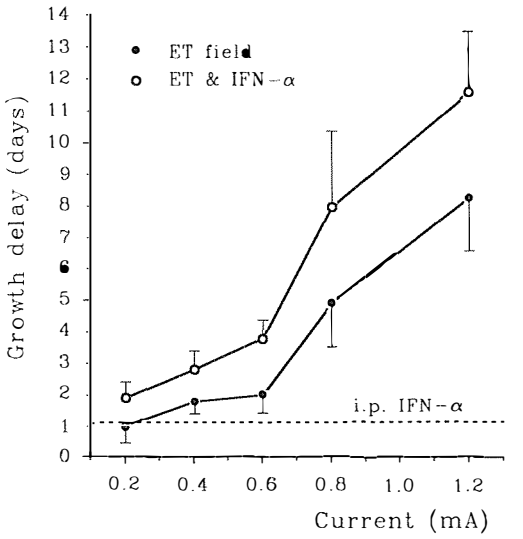

Figure 4. Tumor growth delay after electrotherapy or combined modality treatment with electrotherapy and IFN-α. Electrotherapy was performed with different current levels one hour after IFN-α treatment on day 0. The data are presented as arithmetic means and standard deviations of the mean. The tumor growth delay after treatment with IFN-α alone is presented as an average tumor growth delay. Experimental groups consisted of 10 animals.

treatment and more than additive with intraperitoneal treatment.

To determine how IFN-α therapy interacts with escalating electrotherapy doses, combined modality treatment was performed with different direct current levels, ranging from 0.2 mA to 1.2 mA. IFN-α treatment schedule remained the same as in the previous experiment. Relationship between the effectiveness of therapy, presented as tumor growth delay, in relation to electrotherapy at different current levels, is presented in Figure 4. The interaction of IFN-α with electrotherapy was additive up to 0.4 mA and more than additive from 0.6 mA on (Table 1).

Table 1. Tumor growth delay after electrotherapy (ET) alone or in combination with IFN-α.

	Growth Delay (days) ET only	ET + IFN-α[1]
ET 0.2 mA	1.0 ± 0.5 ($\upsilon = 15$)[2]	1.9 ± 0.5 ($\upsilon = 15$)
ET 0.4 mA	1.8 ± 0.4 ($\upsilon = 15$)	2.8 ± 0.6 ($\upsilon = 16$)
ET 0.6 mA	2.0 ± 0.6 ($\upsilon = 18$)	3.8 ± 0.6 ($\upsilon = 18$)
ET 0.8 mA	4.9 ± 1.4 ($\upsilon = 14$)	8.0 ± 2.4 ($\upsilon = 16$)
ET 1.2 mA	8.3 ± 1.7 ($\upsilon = 15$)	11.6 ± 1.9 ($\upsilon = 16$)

[1] Tumor growth delay after intraperitoneal IFN-α treatment was 1.1 ± 0.5 ($\upsilon = 15$)
[2] Tumor growth delay in days (AM ± SD), υ degree of freedom

Discussion

The study shows that electrotherapy and IFN-α treatment interact in control of fibrosarcoma SA-1 tumor growth. More than additive antitumor effect was obtained when electrotherapy was combined with intraperitoneal IFN-α treatment. The interaction between the two treatment modalities increased by escalating current levels.

Electrotherapy is a new treatment modality used in local control of tumor growth.[9, 11, 19] Its antitumor mechanisms are probably multiple: biochemical reactions in the vicinity of the electrodes and influences of electric current directly on tumor cells.[17, 20, 21] Among biochemical reactions are changes of pH and changes of ion composition in extra cellular matrix which all exert influence on cell growth and

survival.[17] Effectiveness of electrotherapy is predominantly dependent on electric current intensity.[11, 19] With currents 1.8 mA a growth delay of approximately 12 days can be achieved on SA-1 tumors, while on B-16 melanoma tumor model even tumor cures can be induced.[19] Nevertheless, after the treatment viable tumor cells remain, which again give rise to a tumor. In order to potentiate effectiveness of electrotherapy, and eradicate the remaining tumor cells, attempts were made to combine electrotherapy with radiotherapy,[22] chemotherapy.[13, 15] and biological response modifiers.[14, 16, 23] In most cases additive or supra-additive effects were obtained.

Our interest was focused on combinations of electrotherapy with biological response modifiers. The studies combining interleukin-2 (IL-2),[14] tumor necrosis factor alpha (TNF-α)[23] and human leukocyte interferon alpha (IFN-α)[16]demonstrated that stimulation of host's defence mechanisms contributes to antitumor effectiveness of electrotherapy. Depending on the biological response modifier used, different arms of the cytokine network are stimulated, but in all cases the effectiveness of electrotherapy was increased.

In our preliminary study we have already tested the combined modality treatment of human leukocyte interferon alpha (IFN-α) with electrotherapy on SA-1 tumor model.[16] In that study IFN-α treatment protocol was the same as in the present study, however, electrotherapy protocol was different. Repetitive electrotherapy treatment was not very effective, therefore, according to later experience we applied the "field" electrotherapy as a single treatment.[17] As demonstrated in the present study, the effect is dose dependent resulting in a moderate antitumor effect at 0.2mA current level, and a significant growth delay at 1.2 mA. Comparison of the IFN-α antitumor effects according to the route of application demonstrated that IFN-α is moderately effective at the doses used. No difference in the antitumor effectiveness of IFN-α was observed, given either locally or systemically. But when combined with cytoreductive electrotherapy, systemic treatment was more effective than local treatment. Although IFN-α was demonstrated to be cytostatic to SA-1 cells *in vitro,* it is very unlikely to reach sufficiently high concentrations in the tumor to exert such an effect, when injected locally or systemically *in vivo.* Therefore, enhancement of the antitumor mechanisms of the organism must be contributing to the supra-additive effect of electrotherapy combined with systemic IFN-α treatment.

The interaction of IFN-α treatment with electrotherapy was dependent on antitumor effectiveness of electrotherapy. With escalating electrotherapy doses also combined modality treatment was more effective. This demonstrates that adjuvant IFN-α treatment was more effective on a smaller tumor burden. The doses used in both treatment modalities were low and no treatment related side effects were observed.

Our study shows that IFN-α and electrotherapy interact in antitumor effectiveness on fibrosarcoma in mice. Combined use of IFN-α and electrotherapy resulted in effective tumor control. Thus, electrotherapy can be used to locally potentiate systemic IFN-α treatment. Further studies are required for possible implementation of the investigated treatment approach in clinical practice.

Acknowledgment

This study was supported by The Ministry of Science and Technology of the Republic of Slovenia, contract No. P3-5252-302. The authors wish to express their appreciation to Srđan Novaković M.Sc., Maja Čemažar B.Sc., Mira Lavrič B.Sc. and Olga Shrestha, all Institute of Oncology, for their helpful suggestions and technical assistance.

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