Radiol Oncol 1999; 33(3): 221-5.

Cryosurgery combined with radiotherapy of tumors in mice

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The aim of this study was to determine antitumor effectiveness of cryosurgery alone and in combination with radiotherapy. Cryosurgery of subcutaneous fibrosarcoma SA-1 tumors in A/J mice was moderately effective treatment. Tumor growth delay was 10.3 ± 3.8 days after 5 minute treatment with nitrogen filled cryo-probe. Shorter treatment times induced less, but dose dependent antitumor effect. In combined treatment, tumors were either first treated by cryosurgery for 3 minutes and then locally irradiated with 10 Gy for 5 minutes, or irradiated first and thereafter treated by cryosurgery. The antitumor effectiveness of combined treatment was sequence dependent; the irradiation of tumors before cryosurgery resulted in better antitumor effect than the irradiation after cryosurgery. These results indicate that radiosensitization may not be always expected, in spite of some reports demonstrating that cryosurgery may have radiosensitizing effect in vivo, and that some other mechanisms may be involved contributing to radiation damage when cryosurgery follows irradiation.

Key words: sarcoma, experimental-surgery-radiotherapy; cryosurgery; mice

Introduction

Cryosurgery is a form of cryotherapy that uses special instrumentation to produce freezing of tissue.[1] Whatever the approach is used, the basic cryosurgical technique has to devitalize the neoplastic tissue by freezing *in situ*. The same volume of the tissue has to be frozen as would have been excised with a conservative local excision. The nature of the tissue response varies with the intensity of the injury; that is, a minor cryogenic injury produces only an inflammatory response whreas greater cryogenic injury produces tissue destruction.[2]

Biological basis of tissue destruction by freezing of tissue is due to a number of factors which can be grouped into two major mechanisms, one immediate, the other delayed.[1,2] The immediate effect is due to injury such as crystallisation of the cells caused by freezing and warming.[3] The delayed effect is due to the progressive failure of microcirculation and ultimate vascular stasis, after the tissue has thawed.

Cryosurgery is being increasingly used in the treatment of malignant diseases as a potential alternative to conventional surgery or irradiation.[1,4-7] Its antitumor effectiveness has been demonstrated in many accessible

Received 20 March 1999
Accepted 19 April 1999

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cutaneous tumors of various types, as well as in the treatment of carcinomas of the pharynx, larynx, trachea, bronchi, lung, oesophagus, liver, as well as vulva, vagina and uterus.[1] Due to the biological basis of freezing effect on tumors, many clonogenic cells may survive in the margins of tumors, which may represent a source of tumor regrowth. Therefore, combined cryosurgery and radiotherapy of tumors may be a potential tool for elimination of the residual disease.

Reports dealing with radiosensitization of the cells with cryosurgery, demonstrate that, in certain conditions, hypothermia may predispose cells to irradiation damage.[8,9] There are only few reports dealing with cryosurgery combined with irradiation of tumors in vivo.[5,10,11] Most of these *in vivo* studies have dealt with radiotherapy as a means to eradicate the remaining viable cells in the tumors after cryosurgery. However, there are no reports comparing the sequencing of cryosurgery and radiotherapy. Therefore, the aim of this study was to determine antitumor effectiveness of cryosurgery on subcutaneous SA-1 fibrosarcoma tumors in mice performed either before or after local tumor irradiation.

Materials and methods

Animals and tumors

A/J mice of both sexes, were purchased from the Institute Rudjer Bošković, Zagreb, Croatia. They were maintained at 21°C with a natural day/night light cycle in a conventional animal colony. The mice were 8-12 weeks old at the beginning of the experiments. The tumor used was fibrosarcoma SA-1 (The Jackson Laboratory, Bar Harbour, ME). SA-1 cells for initiation of subcutaneous tumors were obtained from the ascitic form of the tumors in mice, which were serially transplanted twice per week. Subcutaneous tumors were implanted by injecting 0.1 ml NaCl (0.9%) containing 5×10^5 viable tumor cells under the skin on the rear dorsum. Six to 8 days after implantation, when the tumors reached approximately 40 mm^3 in volume (7 mm in diameter) the mice (5-10 per group) were randomly divided into experimental groups. Experiments were repeated twice.

Cryosurgery and radiotherapy of tumors

Cryosurgery of the tumors was performed by Dewar/gas cylinder filled with liquid nitrogen. After a few minutes, the probe on the cylinder with a diameter of 8 mm was evenly cooled and pressed on the surface of the tumor. During treatment the mice were held in hand, therefore anaesthesia was not necessary. The treatment was performed either for 1, 3 or 5 minutes. The probe was placed on the same spot during the whole treatment with gentle pressure on the tumor not to loose contact.

A Darpac 2000 X-ray unit (Gulmay Medical Ltd. Shepperton, UK), operated at 220 kV, 10 mA, and with 0.55 mmCu and 1.8 mm Al filtration, was used for local tumor irradiation. The tumors were irradiated at a dose rate of 2.1 Gy min^{-1}. A holder for 6 mice was mounted on the X-ray with the aperture for the irradiation of the tumors, the rest of the body of the mice was shielded by lead block, and by the lead holders for the mice. To ensure uniform dose through the tumor volume, the tumors were exposed to irradiation by two opposing treatment fields through each of which 50% of the dose was delivered.

In experiments with combined treatment, the tumors were either irradiated before or after cryosurgery. Interval between the treatments was 5 minutes.

Assessment of antitumor effectiveness

Antitumor effectiveness of cryosurgery and radiotherapy was assessed by measurements of the tumor diameters in three orthogonal directions using Vernier caliper on consecu-

tive days following treatment. Arithmetic means and standard error of the means were calculated for each experimental group. Tumor doubling time was calculated from the growth curve of individual tumors. Tumor growth delay was calculated from the mean tumor doubling time of the experimental groups compared to untreated tumors.

Statistical analysis

Statistical significance was evaluated by modified t-test (Bonferroni t-test) after one way ANOVA had been performed and fulfilled.

Results

Antitumor effect of cryosurgery

Antitumor effectiveness of cryosurgery was evaluated on subcutaneous tumors in mice. The treatment was performed by cryoprobe with a diameter of 8 mm, cooled by liquid nitrogen. The probe was firmly pressed on the tumor for 1, 3 or 5 minutes. Uniformity of the treatment was inspected visually. The results in Table 1 demonstrate that antitumor effectiveness of cryosurgery was dependent on the duration of the treatment. One minute treatment induced 0.8 days tumor growth delay, whereas 3 and 5 minutes 3.8 and 10.3 days, respectively. Significant tumor growth retardation was observed only after a 5 minute cryosurgical treatment ($p<0.05$). It induced early reduction of tumor size which was reflected also in tumor growth curves (Figure 1). The treatment did not result in tumor exulceration or any other side effects.

Table 1. Antitumor effectiveness of cryosurgery on SA-1 tumors in A/J mice

Group	n	Tumor doubling time*
Control	16	2.0 ± 0.2
Cryosurgery 1 min.	14	2.8 ± 0.2
Cryosurgery 3 min.	19	5.8 ± 1.0
Cryosurgery 5 min.	14	12.3 ± 3.8

* Tumor doubling time ± SE

Effect of cryosurgery combined with radiotherapy

Local tumor irradiation was performed either before or after cryosurgery of subcutaneous tumors. The tumors were treated for 3 minutes with cryosurgery, and after 5 minute interval, locally irradiated with 10 Gy, or in inverse sequence, irradiated first and after 5 minutes treated with cryosurgery. Tumor irradiation was effective, antitumor effectiveness, as measured by tumor growth delay was 19.8 ± 7.4 days. When the tumors were irradiated first and cryosurgery was performed thereafter, tumor growth delay was 28.8 ± 6.5 days. However, tumors that were treated by cryosurgery first and then irradiated responded similarly as those treated only by radiotherapy. Tumor growth delay of those tumors was prolonged for 19.1 ± 6.1. This observation is evident also from the growth curves of the tumors (Figure 2). Local tumor irradiation followed by cryosurgery induced early tumor volume reduction, that resulted in overall better antitumor effectiveness than radiotherapy only or cryosurgery followed by radiotherapy. No exulceration of the tumors or other side effects of single or combined treatments were observed.

Table 2. Antitumor effectiveness cryosurgery combined with radiotherapy (RT)

Group	n	Tumor doubling time*
Control	16	2.0 ± 0.2
Cryosurgery 3 min.	19	5.8 ± 1.0
RT 10 Gy	11	21.8 ± 7.4
Cryosurg. + RT	11	21.1 ± 6.1
RT + Cryosurg.	11	30.8 ± 6.5

* Tumor doubling time ± SE

Discussion

Our study shows that cryosurgery is effective when combined with radiotherapy in treatment of tumors. However, antitumor effectiveness of combined treatment was se-

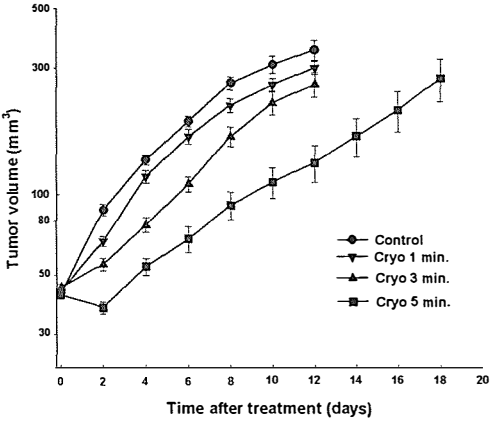

Figure 1. The antitumor effect of cryosurgery on subcutaneous fibrosarcoma SA-1 tumors in A/J mice. Cryosurgery was performed with 8 mm probe cooled by liquid nitrogen for 1, 3 and 5 minutes. Symbols, mean tumor volume; vertical bars, standard error of the mean.

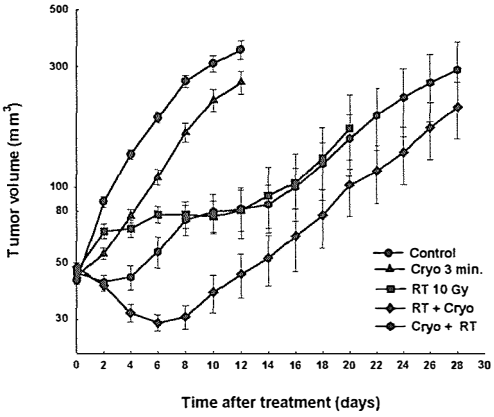

Figure 2. The antitumor effect of cryosurgery combined with radiotherapy on SA-1 tumors in mice. Cryosurgery was performed for 3 minutes and radiotherapy by local tumor irradiation with 10 Gy. The interval between croysurgery and radiotherapy was 5 minutes. Symbols, mean tumor volume; vertical bars, standard error of the mean.

quence dependent. We found that the tumor growth delay was prolonged when tumors were treated with irradation before cryosurgery. The inverse combined treatment did not differ significantly compared to irradiation alone.

Cryosurgery is being increasingly considered as a treatment of choice for a number of malignant skin tumors.[1] Moreover, better understanding of the mechanisms of tissue injury by cryosurgery has lead its way into broader clinical practice for treatment of the head and neck and gyneacological tumors. It is known that tissue damage by freezing depends on both, freeze and thaw rates; in many instances, rapid freezing and slow thawing should be used.[2] Injury is increased by repeating freeze-thaw cycles.[3] The best results of cryosurgery have been obtained in the treatment of malignant skin lesions.[1] The overall cure rates obtained by cryosurgery compare favourably with those obtained by other treatment modalities.

The depth of freezing is approximately the same as the lateral spread of frost from the edge of the probe. However, when the volume of the tumor hampers the optimal freezing, cryosurgery can not be performed adequately. These advanced bulky tumors may require combined therapeutic technique, such as combined cryosurgery and radiotherapy. It can be predicted that cryosurgery may deal better with central portion of the tumor, that tends to be radioresistant, while radiotherapy would deal better with the peripheral parts of the tumor that are more radiosensitive due to better oxygenation.

However, there have been only limited studies of hypothermia dealing with response to subsequent irradiation, either to cells or tissues in vivo.[5,8,9] Generally, enhanced radiosensitivity of cells and tumors after hypothermia have been observed, but the magnitude was dependent on cell line used, cooling temperature, duration, and rewarming interval before irradiation. In this study, the importance of sequencing was examined, i.e. local irradiation of tumors either before or after cryosurgery. This is important, since the rationale of tumor irradiation after cryosurgery bears the notion that cryosurgery may predispose cells to radiation damage. However, cryosurgery after irradiation can have the rationale in the potentiation of sublethal radiation damage of cells.

Although the aim of this study was not to investigate the underlying mechanisms of antitumor effectiveness of combined cryosurgery and radiotherapy, the results show that in our experimental design, cryosurgery was more effective when given after radiotherapy.

Acknowledgements

This work was supported by the Ministry of Science and Technology of the Republic of Slovenia.

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