THE EFFECT OF HIGH INTENSITY ULTRASOUND ON THE GROWTH AND METASTASIZATION OF INOCULATED BROWN-PEARCE TUMORS IN RABBITS

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The action of ultrasound on malignant tumors has attracted the attention of numerous research workers during the last decades. A number of workers abroad [9, 10, 13, 16-19], for instance, have tried out the action of low intensity ultrasound (2-4 w/cm^2) on malignant tumors in animals. The results of these investigations are extremely contradictory. Attempts to use low intensity ultrasound in the treatment of malignant tumors in man, especially melanoma and sarcoma, have not been successful. Only individual workers [12, 17, 18] report the effective action of low intensity ultrasound on cancer of the skin in man. However, these findings were not confirmed by other authors [7, 8, 11, 14, 15].

In 1951 A. K. Burov put forward the hypothesis that these negative results are not due to the nature of the ultrasound but to the duration of its action at a low intensity, in consequence of which the thermal effect predominates. In the opinion of this author, it is possible by the use of high intensity ultrasound in short exposures to obtain positive results by their action on malignant tumors in animals and man [2, 3]. A. K. Burov constructed powerful ultrasonic apparatus capable of producing ultrasonic waves with an intensity of the order of 350 w/cm^2 with flat quartz plates as a continuous system, and up to 500 w/cm^2 as a pulsed system, i. e. ultrasound of ten times the limiting intensity obtained by other workers, and of 100 times that usually used by foreign workers in biological experiments.

The aim of our investigation was to study the effect of high intensity ultrasound on the growth and character of metastasization of malignant Brown-Pearce tumors in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits of the chinchilla breed, males, 2.5-3 kg in weight. The tumor

TABLE 1

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intramuscular Inoculation, First Group of Experiments)

Group of rabbits	No. of rabbits	Tumor present			Tumor absorbed
		widespread metastasisation	solitary metastases	metastases absent	
Experiment	41	9	2	3	27
Control	15	11	0	0	4

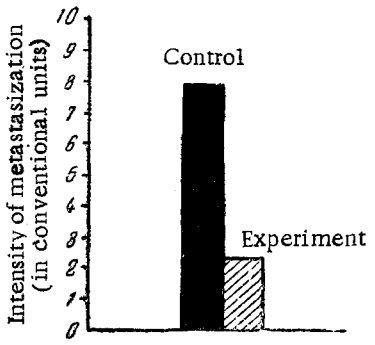

Fig. 1. Intensity of metastasization of a Brown-Pearce tumor in irradiated and control rabbits.

tissue was finely macerated and mixed with physiological saline in a proportion of 1:3, and this material was injected in a dose of 0.8-1 ml intramuscularly, intratesticularly, and into the testis brought down under the skin of the thigh and fixed in situ with a suture. This last method was undertaken to provide maximum transmission of ultrasonic energy to the tumor.

Animals were selected for the experiment on the 7th, 9th, 10th and 15th days after inoculation with the tumor, when the tumor nodule had reached a size of 4.5 × 3.5 × 2 cm. At these times, as a rule, at necropsy metastases could be seen with the naked eye in the internal organs. Survival experiments were carried out. In individual cases the observations lasted for 2-3 years. Rabbits killed at the end of the experiment, and also those dying in the course of the experiment, were subjected to thorough pathological anatomical examination. The antitumor activity of the ultrasound was determined by comparison of the experimental and control groups of animals, using the following criteria: the number of rabbits in which the tumors were becoming absorbed, the rate of growth of the tumor (according to its mean diameter), the duration of life of the animals,and the intensity of metastasization (in conventional units).

Altogether 204 rabbits took part in the experiment; 136 of them were irradiated under different conditions, and 68 acted as controls.

EXPERIMENTAL RESULTS

Depending on the method of inoculation and the conditions of action of the ultrasound, the experiments could be subdivided into 5 main groups.

In the first group of experiments (41 experimental and 15 control rabbits) we tested a single exposure to the action of ultrasound with a frequency of 718, 750, 1000 and 1500kcps, an intensity of 22-35 w/ cm^2 and exposure time of 30-75 seconds (given fractionally) on a Brown-Pearce tumor inoculated intramuscularly (contact method of ultrasonic irradiation). Under the conditions of its action as enumerated above the effectiveness of the ultrasound was approximately the same in the individual experiments, and so we will consider the analysis of the results as summarized in Table 1.

The difference found between the experimental and control values is statistically significant, since T = = 3.92, i. e. is greater than 3.*

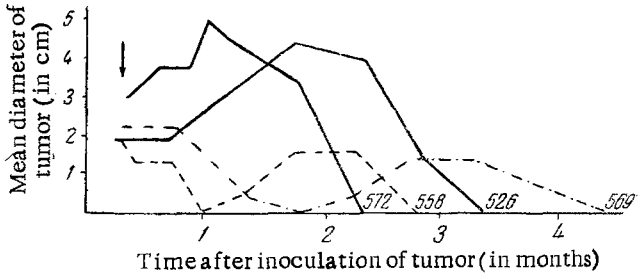

Fig. 2. Curves showing absorption of a Brown-Pearce tumor in irradiated and nonirradiated rabbits.
— nonirradiated rabbits (Nos. 572, 526); - - - irradiated rabbits (Nos. 558, 568); the arrow indicates ultrasonic irradiation.

* T is the significance of the difference, calculated according to the formula quoted in A. A. Sapegin's "Variational Statistics" [6], where the difference is significant if T > 3.

TABLE 2

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Inoculation into the Testicle, Second Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasisation	Tumor present: restricted metastasisation	tumor absorbed
Experiment	1 500	35	4×15	5	1	3	1
	1 500	35	45	6	4	1	1
Control	Not irradiated			10	10	0	0

* In 4 rabbits, the nonirradiated metastases were absorbed.

TABLE 3

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intratesticular Inoculation, Third Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasization	Tumor present: restricted metastasization	tumor absorbed
Experiment	1 500	27—37	17	13	4	3	6[1]
	1 500	27—37	12	12	11	0	1
Control	Not irradiated			16	15	1	0

* In 4 rabbits the nonirradiated metastases were absorbed.

As seen from Table 1, of the 41 experimental animals the tumor was completely absorbed in 27 (65.9%). In 5 animals dying from various causes the tumor disintegrated and was found in various stages of absorption. Metastases from these tumors were either absent or were found in insignificant numbers, in sharp contrast to the number of metastases in the control animals. Thus the number of metastases in 1 experimental rabbit was 2.56 conventional units and in 1 control rabbit – 8 conventional units (Fig. 1). In 73.3% of cases the control rabbits died of metastases.

Prolonged observation of the rabbits showed that the irradiated tumor was absorbed at different times after the procedure – from 7 days to 3½ months.

In the majority of experimental rabbits the tumor was absorbed between 20 and 40 days after ultrasonic irradiation. The process of absorption of the tumor in rabbits treated by ultrasound was, in the majority of cases, sharply distinguished from that of spontaneous absorption, which takes place in tumors inoculated by this means (Fig. 2).

As seen from Figure 2, at the end of the 1st-2nd month after ultrasonic irradiation disappearance of the tumor is observed, whereas in those control rabbits in which regression of the tumor takes place, this is the time when the tumor is at its largest size. Spontaneous absorption of the tumor ends 2-3 months after inoculation, and in place of the original absorbed tumor there arises afresh a thickening which grows for 1½-2½ months and finally is absorbed after 3-4 months.

As we have previously reported [5], in the majority of rabbits in which tumors have absorbed after ultrasonic irradiation, absence of metastases has been observed, and in some cases destruction and absorption of metastases formed in the lungs, kidneys, adrenals and the popliteal lymphatic glands has been noted.

TABLE 4

The Effect of High Intensity Ultrasound on the Growth and Metastasization of a Brown-Pearce Tumor (Intratesticular Inoculation, Fourth Group of Experiments)

Group of rabbits	System of irradiation			No. of rabbits	Results		
	frequency, kcps	power, w/cm²	exposure, sec		Tumor present: widespread metastasization	Tumor present: restricted metastasization	tumor absorbed
Experiment	1 500	80—150	3—1,3	21	10	1	10
Control	Not irradiated			17	16	1	0

A defect of the contact method of ultrasonic irradiation and the systems used was the gross damage to normal tissues (skin, muscles, bones) through which the ultrasound passed.

In the second group of experiments (11 experimental and 10 control rabbits) ultrasonic irradiation was carried out on rabbits with a tumor inoculated intratesticularly, in a water bath. It was found that this system (frequency – 1500 kcps, power – 35 w/cm², exposure – 60 seconds, given fractionally) which causes absorption of an intramuscularly inoculated tumor, slightly inhibits the course of the malignant lesion when inoculated into the testicle. Thus the average duration of life of the experimental rabbit was 81 days and of the control – 47 days; the average number of metastases in 1 experimental rabbit was 7 conventional units, and in 1 control rabbit – 14.5 conventional units. Absorption of the tumor was observed in only one experimental rabbit (Table 2).

Taking into consideration the more malignant course of a tumor when inoculated intratesticularly, we replaced the fractional method in this group of experiments by a continuous exposure (45 seconds). This led to severe necrosis of the irradiated testicle with the tumor and to its subsequent partial amputation. During this process metastasization was not halted and the majority of the animals died from metastases at the same times as the controls (see Table 2).

In the third group of experiments (13 experimental and 11 control animals) a reduction in the time of irradiation to 17 seconds at a frequency of 1500 kcps and a power of 27-37 w/cm² (transmission of energy through water) led to absorption of the tumor inoculated into the testis, both by the usual method and when transposed under the skin. Of the 13 animals the tumor was completely absorbed in 6 (46%) from 1-3-4 months after ultrasonic irradiation (Table 3). Tumor nodules which were palpable before irradiation, along the course of the spermatic cord, were absorbed 2 months after the procedure; metastases in the eyes, kidneys and lymphatic glands, which at first attained a considerable size, disintegrated and were absorbed within 3-7 months after ultrasolic irradiation of the primary tumor nodule. All the control rabbits died from multiple metastases from 1-1½ months after inoculation of the tumor.

However, even an exposure of 17 seconds, at an ultrasound intensity of 27 w/cm² was found to be too long, since it led to severe heating of the surrounding normal tissues (skin, bone) and to consequent trauma. A reduction in the time of irradiation to 12 seconds, with the intensity as before, while it reduced the damage to the normal tissues, it did not produce a positive effect (of 12 irradiated rabbits, 11 died from metastases – see Table 3).

The experimental results shown in Table 3 are statistically significant (T = 4.5).

In the fourth group of experiments (21 experimental and 17 control rabbits) the severe reduction in the length of exposure to 3-1.3 seconds, with a simultaneous increase in the intensity of the ultrasound to 80--150 w/cm² at a frequency of 1500 kcps (irradiation in a water bath) did not damage the normal tissues and led to absorption of the tumor in 40-60% of cases within 1½ -3-4 months after ultrasonic irradiation (Table 4).

Analysis of the results of the experiment as shown in Table 4 showed them to be statistically significant (T = 3.7, i. e. greater than 3).

In a number of the experimental rabbits disintegration and absorption of the nonirradiated metastases

formed in the eyes, kidneys, adrenals, lymphatic glands and bones were observed (these facts were confirmed by pathological anatomical examination [5]).

It was demonstrated by observation of the rabbits which had been cured, over a period of 2-3 years, that there was no recurrence of the tumors. Special experiments which we carried out showed that rabbits in which tumors had absorbed following ultrasonic irradiation were insusceptible to further inoculation of Brown-Pearce tumors [4].

In the fifth group of experiments on 48 rabbits (38 experimental and 10 control rabbits) we tested the action of other frequencies of high intensity ultrasonic irradiation (80 w/ cm^2). The irradiation was carried out in a water bath. It was found that ultrasound of a frequency of 1000 kcps (exposure 2-7 seconds) did not give a positive effect, and of a frequency of 2000 kcps (with the same exposure) led to hemorrhages in the internal organs, the brain and the spinal cord, and caused death of the animals from 1-4 days after irradiation.

On the basis of the results obtained it was established that a single application of intensive ultrasound leads as a rule in 40-50% of cases, and in individual experiments in 60-70%, to absorption of a Brown-Pearce tumor inoculated either intramuscularly or intratesticularly into rabbits.

The fate of the tumor is dependent on the conditions and method of ultrasonic irradiation: a) ultrasonic irradiation at a frequency of 1500 kcps and an intensity of 27-37 w/ cm^2 and with an exposure of 17 seconds leads to absorption of the Brown-Pearce tumor in a number of cases, but at the same time the adjacent normal tissues (skin, bone) suffer damage; b) brief exposure (3-1.3 seconds) to high intensity ultrasound (80-150 w/ cm^2) of a frequency of 1500 kcps selectively acts upon the tumor tissue without harming the adjacent normal tissues.

Absorption of the irradiated tumor is accompanied by disintegration and absorption of the nonirradiated metastases in a number of cases.

SUMMARY

The author studied the effect of ultrasonic waves of high intensity on the growth and metastasis of Brown-Pearce's tumor. The rabbits were acted upon by sound waves in 7-15 days after inoculation of the tumor.

It was shown that single action by ultrasonic waves of high intensity (80-150 w/ cm^2) at the frequency of 1500 kcps of short duration (3-1.3 seconds) results in the resolution of the Brown-Pearce tumor inoculated into the muscle or the testicle in 40-50% of the cases (in certain experiments in 60-70% of the cases) in the surrounding normal tissue is not disturbed under these conditions.

The resolution of the tumor acted upon by the ultrasonic waves is connected in a number of cases with the disintegration of metastases which were not acted upon by sound.

LITERATURE CITED

[1] L. Bergman, Ultrasound and Its Application in Science and Technology (Russian Translation) Foreign Literature Press, Moscow (1956).

[2] A. K. Burov, Doklady Akad. Nauk SSSR 106, 2, 239-241 (1956).

[3] A. K. Burov and G. D. Andreevskaia, Doklady Akad. Nauk SSSR 106, 3, 445-448 (1956).

[4] N. P. Dmitrieva, Biull. Eksptl. Biol. i Med. 6, 60-62 (1957).*

[5] N. P. Dmitrieva, Biull. Eksptl. Biol. i Med. 11, 86-90 (1957).*

[6] A. A. Sapegin, Variational Statistics, Moscow (1935).* *

[7] H. G. Bode and Theismann, Klin. Wschr. No. 28, S. 687 (1950).

[8] V. Buchtala, Brit. J. Phys. Med. v. 15, No. 1, 3-6 (1952).

[9] C. Dittmar, Strahlenther. Bd. 78, H. 2, S. 217-230 (1948).

[10] F. Fanucchi and L. B. Bussi, In book: Der Ultrashall in der Medizin. Zurich, S. 125-130 (1949).

[11] K. Horatz, Der Ultrashall in der Medizin. Zurich, S. 249 (1949).

* See English translation.

** In Russian.

[12] J. Horvath, Cited by L. Bergman, "Ultrasound and Its Application in Science and Technology" (Russian Translation), Foreign Literature Press, Moscow (1956).

[13] K. Namikawa, cited by K. Woeber, Strahlenther. Bd. 79, H. 4, S. 563-576 (1949).

[14] F. Nodl, Strahlenther. Bd. 79, H. 2, S. 289 (1949).

[15] H. W. Schmidt, Ztschr. f. Krebsforsch., No. 56, S. 580 (1950).

[16] K. Woeber, Strahlenther. Bd. 79, H. 4, S. 563-576 (1949).

[17] K. Woeber, Arch. f. Dermat. u. Syph. Bd. 188, H. 6, S. 656-675 (1950).

[18] K. Woeber, Arch. f. Dermat. u. Syph. Bd. 191, S. 400-404 (1950).

[19] K. Woeber, Ztschr. f. Krebsforsch. Bd. 57, H. 5, S. 564-576 (1951).