These investigations revealed a somewhat varied picture of the intensity of the reaction for AG B10 in the biliary capillaries of spontaneous and induced mouse hepatomas. The tumors, as we know, are heterogeneous and consist of a large number of clones. It has been shown, in particular, that heterogeneity of tumor cells with respect to expression of α-fetoprotein is observed in rat hepatomas [7]. The diversity of tumor cells of mouse hepatomas which we found, in relation to the intensity of the reaction for AG B10 and the ultrastructural features of their cytoplasm and biliary capillaries, also probably may reflect the cellular heterogeneity of hepatomas. Correlation was found between disturbance of the ultrastructure of the biliary capillaries of hepatomas and the reduced expression of AG B10.

LITERATURE CITED

1. B. Alberts, D. Gray, J. Lewis, et al., Molecular Biology of the Cell, Garland, New York (1984).
2. V. N. Baranov, V.S. Poltoranina, and A.I. Gusev, Byull. Éksp. Biol. Med., No. 2, 199 (1986).
3. V. N. Baranov, A. S. Gleiberman, V. S. Poltoranina, et al., Biol. Membr., No. 2, 208 (1987).
4. N. I. Kuprina, V. I. Gel'shtein, L. Ya. Shipova, and T. D. Rudinskaya, Tsitologiya, No. 9, 1102 (1982).
5. T. D. Rudinskaya, N. I. Kuprina, A. K. Yazova, et al., Biol. Membr., No. 2, 194 (1987).
6. L. Ya. Shipova and N. I. Kuprina, Immunologic Aspects of Developmental Biology [in Russian], Moscow (1984).
7. T. L. Eraiser and L. S. Khamsina, Int. J. Cancer, **42**, 633 (1988).
8. H. Ishii, H. Sususki, K. Kobayashi, et al., Tohoku J. Exp. Med., **151**, 339 (1987).

USE OF MAGNETICALLY CONTROLLED MICROCAPSULES IN COMBINATION (CHEMOTHERMOMAGNETO-) THERAPY OF EXPERIMENTAL TUMORS

V. A. Volkonskii, L. E. Kholodov, E. K. Kazarina, and V. F. Gudov

UDC 616-006-085.277.3:615.453.4.015.2:615.847.8

KEY WORDS: tumor, magnetically controlled capsule

The main obstacle to the use of antitumor preparations (ATP) in clinical oncology is the nonselectivity of their action, which gives rise to serious levels of poisoning and ultimately lowers the natural resistance of the organism to the development of the tumor process. Often the depression of immunity as a result of chemotherapy leads (after a short remission) to recurrences and to the development of latent metastases. One way of obtaining selective accumulation of ATP in the zone of tumor growth is to use magnetically controlled microcapsules (MC), covered with a biocompatible membrane, and which may contain various ATP [1-4, 5]. In this way the concentration of an ATP in the zone of tumor growth can be greatly increased, the duration of action of the ATP lengthened, and the total therapeutic dose of the ATP substantially reduced. We know that chemotherapy of tumors is most effective when combined with hyperthermia. Meanwhile the use of hyperthermia is limited by the impossibility of obtaining a strictly local rise of temperature in the zone of tumor growth. The use of magnetically controlled MC in conjunction with a high-frequency field enables strictly local hyperthermia to be attained in the tumor tissue, as a result of the hysteresis effect in the ferromagnetic material of MC, and this sensitizes the tumor cells to the action of ATP incorporated in MC.

Sector of Neuromagnetics, Therapeutic Substances Research Institute, Ministry of the Medical and Biological Industry of the USSR, Moscow Region.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 295-296, March, 1990. Original article submitted September 20, 1989.

0007-4888/90/0003-0382$12.50

The aim of this investigation was to study the possibility of using magnetically controlled MC and to evaluate their efficacy in the treatment of experimental tumors.

EXPERIMENTAL METHOD

Experiments were carried out on 40 mature male Wistar rats weighing 180-200 g, bred at the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, which were divided into four groups, with 10 animals in each group: 1) control, 2, 3, and 4) experimental. All the rats were inoculated in the middle of the tail subcutaneously with a Walker's carcinosarcoma (0.5 ml of inoculate, containing on average 400,000-500,000 tumor cells per rat). The take rate of the tumor was 100%. The volume of the tumor in each animal was measured by plethysmography. On the 4th day after transplantation, when the volume of the tumor had reached 1034 ± 110 mm^3, 1 ml of physiological saline was injected into the ventral caudal vein of the rats of group 1, and 0.4 mg of carminomycin (dose 2 mg/kg) in 1 ml of physiological saline was injected into the rats of group 2 daily for 5 days. Animals of group 3 received a suspension (in 1 ml of physiological saline) of 150 mg MC with ferromagnetic core of reduced iron, covered with an albumin coat, which contained a sessional therapeutic dose of carminomycin, namely 0.4 mg. The MC were obtained by the method of Widder et al. Their diameter was 2-2.6 μ. Before injection of the suspension of MC a permanent magnet was laid on the surface of the tumor, with a magnetic field of intensity of 6000 Oe. Confirmation that the MC were in the venous bed in the zone of tumor growth was obtained by means of roentgenograms. An "Élektronika" microfocal x-ray apparatus was used. MC carrying 0.4 mg of carcinomycin also were injected into the rats of group 4, and localized, just as in group 3, but the tail of these rats was placed in an induction coil of a high-frequency generator (the IKV-4 apparatus with output power of 60 W) 3 and 72 h after inoculation. In this way a temperature of 40°C was produced in the tumor tissue and maintained for 30 min. A thermocouple was used as the temperature transducer. The significance of differences in the efficacy of treatment between the groups of animals was determined by the chi-square test.

EXPERIMENTAL RESULTS

The animals of group 1 (control) died 21 ± 1 days after inoculation of the tumor when its volume was 3920 ± 200 mm^3 carminomycin in group 2 for 5 days resulted in a complete remission in two animals. Incidentally, three rats of this group died on the 17th-19th day, when the tumor had reached a relatively small size, on average 2788 mm^3. Death of these rats was evidently associated with liver damage as a result of increased toxic effects of the tumor and the course of chemotherapy. Growth of the tumor in the remaining five animals was retarded and their survival period was increased by 7-9 days compared with the control rats. In group 3, the tumor completely regressed in six animals 3 days after injection of MC. Stabilization of the volume of the tumor for 6 days after injection of MC was observed in another four rats. The tumor then continued to grow, and caused the animals' death. Nevertheless, their survival period increased by 21 ± 2 days compared with rats of the control group. The efficacy of tumor chemotherapy in group 3 was significantly higher than in group 2 ($p < 0.05$). In group 4, intravenous injection of MC containing a sessional dose of carminomycin (0.4 mg), in conjunction with two sessions of local hyperthermia, had the greatest antitumor effect. In nine rats, for instance, complete remission was observed. In one animal growth of the tumor was greatly retarded. On the 34th day, when the tumor was relatively small in volume, the rat died evidently because of the development of metastases. The effect of antitumor therapy in group 4 was significantly better than in group 2 ($p < 0.01$) and also in group 3 ($p < 0.05$). The volume of the tumor in the terminal stage in all animals of groups 1, 2, and 3 which died averaged 3891 ± 220 mm^3.

It is interesting to note that the injected MC were securely fixed to the walls of the veins under the influence of the permanent magnetic field. For instance, 20 sec after injection and localization of the MC the external magnet could be removed without the risk of "leaking" of the MC from the blood stream. Even 25 days after localization of the MC in the walls of the veins, individual concentrations of iron particles could be clearly seen on the roentgenogram. These ferromagnetic particles probably become encapsulated by connective tissue in the body. Animals with a complete remission remained under observation for 120 days, and throughout this period, no recurrence of the tumor was observed.

The considerable therapeutic effect in group 3 (60% of complete remission) was evidently caused by the increased concentration of ATP in the tumor tissue when MC were used, compared with a course of chemotherapy (group 2); this effect was observed, moreover, after a fivefold decrease in the total dose of carminomycin. The results in group 4 (90% of complete remission) were even more impressive, due to sensitization of the tumor cells to the action of carminomycin as a result of ses-

sions of local thermomagnetic hypothermia in the zone of tumor growth. The possibility cannot be ruled out that the strong antitumor effect, leading to death and lysis of the tumor cells, was responsible for stimulation of antitumor immunity.

Thus the use of magnetically controlled MC in the treatment of solid tumors can make the action of ATP much more effective. The combined use of MC carrying ATP with thermomagnetic hyperthermia at 40.5°C enables the sensitivity of tumor cells to the action of ATP to be enhanced strictly locally.

LITERATURE CITED

1. C. F. Driscoll, R. M. Morris, and A. F. Senycei, Microvasc. Res., **27**, 353 (1984).
2. S. Fujimoto, M. Muazaki, F. Endon, et al., Cancer (Philadelphia), **56**, 2404 (1985).
3. T. Kato, Japanese Patent 51411181.
4. T. Kato, R. Nemoto, H. Mori, et al., Appl. Biochem. Biotechnol., **10**, 199 (1984).
5. K. J. Widder, Pharm. Sci., **68**, 79 (1979).