Blood Flow-interrupting Hyperthermic Chemotherapy on Established Autochthonous Mouse Sarcoma Induced by 3-Methylcholanthrene

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ABSTRACT

Established autochthonous sarcomas induced in the hind limbs of mice by 3-methylcholanthrene were treated with our method of “regional blood flow-interrupting hyperthermic chemotherapy.”

After administration of an injection of 20% of the dose of carbazilquinone (CQ) lethal for 50% of the animals (1 mg/kg i.v.), followed by temporary interruption of blood flow of the tumor-bearing limbs and warming of the limbs at 37°C for 60 min, we found that the tumor-bearing mice survived significantly (p < 0.001) longer than the control mice treated with CQ injection alone. One of 23 mice treated thusly (4.3%) survived without any tumor growth for 24 weeks, and the animal walked with an almost normal gait, using the treated limb. With injection of 12.5% of the dose of CQ lethal for 50% of the animals (0.62 mg/kg), warming of the region for 30 min at 41°C was more effective than warming for 60 min at 37°C in the present chemotherapeutic system. The side effects on regional normal tissues, induced by the treatments, were almost the same between the two chemotherapeutic conditions.

Nitrogen mustard N-oxide and mitomycin C were also tested for comparison in the same experimental system. Nitrogen mustard N-oxide revealed remarkable antitumor effects, but its side effects on regional normal tissues were more severe than those of CQ. Mitomycin C showed fewer antitumor effects than did CQ.

INTRODUCTION

In our previous paper (2) we reported on the remarkable chemotherapeutic effects on transplanted tumors in mice, which were obtained by a chemotherapeutic system consisting of an i.v. injection of an antitumor drug, followed by temporary interruption of blood flow to the tumor-bearing region and simultaneous warming of the region for a certain period of time. The conception of the chemotherapeutic system was prompted by our discovery that the action of a drug such as CQ could be potentiated in the acidic condition induced by cancer cell glycolysis (5).

In the present study we tested the same chemotherapeutic system on chemically induced autochthonous sarcomas to simulate the actual clinical tumor condition so that we might examine its antitumor effect on autochthonous tumors, the mortality due to the treatments, and the side effects on regional normal tissues.

MATERIALS AND METHODS

Tumor Induction. A 0.1-ml solution of 0.1% (w/v) MC in olive oil was injected i.m. twice at weekly intervals into the right thigh of each 6- to 9-week-old female CF1 mouse. All MC-injected mice were carefully observed and palpated weekly for 6 months at the site of the injection to detect tumor growth. When a tumor had grown to palpable size in a mouse and was confined to a region distal to the inguinal ligament, the size of the tumor was measured. Only mice bearing tumors that did not exceed 7 x 8 mm in 2 orthogonal diameters of the tumor-bearing limb were used in this study; these mice were randomly divided into control and experimental groups.

Antitumor Drugs and Treatment. CQ (NSC 134679; carbazilquinone or Esquinon; Sankyo Co., Ltd., Tokyo, Japan), NMO (Nitromin; Yoshitomi Pharmaceutical Industry, Ltd., Osaka, Japan), and MMC (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) were used as antitumor drugs. The same method was used to administer the antitumor drug, apply the tourniquet, and warm the constricted tumor-bearing limb as was described previously (2). The treatments were performed 1/week for 3 weeks.

Measurement. The antitumor effect of each therapy was evaluated by the prolongation of the survival time of test mice compared with that of control mice. The observation period lasted 24 weeks after the first therapy. Mice found dead within 1 week after the therapy were excluded from the data of survival time but were included in the data of mortality. Significance of difference in survival time between the control and the test groups was determined by the standard t test.

RESULTS

Chemotherapy with CQ. When the tumor-bearing mice were treated with 20% of the LD50 (1 mg/kg) of CQ, combined with application of the tourniquet to the tumor-bearing limb 2 min after the drug injection and warming of the constricted limb for 60 min at 37°C, the mean survival time of the test group was 101.4 ± 7.6 (S.E.) days, which was significantly (p < 0.001) longer than that of a group in which 20% of the LD50 of CQ was administered alone.
(59.8 ± 4.4 days). Notably, 1 of 23 mice treated thusly (4.3%) survived throughout the observation period of 24 weeks with complete regression of the tumor (Table 1, Group A). The joints in the treated limb were not badly damaged; the animal using the treated limb could walk with an almost normal gait. CQ injection alone produced a slight but insignificant increase in survival time compared with that of the untreated control group (Table 1, Group A).

An experiment was also carried out to test the effect of hyperthermia (41°). The duration of interruption of blood flow to the tumor-bearing limb was shortened to 30 min, and the dose of CQ was reduced to 12.5% of the LD₅₀. As shown in Table 1, Group B, 4 of 37 of the treated mice (10.8%) survived without tumor growth for 24 weeks in the test group; the mean survival time was 97.2 ± 7.4 days. The results were superior to those obtained in a group in which 12.5% of the LD₅₀ of CQ was used, combined with temporary interruption of the regional blood flow for 60 min at 37° (Table 1, Group C). As to the side effects on the regional normal tissues induced by the treatments, almost the same degree of impairment was observed in both Groups B and C (Table 1). The mortality by the treatments was different, however, between the 2 groups; it was 29.7% in the former group, in which 12.5% of the LD₅₀ of CQ was used with the interruption of regional blood flow for 30 min at 41°, and 11.1% in the latter group.

NMO. Similarly, NMO was tested against established MC-induced tumors of mice. If the drug was used at 20% of the LD₅₀ (15 mg/kg), as in the case of CQ, followed by application of a tourniquet to the tumor-bearing limb for 60 min at 37°, the mortality of the mice was as high as 70.8%. Therefore, under the condition of the interruption of regional blood flow for 60 min at 37°, the dose of NMO had to be reduced to 10% of the LD₅₀. When these chemotherapeutic conditions were applied to the tumor-bearing mice, a prolonged survival resulted; i.e., the survival period (122.7 ± 11.6 days) was significantly (p < 0.001) longer than that of the untreated control group and even that of a group in which 20% of the LD₅₀ of NMO was injected alone without applying the tourniquet and warming the limb (Table 1, Group D). Three of 20 mice treated thusly (15%) survived throughout the 24 weeks without any tumor growth.

As judged from the rate of tumor regression, NMO seemed to reveal a better chemotherapeutic efficiency than did CQ. However, the side effects of the former on regional normal tissues proved to be much more severe than those of the latter; i.e., a marked skin erosion at the toes was frequently detected in the NMO group, whereas no skin erosion was seen in the CQ group. Severe atrophy of the limbs was another marked side effect observed in the NMO group, which was always accompanied by notable ankylosis of the hip and the knee joint. However, no ankylosis was seen in the CQ group.

MMC. MMC was also tested against established MC-induced tumors by our therapy system. Mice were injected with 20% of the LD₅₀ (1 mg/kg i.v.) of MMC, followed by restricting regional blood flow after the drug injection and warming the constricted limb for 60 min at 37°. As shown in Table 1, Group E, the treated group revealed a slightly increased survival time compared with that of the control group (60.8 ± 4.3 days versus 37.3 ± 3.1 days). However, there was neither complete tumor regression in the test group nor significant difference in the survival time between the test group and a group in which 20% of the LD₅₀ of

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<th>Experimental group</th>
<th>Drug</th>
<th>% of LD₅₀</th>
<th>Time of tourniquet application (min)</th>
<th>Local warming temperature (°)</th>
<th>No. of animals</th>
<th>No. of animals dead due to treatment</th>
<th>Av. life span (days)</th>
<th>No. of cures</th>
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| a Numbers in parentheses, percentage. |
| b Mean ± S.E. |
| c p < 0.001 (f test), compared with each drug control group. |
| d p < 0.001 (f test), compared with each untreated control group. |
MMC was administered alone. These results indicate that in the present system MMC is inferior to both CQ and NMO in antitumor effectiveness.

DISCUSSION

Thus far, experimental therapy of any kind applied to chemically induced or spontaneously established autochthonous tumors in animals has not been attempted very often but, when attempted, it has met with failure in most cases. Typical of such studies was the study on the effect of steroids on MC-induced tumors in mice, in which no antitumor effect was observable (6). The study that has thus far produced the best results may be the study by Alexander et al. (1), who reported that nucleic acids obtained from immune lymphocytes of sheep had a curative effect on rat autochthonous sarcoma (1). For its clinical use, however, there remains the problem of how graft versus host reaction could be suppressed in this kind of immune therapy.

As has been reported in this paper, blood flow-interrupting hyperthermic chemotherapy revealed remarkable therapeutic effects on MC-induced autochthonous tumors in mice. However, the problem exists of whether the tumors induced in all of the mouse limbs in this study were actually the definite and uniform type of sarcoma, since no biopsy was performed on them. The incidence of tumor induction by MC treatment, however, was over 80% in this study, which nearly coincides with the figures reported by other investigators (7). In all control series of the present study, all of the mice developed tumors and died of sarcoma within the 24 weeks of observation. From these facts it may also be safely concluded that sarcoma was actually induced in the tumor-bearing limbs of the test groups in our experiments. In each test group, however, the curative effects on tumors in individual mice were varied; i.e., some tumors remained regressed for a long period of time, whereas others did not respond to the same therapy. It may be possible, however, that some of the tumors that did not respond to the chemotherapy could have already infiltrated into the trunk when the therapy was applied to the mouse, because the effectiveness of the present therapy was by nature dependent on the localization of the tumor and was strictly confined to the region distal to the inguinal ligament. It might also be possible that, although a tumor had regressed due to the therapy, another tumor was newly induced by the MC that remained in loco during the long period of observation.

Among the various chemotherapeutic agents tested in this study, CQ was the most recommendable both for its remarkable antitumor effects and for its fewer side effects. NMO was inferior to CQ for its severe side effect on regional normal tissues. MMC could not produce results as good as those of CQ in antitumor effects. With CQ, warming of the region for 30 min at 41° was more effective than was warming for 60 min at 37° in the present chemotherapeutic system. On the other hand the side effects of this drug on regional normal tissues induced by restricting regional blood flow with a tourniquet and warming the limb for 30 min at 41°, were the same as those induced by the same treatments with the different warming conditions of 60 min at 37°. Warming at a higher temperature (42.5°), however, resulted in increased mortality (90%), even when the CQ was reduced to 12.5% of the LD50 and the time of the blood flow interruption was shortened to 30 min. In this case the treated limbs of a few survivors were so severely damaged that total necrosis or mummification was frequently observed.

As to the mechanism involved in the remarkable results obtained in the chemotherapeutic system that consists of CQ injection followed by application of a tourniquet and warming of the region for 30 min at 41°, a few possibilities may be envisaged. The warming temperature of 41° may have increased the anaerobic glycolysis of the tumor cells and, consequently, the decreased intracellular and extracellular pH resulting from the production of lactic acid may have enhanced the action of CQ on tumor cells (5). According to Dickson and Ellis (4), heating at 40° produced an increase in O2 uptake and CO2 production in Yoshida sarcoma. The response pattern of MC-induced autochthonous tumor to the elevated temperature of 41° in the present study may well be considered analogous to their findings. The fact that CQ is a temperature-dependent drug might also be considered an additional factor in enhancing its effects in a short time.

The effect of interruption of blood flow and hyperthermia alone without chemotherapy was not studied in this experiment. As reported in a previous study, however, the treatments without chemotherapy could not produce any effects even on the transplanted tumors in mice (2). Autochthonous tumors have generally been known to be more resistant to chemotherapy than have transplanted tumors. Therefore, a control experiment on the effect of the treatments without chemotherapy on autochthonous tumor was considered to be unnecessary for any of the above experiments.

In the treatment with NMO, the same dose of the drug (20% of the LD50) as that of CQ in the same chemotherapeutic system produced a high mortality. This result was not in agreement with that obtained in our previous study, which did not show as high a mortality in mice that bore transplantable tumors and received the identical treatments (2). The relatively high mortality obtained in the present study (regardless of the drugs used) compared with that in the previous study may be related to a reduced immune status of the host. All of the mice used in this study were treated with carcinogen, so that they could have been in an immunosuppressive state (3), which could have further been enhanced by the side effects of the chemotherapy. Possibly, therefore, an infection could have greatly contributed to the high lethality of the mice in the present study. The results obtained in this study, however, seem to be satisfactory when compared with other results reported thus far, and it seems reasonable to assume that some types of human cancer could be treated with a similarly devised therapy system.

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REFERENCES


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