



Title	Radiosensitizing action of ametohepazon on the irradiated tumor tissues I. Combined therapy on Yoshida sarcoma
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特別掲載

Radiosensitizing Action of Ametohepazon
on the Irradiated Tumor Tissues

I. Combined therapy on Yoshida sarcoma

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Ametohepazon の放射線増感作用について

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七員環化合物の一つである Ametohepazon に放射線増感作用のあることが、林らによつて報告されている。Ametohepazon は元来、鎮痛、解熱剤として三共中央研究所において合成、開発されたものである。本剤の特色としては、投与が簡便であり、かつ副作用がほとんど認められないことである。この点、5-FU などの増感剤とは異なる点である。

我々は、増感の機序を明らかにすべく吉田肉腫を用いて2, 3の実験を行ない、これまでに記載された種々の増感剤とは機序を異にすることを知

つた。

1. Ametohepazon 単独では抗腫瘍作用はほとんど認められない。

2. 放射線と併用して、はじめて抗腫瘍作用を発揮する。

3. 照射24時間後に Ametohepazon を投与しても有効である。

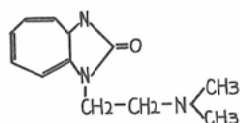
4. 作用機序の詳細はわからないが、放射線障害からの回復を直接、間接に阻害するものである。

5. 投与方法としては、局所注入の方が全身投与より優れている。

Agents which can modify biologic effects of ionizing radiation¹⁾ are called radioprotectors or radiosensitizers when applied to radiotherapy. It was oxygen²⁾³⁾⁴⁾ which was first used as radiosensitizer, and workers are now most strongly interested in it and attempting its clinical application. Among many others which have been introduced, there are BUdR⁵⁾⁶⁾⁷⁾, 5-FU⁸⁾⁹⁾, and Actinomycin D¹⁰⁾, in all of which the action mechanism is known, and Radioplex¹¹⁾, in which the action mechanism is unknown. Although each of them has some noticeable effects, there is still much to be improved by future studies, some having strong side effects and others requiring difficult technique in application.

It was previously discovered by Hayashi and Asano¹²⁾ that Ametohepazon (Fig. 1), a seven-membered ring compound, had radiosensitizing effect. That some of these compounds have anticancer action was reported earlier¹³⁾¹⁴⁾¹⁵⁾¹⁶⁾. Colchitin which Dustin discovered in 1934 is well known as mitotic poison, and as early as 1940 Brues¹⁷⁾ examined for the effect of its combination with radiation. But it has not

Fig. 1 Ametohepazon $C_{12}H_{16}ON_3$ Mol. Wt. 217.26
1-(2-dimethylaminoethyl) cycloheptimidazol-2 (1H)-one



yet been applied clinically to date, since it has strong side effect.

According to Hayashi, Ametohepazon is characterized by near absence of side effect and easiness in application. But the mechanism of its sensitizing action is not yet clear. In view of this, we examined sensitizing effect of Ametohepazon on Yoshida sarcoma, which have been used for many years in studies on cancer. And as the result it was found out that Ametohepazon exerted scarcely any effect when applied alone, but that its effect was manifested only when it was administered in combination with radiation. It was further observed that Ametohepazon was effective even when given as late as 24 hours after irradiation.

Materials and Methods

Donryu strain male rats weighing about 120 g, were used.

Yoshida sarcoma was supplied by the Sasaki Laboratory and was used after several passages through Donryu rats in this department.

Ametohepazon was product of Central Research Laboratories, Sankyo Co., Ltd., and had passed the aseptic test. It was used as a 5% solution.

Topical injection of Ametohepazon: 10^6 cells of Yoshida ascites sarcoma were transplanted intramuscularly into the right femur of the Donryu rat, and on the 4th day after the transplantation, 100 mg/kg of Ametohepazon was injected into the tumor tissue. Then the right femur was irradiated with a dose of 300 R, and thickness of the femur was daily determined until the death of the animal, to be used as a measure for assessing the effect. The control group were injected with saline solution of pH 6.4 before irradiation.

To see effect of time interval between Ametohepazon injection and irradiation, the animals were divided into 4 groups, and Ametohepazon was given at 24 and 0 hours before and 0 and 24 hours after irradiation, respectively, in these groups. And curves of tumor regression and recidivation were obtained by determining the thickness of the muscle. From time to time the animals were sacrificed for histological examination. The dose of irradiation was 500 R.

Systemic administration of Ametohepazon: 10^6 cells of Yoshida sarcoma were transplanted intramuscularly into each of the right and the left femur, and on the 4th day after the transplantation, 100 mg/kg of Ametohepazon was intraperitoneally injected. Then only the right femur was irradiated with 500 R. The effect was assessed by the degree of DNA synthesis inhibition using ^3H -thymidine. At 1, 6, and 24 hours after radiation, $1 \mu\text{Ci/g}$ of ^3H -thymidine was intraperitoneally injected, the animals were sacrificed 45 minutes later, and after formalin fixation, paraffine embedding and sectioning, autoradiograph was made by the dipping method using Sakura NR-M₂ emulsion. Also the samples were stained with methylgreen-pyronine for microscopical observation.

In vitro mixing of Ametohepazon with Yoshida sarcoma cells: To 3×10^7 Yoshida sarcoma cells were

added 10 mg of Ametohepazon and 3 ml of Eagle's solution, and after mixing well, the whole was allowed to stand in test tubes at 37°C for 6 hours. The mixture then received 1,000 R of radiation, and each 0.1 ml of it was intraperitoneally transplanted to the animals. And the effect was assessed by survival days and death rate. The control consisted of 3 groups—one irradiated without mixture of Ametohepazon, and the other two non-irradiated with and without mixture of Ametohepazon.

Radiation was made with the Toshiba's KXC-18: Tube voltage, 180 kv; tube current, 20 mA; filter plate, 0.5 Cu + 0.5 Al; F.S.D., 18 cm; dose rate, 209 R/m; and half value layer, 1 mm Cu.

Results

1. Effect of intra-tumorous injection of Ametohepazon plus irradiation: Ametohepazon alone could not inhibit proliferation of tumor cells, making no difference from the control, whereas the combination treatment produced evident effect (Fig. 2).

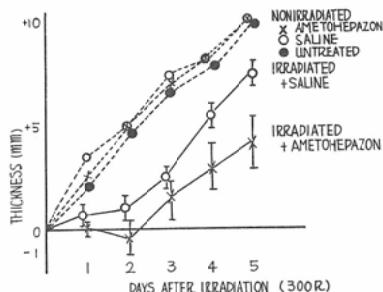


Fig. 2 Effect of intra-tumorous injection of Ametohepazon plus irradiation

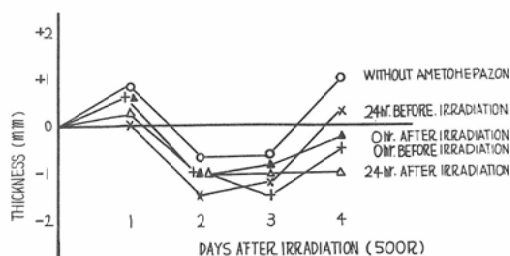


Fig. 3 Effect of time interval between Ametohepazon injection and irradiation

Effect of time interval between Ametohepazon injection and irradiation: At 2 days after irradiation no change was visible between the 4 groups; but at 4 days after irradiation (8 days after the transplantation), restoration above the pre-irradiation thickness was seen in the saline-given control and the group given Ametohepazon 24 hours before irradiation, while the restoration was delayed in the other 3 groups (Fig. 3). Longer observation was impossible in the present experiments, since the host animals died of lung metastases at about 8 days following the tumor transplantation. The noteworthy result in the present experiments was that the effect of the combination treatment was visible even when Ametohepazon was topically injected 24 hours after irradiation. Histologically, the temporal change was nearly the same in all the groups. Below are given, for example, changes in the group given Ametohepazon immediately before irradiation: At 24 hours after irradiation, there were edematous change and cellular infiltration at the site of tumor, and softening and degeneration were seen in tumor cells which were in contact with Ametohepazon (Fig. 4). At 48 hours, softening and degeneration were advanced further, and tendency of confined fibrosis was seen (Fig. 5). At this time yellowish grey tint could be discerned macroscopically, which was different from reddish tint in the saline-given control (Fig. 6). At 3 days after irradiation, tumor tissue, not in contact with Ametohepazon or in the control group, showed perfect repair and remarkable proliferation (Fig. 7), whereas the tissue which was in contact with Ametohepazon, showed still more advanced fibrosis (Fig. 8). Repair rate in terms of muscular thickness was high in the saline-given control, but low in the experimental groups except one which were given Ametohepazon at 24 hours before ir-

radiation (Fig. 9). This is because a large amount of other elements than tumor cells were contained in the tumors of the groups of combined treatments. In the non-tumor tissue, softening of muscle and cellular infiltration were slightly observed (Fig. 10), but there was no remarkable change in other organs.

2. Effect of systemic injection of Ametohepazon plus irradiation: Since no clear difference was hard to observe in terms of muscular thickness, inhibition of DNA synthesis as seen in autoradiograph was used as a measure in the evaluation of the effect.

Labeling index (L. I.) was 57% when Ametohepazon alone was given, which was scarcely different from the result in the saline given control. But when Ametohepazon was supplemented by radiation, evident difference was produced (Table 1).

Table 1 Uptake of ^3H -thymidine of tumor cells irradiated with or without Ametohepazon

time after irradiation	labeling index		grains per labeled cell		grains per 100 cells	
	with	without	with	without	with	without
1 hours	0.8%	6%	4.3	5.0	3	30
6	0	20	0	7.1	0	142
24	14	32	9.8	11.4	137	364
—	57	57	11.9	11.8	678	671

Table 2 Effect of Ametohepazon in *in vitro* irradiation of Yoshida sarcoma

Group	survival rate	survival days
Ametohepazon with irradiation	2/4	17, 18, —, —
irradiation alone	0/4	13, 14, 21, 24
Ametohepazon alone	0/4	7, 9, 11, 12
untreatment	0/4	9, 10, 12, 13

In terms of grain count (G. C.) of labeled cells, the value was generally low in the combination treatment than in the control (Figs. 11—14).

3. Effect of Ametohepazon in *in Vitro* irradiation of Yoshida sarcoma: Survival cases (100 days) were found only in the group of Ametohepazon administration plus irradiation. The cure in these 2 survival cases could not be spontaneous one induced by participation of immunizing factor since the intraperitoneal puncture sample demonstrated no tumor cells at the time of the transplantation. When Ametohepazon alone was given, no difference was observed from the nontreated group either in survival rate or in survival days (Table 2).

Discussion

Effect of Ametohepazon in combination with radiation was visible whether it was topically injected in tumor, or systemically given or added *in vitro* experiment. Ametohepazon alone produced scarcely any inhibitory effect on growth of tumor cells. In other words, Ametohepazon exerted scarcely any anti-cancer effect when given alone, and produced the effect only in combination with irradiation. Therefore it can be classified as radiosensitizer.

1. Experimental system

In assessing the effect of radiosensitizer, the most important point is the selection of an adequate measure. And special care must be taken with the agent which, like Ametohepazon, does not exert effect when given singly. The most simple and effective measure for experimental tumor will be cure rate. But depending on the kinds of animal and tumor, immunological reaction may operate, and depending on the site of tumor transplantation, secondary factors such as ulcer and infection may be involved, which will eventually make the evaluation difficult¹⁸⁾. Taking these into consideration, we transplanted Yoshida

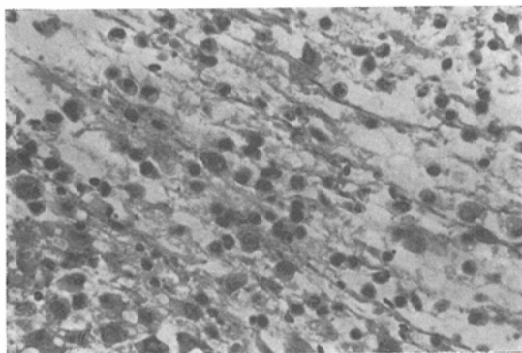


Fig. 4 At 24hr. after irradiation, cellular infiltration and edematous change are seen.

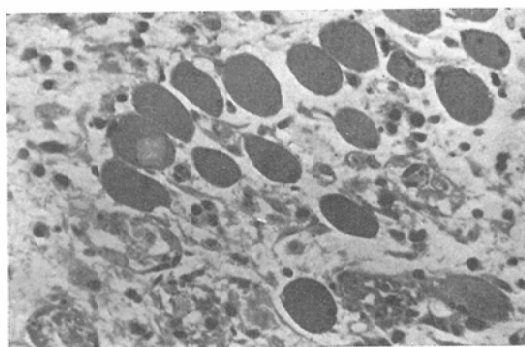


Fig. 5 At 48hr. after irradiation, softening, lysis and fibrosis are seen.

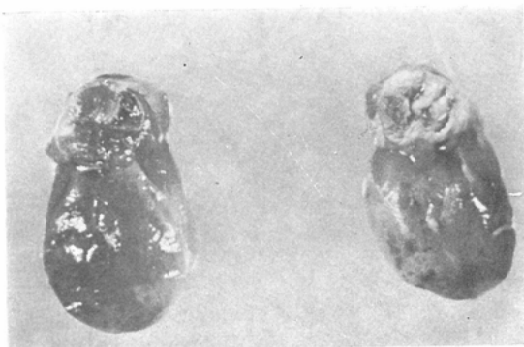


Fig. 6 At 48 hr. after irradiation, the left irradiated with saline shows reddish tint, the right with Ametohepazon shows yellowish grey tint.

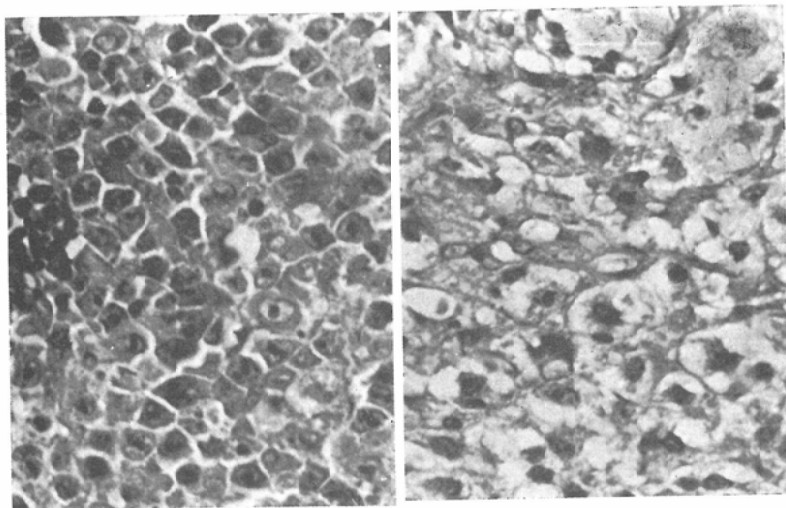


Fig. 7,8 At 3days after irradiation, tumor tissue in the control group shows perfect repair (the left) and the tumor tissue in contact with Ametohepazon shows more advanced fibrosis (the right).

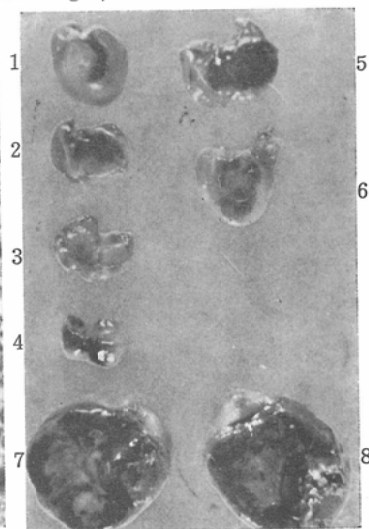


Fig. 9 At 2days after irradiation,
1. Irrad. 24hr. after Ameto.
2. Irrad. 0hr. after Ameto.
3. Irrad. 0hr. before Ameto.
4. Irrad. 24hr. before Ameto.
5. Irrad. without Ameto.
6. Irrad. 0hr. before saline
7. Administered Ameto. alone
8. Untreated

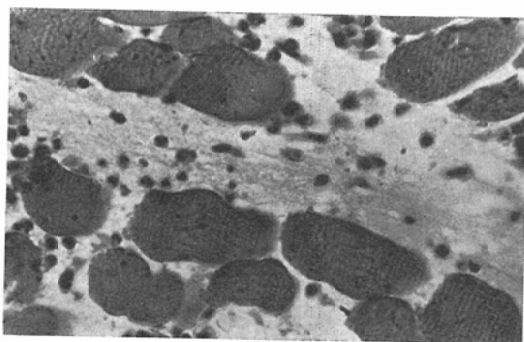


Fig. 10 The non-tumor tissue, in contact with Ametohepazon, shows softening of muscle and cellular infiltration slightly.



Fig. 11 Grain counts of labeled cells 1hr. after irradiation

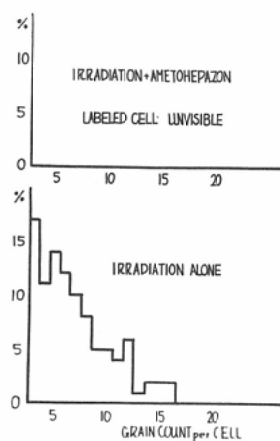


Fig. 12 Grain counts of labeled cells 6hr. after irradiation

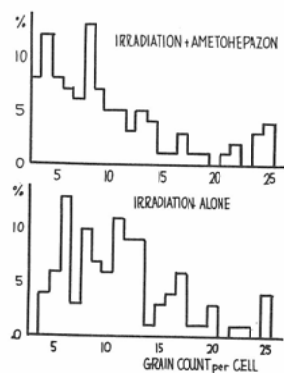


Fig. 13 Grain counts of labeled cells 24hr. after irradiation

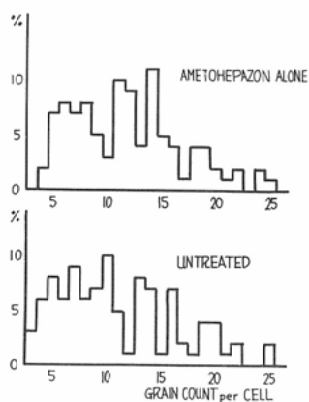


Fig. 14 Grain counts of labeled cells Non-irradiated

sarcoma intramuscularly into the femur of Donryu strain rat, and used, as the measure, the muscular thickness, which was demonstrated by repeated preliminary experiments to increase almost linearly in proportion with proliferation of tumor¹⁹⁾. In the *in vitro* radiation experiments, the survival rate was used as the measure. In this case, ascites sample was taken from time to time in order to check for the participation of immunization and other secondary factors.

2. Action mechanism of Ametohepazon

Ametohepazon was initially developed as analgesic and antiphlogistic²⁰⁾²¹⁾, and later found by Hayashi et al¹²⁾ to have radiosensitizing action. But it is not yet known what mechanism underlies this action. From a fact disclosed by the present experiment that Ametohepazon exerted the sensitizing effect even when applied at 24 hours after irradiation, it can not be considered to have radical participation like oxygen²²⁾, DTBN²³⁾ and Synkavit²⁴⁾. Nor can it have the same underlying mechanism as BUDR, which is assumed to exert the sensitizing effect by being incorporated into DNA before irradiation.

Histological examination after topical injection of Ametohepazon revealed succession of edematous degeneration, cellular reaction, softening necrosis, and fibrosis, which is similar to the reactive process to foreign body. It can therefore be considered that the reaction to foreign body induced by Ametohepazon may indirectly block the repair of cells damaged by radiation. In support of this view, there is change, though slight, similar to reaction to foreign body after injection of Ametohepazon into tumor without irradiation. However, the inhibition of DNA synthesis and the delay in the repair, which were observed after systemic injection of Ametohepazon, can not be ascribed for temporal reason, to the reaction to foreign body. Also in the *in vitro* experiment there seems to be very poor reason to justify the participation of the reaction to foreign body. Nevertheless the direct action of Ametohepazon can not be excluded completely, since difference was produced by its administration in the survival rate. In other words, Ametohepazon can be considered to exert inhibitory action, either direct or indirect on the repair of damaged tumor cells, though it is ineffective for undamaged ones.

To date, numerous radiosensitizers have been introduced, but their action mechanisms are varying. In 1963, Bagshaw²⁵⁾ classified actions of radio-sensitizers into 4 types: (1) Sensitization, (2) Augmentation, (3) Potentiation, and (4) Additivity. According to him, the action of Ametohepazon, which is assumed to consist in blocking repair of radiation damage, should belong to potentiation like actions of antimetabolites such as 5-FU and Methotrexate⁹⁾ and of SH inhibitor such as Iodoacetamide²⁶⁾. But different from 5-FU and Methotrexate, Ametohepazon has not anti-cancer action, and any comparable drug can be found among Bagshaw's potentiators.

3. Method of administration of Ametohepazon

Comparison between systemic and topical injection revealed that difference was not conspicuous in terms of muscular thickness after one time intraperitoneal injection but that difference was discernible in terms of DNA synthesis inhibition in early post-irradiation period. According to this, the topical injection is considered more effective when the dose is same. This is in agreement with result of depilation experiment by Hayashi et al²⁷⁾. By the topical injection, however, thorough penetration of the drug in the tumor tissue, consequently, radical cure of tumor can not be expected. And in this respect, the systemic injection, which allows the drug to be carried into tumor by means of blood flow, is considered more efficacious.

In the *in vitro* experiments, the group of the combined treatment gave 50% survival (100 days) against

0% in the group of Ametohepazon or radiation alone. In this way, difference was prominent in number of survival, but when viewed in terms of survival days of dead cases, difference was indiscernible. It seemed therefore better to be highly deliberate in assessing the effect in the *in vitro* experiment.

In experiments with Yoshida sarcoma and Donryu strain rats, length of observation is limited. In view of this we are now attempting long term observation of the effect of Ametohepazon by the use of mammary cancer, spontaneously produced in C₃H strain mice.

Conclusion

With Yoshida sarcoma, radiosensitizing action of Ametohepazon was investigated with the following results:

- 1) When given alone, Ametohepazon scarcely exerted anti-cancer action.
- 2) Its anti-cancer action was manifested only in combined application with irradiation.
- 3) According to histological measure of evaluation, the topical administration was more effective than the systemic administration.
- 4) Ametohepazon was effective even when applied as late as 24 hours after irradiation, thus indicating difference in action mechanism from other radiosensitizers.
- 5) As for the action mechanism, details are not yet known, but Ametohepazon seems to impede either directly or indirectly the repair of tumor cells damaged by irradiation.

Acknowledgement

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