

Studies on Hyperthermic Chemotherapy for Cancer of the Esophagus—Especially the Intraluminal Administration with Perfusion of BLM Containing Warmed Saline Solution

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Introduction

Recently, surgical treatment of esophageal cancer has progressed, however, inoperable or recurrent cases are not necessarily few. Multidisciplinary therapies are required for these cases. These treatments consist of chemotherapy, radiotherapy, and immunotherapy. Also hyperthermia is found to have a benefit in the treatment of human cancers. It was reported 100 years ago that heat treatment exerted destructive effects on cancer⁶⁾. Recently many investigations have supported this treatment. The purpose of this paper is to report on fundamental experiments for clinical use of hyperthermic chemotherapy for esophageal cancer.

The method to generate hyperthermia is one of the important problems for the clinical application of hyperthermia. Heat generating systems have been developed rapidly. In this experiment tissue heating was attained by perfusion of warmed saline solution because the system was simple.

Further, a synergistic antitumor effect of hyperthermia in combination with chemotherapeutic drug has been demonstrated. A marked effect of Bleomycin (BLM) has been observed on cancer of the esophagus. Many investigations^{9,16,18,19)} suggested that local hyperthermia could potentiate BLM cytotoxicity. However, relatively few studies¹⁸⁻²⁰⁾ in vivo have been reported in the past. Further both systemic and regional administrations of BLM are used in clinical trials, and it is conceivable that there are different pharmacokinetics respectively. For the purpose of designing appropriate clinical use of hyperthermic chemotherapy, this study was done in animal systems. A synergistic antitumor effect and appropriate administration method of BLM in combination with local hyperthermia was investigated by the BLM level of the esophageal mucosa in each administration method.

Even local hyperthermia may affect blood flow³⁾, therefore, it was a study of what effect the blood flow had on the synergistic effect.

Key words: Hyperthermia, Chemotherapy, Esophageal cancer, Bleomycin, Intraluminal administration.

索引語: 温熱療法, 化学療法, 食道癌, ブレオマイシン, 内腔内投与.

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This study also evaluated a histological effectiveness following hyperthermic chemotherapy of carcinoma tissues in rats induced by carcinogen.

Materials and Methods

Experiment 1

The catheter was designed to perfuse warmed solution in the esophageal lumen for clinical use. It is 18 Fr. 4-way catheter and consists of inlet and outlet tubes and two balloons which make the blind lumen of the esophagus. The distance between two balloons is 6 or 13 cm. It is able to perfuse warmed solution in this lumen. Warmed saline solution is heated using a warming coil placed in water bath. The system is driven by a pump (Sarns S 10 KII). A thermistor probe is placed on the outlet side between two balloons of the catheter in order to measure temperature of solution retained in the lumen of the esophagus (Fig. 1).

This system was used in 3 normal adult mongrel dogs and temperature was recorded at 3 sites of the esophagus. Dogs were anesthetized by intravenous injection of Pentobarbital sodium at a dose of 25 mg/kg and the respiration was controlled by intratracheal intubation with a respirator. Right thoracotomy was performed through the 6th intercostal space. Thermistor probes were placed in the esophageal mucosal layer and the esophageal muscular layer. A 4-way double balloon catheter was inserted from the mouth into the lumen of the esophagus and double balloons were inflated by air. Warmed saline solution was perfused by a pump at a flow rate of 50 ml/min for 1 hour. Temperature of each layer was measured by a thermistor (Bailey TH-6) throughout the experiment (Fig. 2).

After 1 hour, the dog was sacrificed and the esophagus was resected. The specimens were fixed in 10% formalin solution and 4 μ sections were cut and stained with Hematoxylin and Eosin

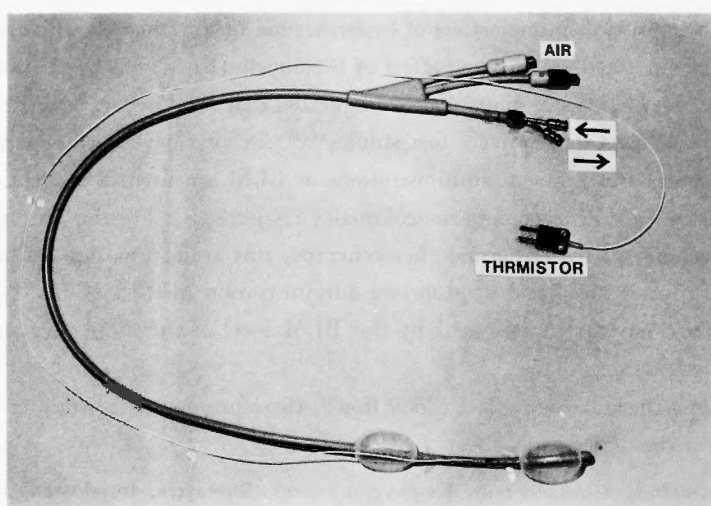


Fig. 1. FOUR WAY DOUBLE BALLOON CATHETER

A thermistor probe was placed on the outlet side between two balloons of the catheter.

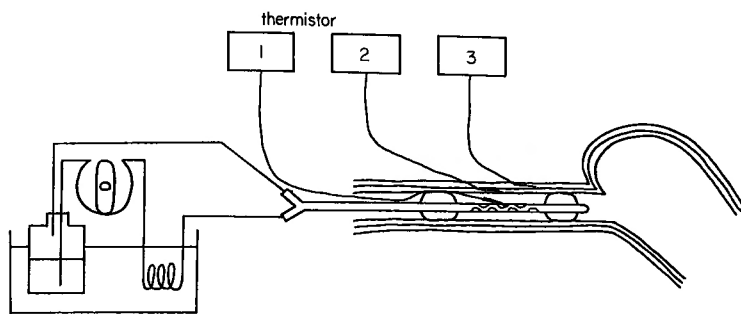


Fig. 2. Perfusion system

Perfusion of the warmed saline solution using 4-way double balloon catheter
 temperature monitor: 1 fluid in the esophageal lumen
 2 mucosal layer of the esophagus
 3 muscular layer of the esophagus

as usual. The histological changes following perfusion of warmed saline solution was studied.

Experiment 2

BLM was dissolved with saline solution at a concentration of $250 \mu\text{g/ml}$ and 5 ml of this BLM containing solution was placed in each test tube. The test tubes were immersed in water bath. The temperature of the water bath was 20, 40, and 80°C . After 0, 30, 60, and 120 minutes at each temperature the concentration of BLM was measured by bioassay, the Band Culture Method²⁶⁾, using bacillus subtilis PCI 219.

Experiment 3

Experiment was carried out in 24 normal adult mongrel dogs. Dogs were anesthetized by intravenous injection of Pentobarbital sodium at a dose of 25 mg/kg and the respiration was controlled by intratracheal intubation with a respirator. Local hyperthermia was produced by perfusion with warmed saline solution. Right thoracotomy was performed through the 6th intercostal space. A 2-way catheter (Fr. 18) was inserted from the mouth into the lumen of the esophagus. The esophagus was ligated at two sites approximately 10 cm apart to prevent leakage of

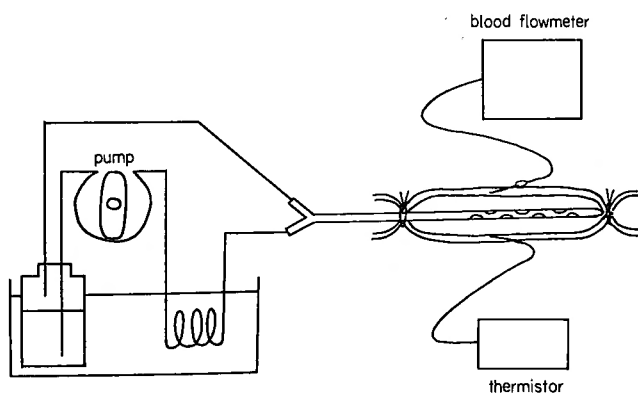


Fig. 3. Experimental method

The esophagus was ligated to prevent leakage of the solution.

solution and the blind lumen of the esophagus was made. Warmed saline solution, which was heated using a warming coil placed in water bath, was perfused in this lumen by a pump (Sarns S 10 KII). Flow rate of the saline solution was 50 ml/min. A probe of thermistor (Bailey TH-6) which was placed in the esophageal submucosal layer was utilized to measure the temperature of the esophagus. The temperature of the esophagus was measured throughout each experiment (Fig. 3).

Two different methods of administration of BLM were used: (a) Systemic administration with intravenous injection, (b) Local administration with intraluminal perfusion of BLM containing saline solution. BLM was intravenously administered at a dose of 1, 2, and 4 mg/kg and was intraluminally administered at a dose of 0.1, 0.2, and 0.4 mg/ml into 6 groups of dogs, respectively. In each group the temperature of the esophagus was maintained at 37°C for control groups and 43°C for hyperthermic groups. BLM was administered intravenously immediately after beginning of perfusion. After 1 hour at 37°C or 43°C, respectively, the esophageal mucosal layer was resected. The resected tissue was weighed, rinsed, cut into small cubes, and homogenized with twice the volume of physiological saline solution. This homogenate was kept in a refrigerator at 4°C for 24 hours. The supernatant fluid was used for the measurement of BLM concentration by bioassay, the Band Culture Method²⁶⁾.

A cutdown tube was inserted into the azygos vein and the azygos vein blood was collected at 5, 10, 20, 30, and 60 minutes after intravenous injection of BLM or beginning of perfusion of BLM containing saline. The concentration of BLM was measured by the same method²⁶⁾. A concentration-time-curve of BLM was simulated using least squares nonlinear regression analysis with a microcomputer³⁴⁾.

Simultaneously, blood flow of the esophagus was measured by a hydrogen gas clearance flowmeter²⁾ (UNIQUE MEDICAL CO. LTD. PHG 201); the probe was placed in the submucosal layer of the esophagus. Hydrogen gas was inhaled at a dose of 0.8 l/min (5-6%) through the intratracheal tube.

Experiment 4

Male Donryu strain rats weighing 100 g were used. Solution of 0.003% of N-Methyl-N-Amyl-Nitrosamine was given to the rats as drinking water for 12 weeks and after these periods the rats were given tap water for 14 weeks^{11,25)}. Hyperthermic chemotherapy was performed for these rats which had esophageal cancers induced by the above mentioned method. The effects of hyperthermic chemotherapy were histologically studied.

Local hyperthermia was produced by perfusion with warmed saline solution. BLM was administered into the lumen of the esophagus using perfusion of BLM containing warmed saline solution. Rats were anesthetized by intraperitoneal administration of Pentobarbital sodium at a dose of 50 mg/kg. Under laparotomy, gastrotomy was made and a ϕ 1.5 mm silicon tube was placed in the esophageal lumen through a ϕ 3 mm sheath tube which was inserted into the lower esophagus inversely from the stomach. The cervical esophagus was exposed and ligated to prevent leakage of solution. BLM containing warmed saline solution, which was heated using a warming

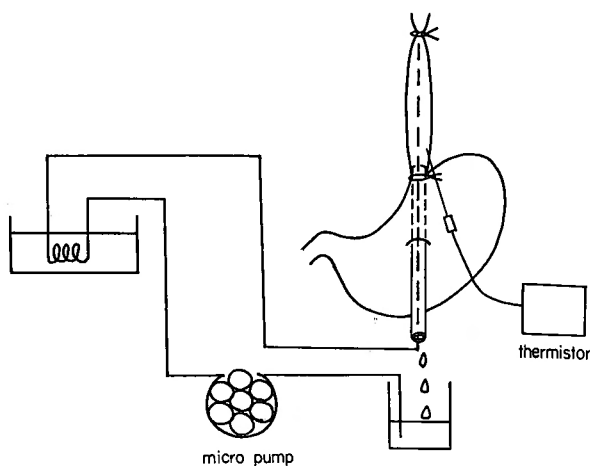


Fig. 4. Experimental method
BLM containing saline solution was perfused in the esophagus by a micro pump.

coil placed in water bath, was infused into the lumen of the esophagus through a silicon tube by a micro pump (EYEL Δ micro tube pump MP-3). The solution was drained using a sheath tube. Collected solution was reinfused and perfusion of solution was carried out in this way. BLM was contained in the solution at a concentration of 0.3 mg/ml. Flow rate of the solution was 15.3 ml/min. Temperature of the esophagus was measured throughout experiment by a thermistor (Bailey TH-6) probe which was placed in the lower esophageal wall (Fig. 4). The duration of perfusion was 30 or 40 minutes and esophageal temperature was maintained at 43°C during perfusion.

Rats were divided into two groups after hyperthermic chemotherapy. One was immediately sacrificed, the other was released the ligation of the cervical esophagus and survived. It was sacrificed after 4 days. The esophagus and stomach were resected. The specimen of the esophageal tumor was fixed in 10% formalin solution and 4 μ sections were cut and stained with Hematoxylin and Eosin as usual. The effects of hyperthermic chemotherapy were studied histologically.

Statistical Analysis⁸⁾

BLM levels from the different groups were compared with a two-way analysis of variance. The data of blood flow were analyzed using Student's t-test for unpaired data. Results were expressed as the mean (95% confidence interval).

Results

Experiment 1

Perfusion of warmed solution could be performed using this system. There was little leakage during perfusion. Each temperature of the solution in the esophageal lumen, the mucosal and muscular layers of the esophagus was shown in Fig. 5. The respective temperatures were raised rapidly when the perfusion was started, and maintained constantly. Temperatures were easy to control. Temperature appeared in an order of solution in the esophagus > the mucosal layer >

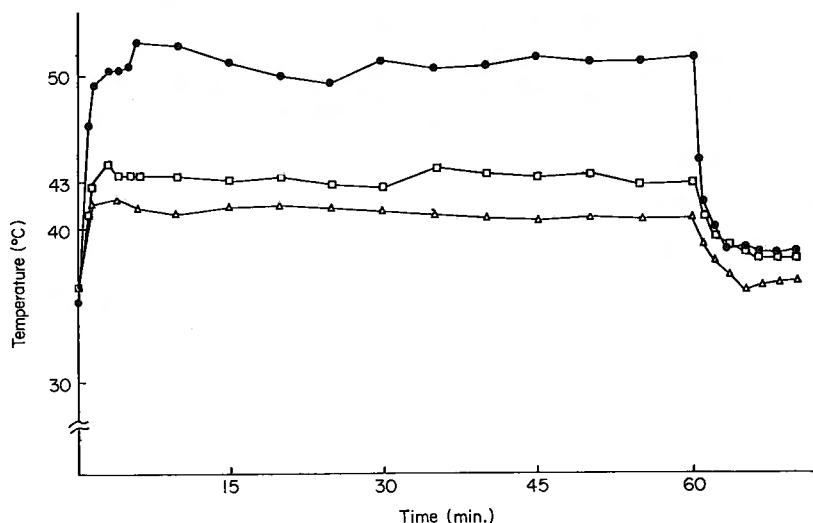


Fig. 5. Temperature profile during perfusion of warmed saline solution
Thermistor probes were placed at 3 locations.
● temperature readings from probe in the lumen of the esophagus
□ temperature readings from probe in the mucosal layer of the esophagus
△ temperature readings from probe in the muscular layer of the esophagus

the muscular layer. Temperature distribution spread superficially. Each temperature ran parallel to the other. If the temperature of retained solution in the esophagus was controlled the temperature of the mucosal layer could be maintained constantly at 43°C. Histological sections obtained after perfusion of warmed saline solution showed blister in the epithelium, edema of the mucosal, submucosal, and muscular layers, and dilatation of the lymphatic vessels. Although, irreversible damage was not seen (Fig. 6).

Experiment 2

On different heating temperature and duration of heating, statistically no significant difference of BLM concentration was shown (Fig. 7).

Experiment 3

1. On systemic administration of BLM (intravenous injection) BLM level of the esophageal mucosa was increased with increase in administration dose ($p < 0.001$). However, there was no statistically significant difference between the hyperthermic groups and the control groups (Fig. 8).

2. On local administration of BLM (perfusion of BLM containing saline solution) BLM level of the esophageal mucosa was increased with increase in concentration of solution ($p < 0.001$). In the hyperthermic groups, BLM level of the esophageal mucosa was significantly increased ($p < 0.001$) (Fig. 9).

3. When BLM concentrations of azygos vein blood were analyzed, the data fitted a biexponential function or a monoexponential function. BLM level was decreased exponentially following intravenous administration. BLM was not detected in the intraluminally administered groups.

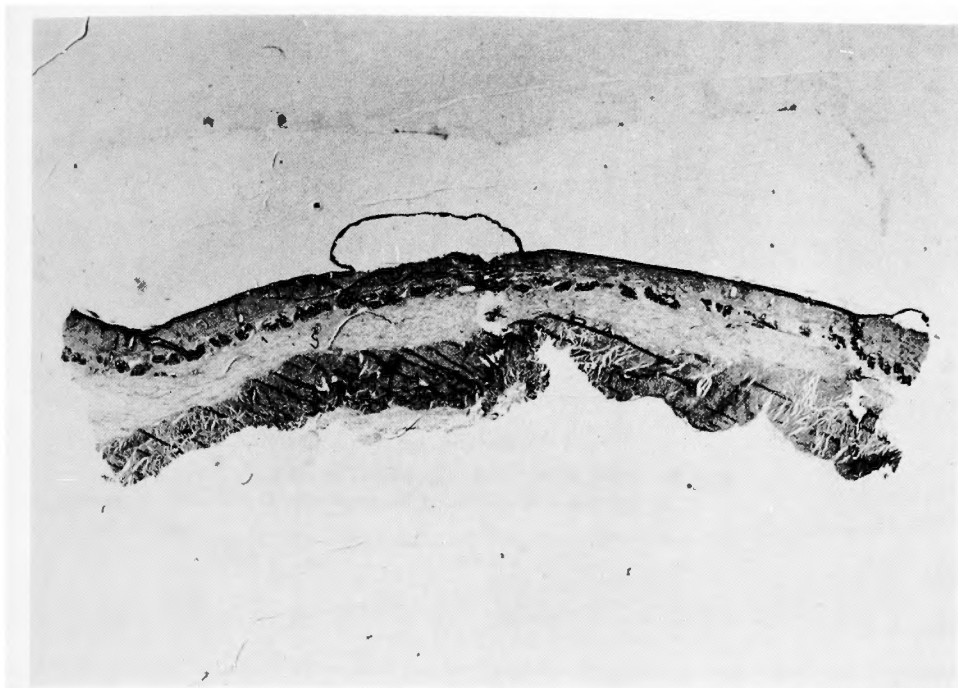


Fig. 6. Histological section after the 60 minutes periods of heating at 43°C. Irreversible damage was not observed. H.E. stain ($\times 1$)

4. On intravenous administration there were different patterns of the concentration-time-curve in each dog (Fig. 10-1, 10-2, 10-3).

5. Blood flow of the esophagus showed statistically significant increase in the hyperthermic groups 44.6 (39.0-50.2) ml/min/100 g in contrast to the control groups 32.7 (29.5-35.9) ml/min/100 g ($p < 0.001$) (Fig. 11). Although, multiple regression analysis showed that there was no correlation between blood flow and BLM level of the esophageal mucosa in both administration methods

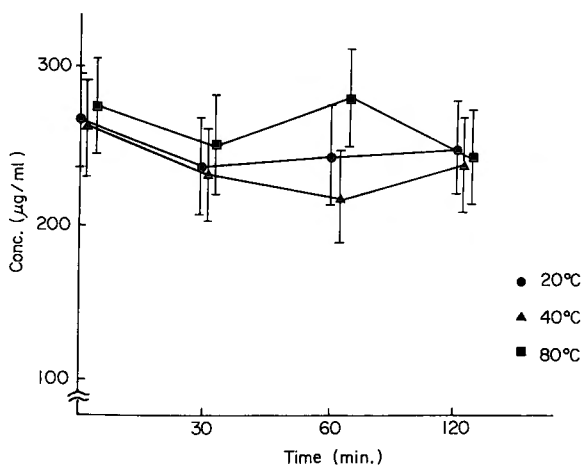


Fig. 7. Influence of heating on BLM activity

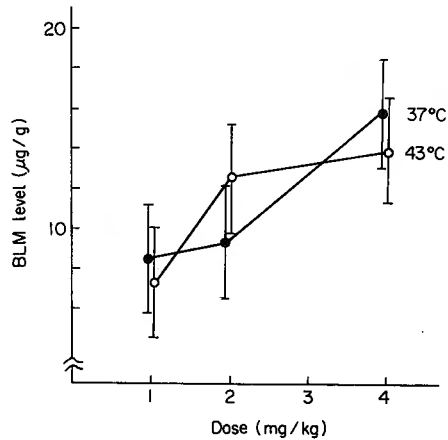


Fig. 8. BLM levels in the esophageal mucosa BLM was administered intravenously.

of BLM.

Experiment 4

Among 7 rats which had esophageal cancers, one rat was sacrificed immediately after hyperthermic chemotherapy and 3 rats were sacrificed 4 days after this therapy. Histological sections obtained from these rats were studied. Immediately after therapy, although the stroma was infiltrated with inflammatory cells, degeneration of cancer cells could not be shown. Four days after therapy, marked coagulation necrosis was recognized in the area in which it seemed to have been presented the cancer and viable cancer cells could not be seen (Fig. 12). Necrosis of blood vessels was seen around the area of coagulation necrosis (Fig. 13). Also around these areas inflammatory cell infiltration including eosinophils was shown.

Three rats among 7 rats which had esophageal cancers were sacrificed without therapy and

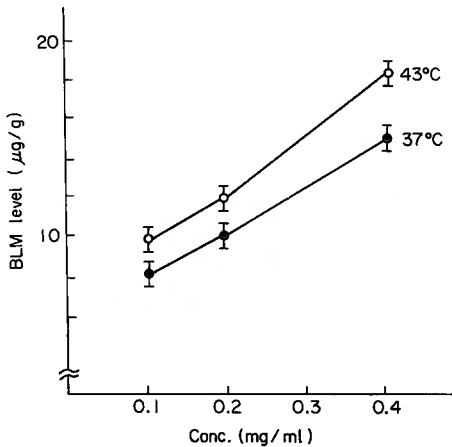


Fig. 9. BLM levels in the esophageal mucosa BLM was administered intraluminally.

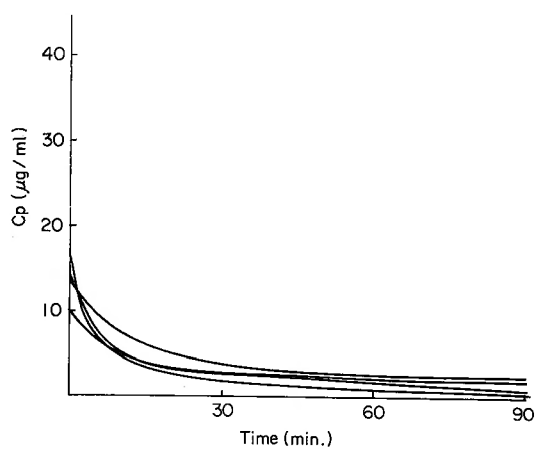


Fig. 10-1. Concentration-time-curves of BLM administered doses: 1 mg/kg

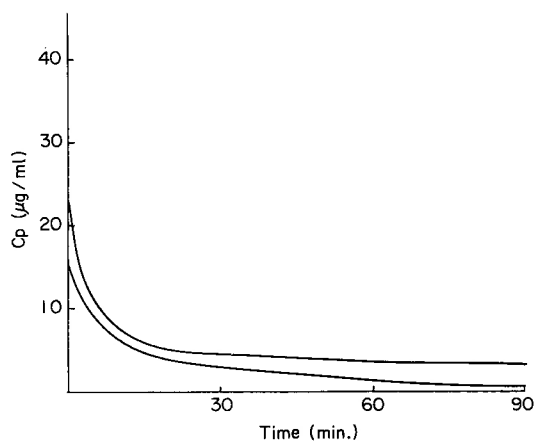


Fig. 10-2. Concentration-time-curves of BLM administered doses: 2 mg/kg

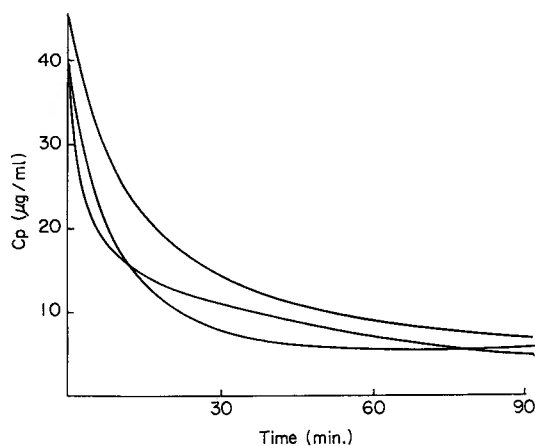


Fig. 10-3. Concentration-time-curves of BLM administered doses: 4 mg/kg

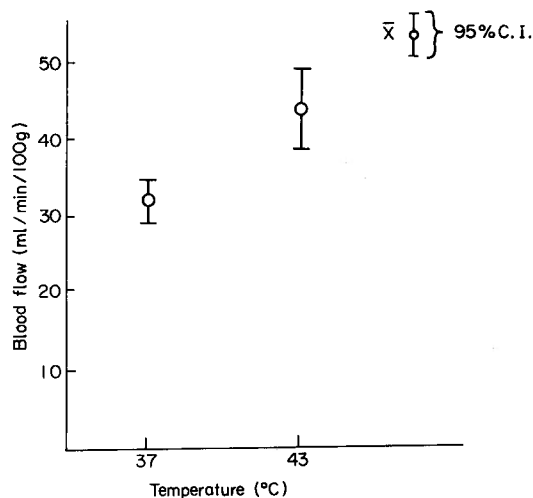


Fig. 11. Blood flow of the esophagus

histologic sections were studied as controls. No coagulation necrosis and no degenerative changes of blood vessels were seen in these specimens (Fig. 14).

Among 5 rats which had papillomas, one rat was sacrificed immediately after hyperthermic chemotherapy and 3 rats were sacrificed 4 days after this therapy. Histological sections obtained

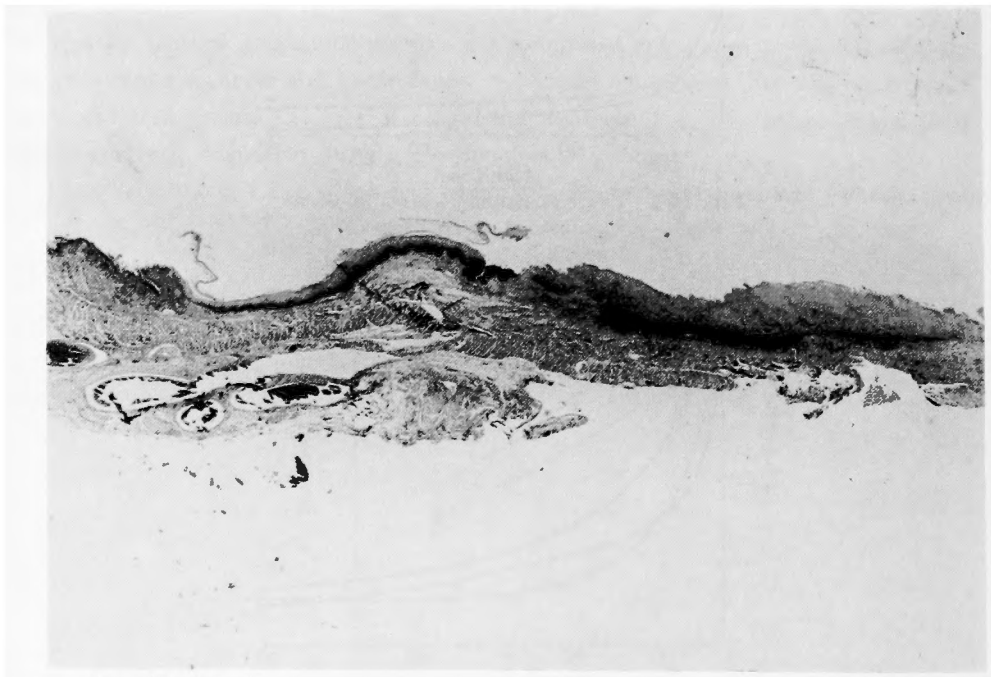


Fig. 12. Histological appearance 4 days after 30 minutes period of hyperthermic chemotherapy. Coagulation necrosis of the tumor was observed. The normal esophageal tissue was not affected. H.E. stain ($\times 3.5$)

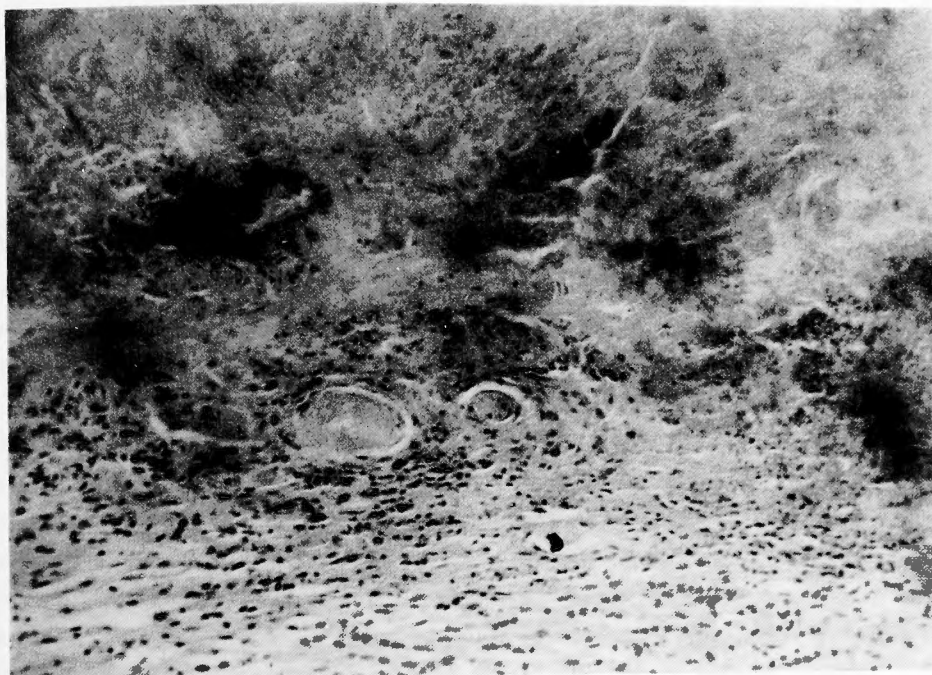


Fig. 13. Histological appearance 4 days after 30 minutes period of hyperthermic chemotherapy. Necrotic blood vessel was observed. H.E. stain ($\times 100$)

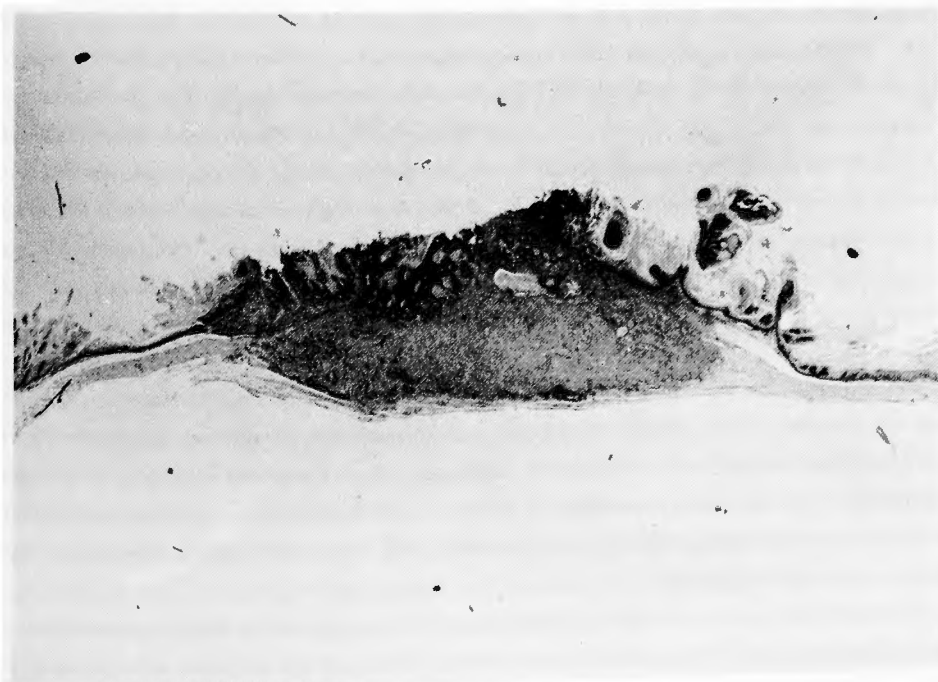


Fig. 14. Squamous cell carcinoma induced by MNAN. Hyperthermic chemotherapy was not performed. H.E. stain ($\times 1.5$)

from these rats were studied. Congestion of the blood vessels in the mucosal and submucosal layers was shown, however, degenerative changes of tumor cells were not seen. One rat among 5 rats was sacrificed without therapy and histologic section of this rat showed no congestion of blood vessels.

There was no histological alteration in areas of the normal esophageal tissue after hyperthermic chemotherapy (Fig. 12).

Therefore, it was histologically conclusive that cancer cells were selectively destructed following hyperthermic chemotherapy.

Discussion

Hyperthermia have been shown by in vitro and animal studies to be selectively lethal to cancer cells.

The appropriate hyperthermic system is desirable for clinical use. Technology in this field has expanded rapidly. The methods with the most promising potential for inducing local heating are those involved 1) radiofrequency (RF), 2) microwaves, and 3) ultrasound. However, perfusion of warmed solution is one of the useful methods because the system is simple.

Hyperthermic therapy using perfusion of warmed solution was performed by WESTERMARK³³⁾ in 1898 for cancer of the uterine cervix. Succeedingly COCKET⁵⁾, LUNGLMAYR¹⁷⁾, and KUBOTA¹⁴⁾ et al. carried out this therapy for bladder cancer and LAW¹⁵⁾ reported that it was used for gastric cancer.

YASUMOTO³⁵⁾ invented a specially designed double balloon catheter for intraluminal administration of BLM to the esophagus. The author improved this catheter for perfusion of warmed solution in the lumen of the esophagus. Hyperthermic therapy requires the monitoring of the tissue temperature. It is difficult to directly measure the tissue temperature using this system. In order to be approximated closely to the tissue temperature, the temperature of intraluminal fluid was measured in this system. One to 2°C differences between temperature of the mucosal layer of the esophagus and that of the muscular layer near the tunica adventitia were observed in this study. Also differences between temperature of the solution in the esophagus and that of the mucosal layer were observed. Temperature distribution spread superficially as reported previously¹²⁾.

It was thought that atmospheric temperature and thoracotomy state affected the differences in these temperature. SUGIMOTO²⁹⁾ et al. studied temperature distribution at various depth of the tissue in hyperthermia for bladder cancer using perfusion of warmed solution. They reported that the decline in temperature was milder in cancer than in normal tissue. Accordingly, it seemed that differences in these temperatures could be decreased when thoracotomy was not performed and this system used to cancer.

In this study using normal dogs local hyperthermia was generated with our designed system and irreversible damage of the esophagus was not seen after perfusion for one hour using 50-60°C solution. Therefore, the system was determined to be safe for clinical procedure.

BLM is an antineoplastic antibiotic complex isolated in 1962 from fermentation products of

*Streptomyces verticillus*³¹⁾. This drug is shown to be effective against a squamous cell carcinoma¹⁰⁾, therefore, it has been used as a chemotherapeutic agent for cancer of the esophagus²⁴⁾.

It has been reported that hyperthermia increased cytotoxicity of some chemotherapeutic agents. Effect of combined treatment with BLM and hyperthermia on original Chinese Hamster HA 1 cells was reported by HAHN⁹⁾, et al. The sensitivity of cells exposed in vitro to BLM was only mildly increased at 41°C over that seen at 37°C, however, at 43°C a marked synergism between the effects of hyperthermia and BLM was observed. This synergism could also be demonstrated to occur in solid tumors in vivo^{18,19)}. This synergistic effect was abolished when the two modalities were given 4 or 24 hours apart^{18,19)}. LIN et al.¹⁶⁾ suggested using V 79 Chinese Hamster cells that more effective cell killing could be achieved if the cells were exposed to heat before or simultaneously with BLM administration. According to these results, the 43°C temperature was selected and BLM was administered simultaneously with hyperthermia in this study.

It has been shown that BLM administration into the lumen of the esophagus was effective on the esophageal cancer^{27,35)}. In order to investigate potency of synergistic effect between hyperthermia and BLM in different administration methods of BLM, this study was carried out with intravenous administration and intraluminal administration using perfusion of BLM containing solution.

BLM was exposed to high temperature when it was dissolved in hot saline solution and administered into the lumen of the esophagus. It was demonstrated that the period of time which caused 15% decline of activity of BLM was 39.2 hours when BLM was dissolved in saline solution and the temperature of the solution was maintained at 79.6°C³⁰⁾. It has been reported that BLM is stable to heat. BLM was not inactivated when it was maintained at 20, 40, and 80°C for 2 hours in this study. It was in agreement with the above notion. Accordingly BLM could be administered dissolved in warmed saline solution.

The mechanism of enhancement of the actions of antitumor drugs by hyperthermia probably varies with different drugs, and may relate to 1) membrane permeabilities to drugs, 2) increased thermal activation of cytotoxic process, 3) inhibition of recovery from drug-induced potentially lethal damage, and other undefined mechanisms²²⁾. BRAUN⁴⁾ et al. described that no increase in [¹⁴C] BLM uptake was observed at 43°C over that at 37°C, and this increased cytotoxicity was not correlated with a gross change in cell permeability to BLM. MIZUNO²³⁾ et al. reported that the cellular uptake of ³H-Pepleomycin, a potent antitumor agent derived from BLM, was not increased by hyperthermia and the increased cytotoxicity was not correlated with a change in membrane permeability to the drug. In this study when BLM was administered into the lumen of the esophagus, the more BLM in the esophageal mucosa was observed in the hyperthermic group. It seemed at first glance that the result of this study contradicted those authors' findings.

Anticancer agents can be measured by the following : radioactivity using isotope, ultraviolet rays, colorimetry, titration, bioassay. Bioassay is characterized by the fact that inactivated substance, anabolic substance, and catabolite of drug are not detected. According to UMEZAWA³²⁾ et al., tissue levels of BLM measured by bioassay did not always agree with those measured by radioactivity using ³H-BLM. They explained this by assuming that the rate of BLM inactivation

varied with each organ. Tissues of the organs inactivate BLM. MIYAKI²¹⁾ et al. suggested that in using BLM-sensitive and -resistant rat ascites hepatoma cells the sensitivity to BLM of the 2 hepatomas was dependent on the amount of BLM-inactivating enzyme and thus on the amount of active BLM in the cells. They also described that the difference in permeability to [¹⁴C] BLM was not significant. UMEZAWA³²⁾ et al. extracted the BLM-inactivating enzyme from homogenate of the liver of mice and reported that the optimum temperature of this enzyme was 37°C. In this study BLM level was increased significantly in hyperthermic group when BLM was administered into the lumen of the esophagus. It was measured by bioassay, therefore, it was experimentally proved that the level of BLM in the active form was significantly higher in hyperthermic group. It was conceivable that the activity of BLM inactivating enzyme was suppressed by hyperthermia. LIN¹⁶⁾ et al. also suggested the heat-induced suppression of cellular BLM inactivation activities. It was reported that the increased cytotoxicity of cells to BLM was related to the hyperthermic inhibition of repair of potentially lethal damage induced by BLM^{4,13)}. It was even thought that high level of active BLM caused by heat-induced suppression of BLM inactivating activities was one of the factors in synergistic effect in hyperthermic chemotherapy.

In systemic administration, differences of BLM level of the esophageal mucosa between the hyperthermic group and the control group could not be detected. The excretion into the urine is the main cause for the disappearance of BLM from the body⁷⁾. In this study slight differences of excretion of BLM were observed in each dog. A possible explanation was that the diversity of excretion of BLM in each dog was the major factor in the BLM level of the esophageal mucosa and the discrepancy in temperature was the minor factor in systemic administration. Because the concentration-time-curve suggested that the higher the BLM level of blood was maintained, the more the esophagus could restore BLM.

In intraluminal administration of BLM, it is possible that considerably high concentration of BLM can be maintained constantly for longer periods of time. It was suggested that there might be no side effect because concentration of BLM in blood was so low that BLM in azygos vein blood could not be detected. The effect of hyperthermia on level of BLM in the esophageal mucosa was main and could be presented constantly in this administration method. On the other hand, the effect of hyperthermia on level of BLM was not main and might not necessarily be presented in intravenous administration. Hence, we concluded that local administration (intraluminal administration) may be more effective in hyperthermic chemotherapy of esophageal cancer.

It was reported that although the rise in local tissue temperature led to a significant increase in tissue blood flow, the breaking point in blood flow (the temperature at which blood flow began to decrease) was 41–42°C in tumors and 45°C in some normal tissues³⁾. It was lowered in tumors. Also in this study using a normal dog, blood flow of the esophagus was increased by local hyperthermia. However, multiple regression analysis suggested that there was no correlation between blood flow and BLM level of the esophageal mucosa. It was thought that there was no correlation between the heat-induced suppression of BLM inactivating enzyme and blood flow. Accordingly, it seemed that the result of this study using a normal esophagus could be demonstrated in cancer irrespective of the amount of blood flow.

Experiment 4 was carried out in order to assess histological changes of the carcinoma tissues in rat induced by MNAN following hyperthermic chemotherapy using perfusion of BLM containing warmed saline solution. It was reported that the capillaries and the stroma of transplanted experimental animal tumors were exquisitely thin in comparison with human solid malignant tumors. Such capillaries were unable to withstand to elevated temperatures in the 42–43°C range. Therefore, it has been thought that transplanted experimental tumors are not well suited to serve as models for clinical heat therapy²⁸⁾. Accordingly, in this study the neoplasmas of the esophagus induced by the administration of carcinogen were used. IIZUKA¹¹⁾ et al. and OKAZAKI²⁵⁾ reported that the esophageal carcinoma in rats was induced when MNAN was given in the drinking water at a concentration of 0.003% for 8 weeks. Carcinoma was defined as a tumor which showed invasion to the submucosal layer or to the deeper layer of the esophagus. A tumor which showed slight cellular and nuclear atypism but well presented the basal layer without submucosal invasion or deeper was defined as a papilloma.

No histological changes were seen immediately after hyperthermic chemotherapy. Four days after hyperthermic chemotherapy, typical coagulation necrosis of carcinoma tissues could be observed. It seemed that immediately after hyperthermic chemotherapy, although tissue damage was present, it could not be shown histologically. Coagulation necrosis is commonly produced by cutting off the blood supply¹⁾. In this study degeneration and obstruction of the blood vessels in the tumor was shown. Accordingly it seemed that the disturbance of circulation led to coagulation necrosis. It was thought that these morphologic findings observed in the present study indicated that in addition to the direct effects of heat on tumor cells themselves, the disturbance of the vasculature played an important role in the overall response of a tumor to this treatment. Similar reaction to the normal tissue was observed in the papilloma.

Hyperthermic chemotherapy selectively destructed the carcinoma tissues in this experiment. Therefore, it was indicated that this therapy was valuable for one of the multidisciplinary therapies to cancers.

In experiment 4 the discrepancy between the effect of hyperthermia alone and the effect of hyperthermia with chemotherapy could not be distinguished. Further investigations such as chronological observation of histological changes will be required for clinical use of hyperthermic chemotherapy.

Conclusion

The author studied hyperthermic chemotherapy for esophageal cancer in animal systems. The results were as follows:

- 1) Local hyperthermia of the esophagus could be attained by perfusion of warmed solution using 4-way double balloon catheter.
- 2) BLM was not inactivated by heating.
- 3) Hyperthermia suppressed BLM inactivation activities of the tissue when BLM containing saline solution was administered into the lumen of the esophagus. Accordingly, it was expected that BLM cytotoxicity was enhanced by hyperthermia.

4) Heat-induced suppression of BLM inactivation activities was not elicited in intravenous administration of BLM.

5) Hyperthermic chemotherapy, using perfusion of BLM containing saline solution, selectively destructed the carcinoma tissues in rat induced by carcinogen.

These results led to the following conclusions:

1. Local administration (intraluminal administration) of BLM was more effective in hyperthermic chemotherapy for esophageal cancer. It was thought that one of the mechanisms of this synergistic effect was heat induced suppression of BLM inactivation activities of tissues.

2. As one of the multidisciplinary therapies for esophageal cancer, hyperthermic chemotherapy was valuable.

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References

- 1) Anderson: Pathology, Saint Louis, The C. V. Mosby Company: 91-92, 1971.
- 2) Aukland K, Bower BF, et al: Measurement of local blood flow with hydrogen gas. *Circulation Res* **14**: 164-187, 1964.
- 3) Bicher HI, Hetzel FW, et al: Effects of hyperthermia on normal and tumor microenvironment. *Radiology* **127**: 523-530, 1980.
- 4) Braun J, Hahn GM: Enhanced cell killing by bleomycin and 43°C hyperthermia and the inhibition of recovery from potentially lethal damage. *Cancer Research* **35**: 2921-2927, 1975.
- 5) Cocket AT, et al: Enhancement of regional bladder megavoltage irradiation in bladder cancer using local hyperthermia. *J Urol* **97**: 1034-1039, 1967.
- 6) Coley WB: The treatment of malignant tumors by repeated inoculations of erysipelas—with a report of ten original cases. *Am J Med Sci* **105**: 487-511, 1893.
- 7) Fujita H: Comparative studies on blood level, tissue distribution, excretion and inactivation of anticancer drugs. *Jap J Clin Oncol* **12**: 151-162, 1971.
- 8) Fukuda J: *Rekai enshu: oyo tokei nyumon*. the 19th edition, Nikkan Kogyo Shinbunsha, Tokyo, 1982. (Japanese)
- 9) Hahn GM, Braun J, et al: Thermochemotherapy: Synergism between hyperthermia (42-43°) and Adriamycin (or Bleomycin) in mammalian cell inactivation. *Proc Nat Acad Sci USA* **72**: 937-940, 1975.
- 10) Ichikawa T: Bleomycin, a new antitumor antibiotic—as a specific against the squamous cell-carcinoma. *J Jap Med Asso* **61**: 487-497, 1969. (Japanese)
- 11) Iizuka T, Ichimura S, et al: Esophageal carcinoma in rats induced by N-Amyl-N-Methylnitrosamine. *Gann* **71**: 94-99, 1980.
- 12) Kubota Y, Nishimura R, et al: Hyperthermia using local perfusion. In *Cancer-Hyperthermia* edited by Egawa J, Tokyo, Shinohara shuppan, 106-115, 1982. (Japanese)
- 13) Kubota Y: Hyperthermic therapy of the bladder cancer. 2. The kinetics of cell killing by hyperthermia plus bleomycin. *Nippon Hinyoki Gakkai Zasshi* **72**: 735-741, 1981. (Japanese)
- 14) Kubota Y, Nishimura R, et al: Hyperthermic treatment of bladder cancer: combined hyperthermic treatment with Bleomycin and/or radiation. *J Jap Soc Cancer Ther* **13**: 394-405, 1978.

- 15) Law HT, Pettigrew RT: New apparatus for the indication of localized hyperthermia for treatment of neoplasia. *IEEE Trans Biomed Eng* **26**: 175-177, 1979.
- 16) Lin PS, Peter A, et al: Work in progress: The effect of heat on bleomycin cytotoxicity in vitro and on the accumulation of ^{57}Co -Bleomycin in heat treated rat tumors. *Radiology* **146**: 213-217, 1983.
- 17) Lunglmayr G, Czech K, et al: Experimentelle Untersuchungen über die Wirkung temporärer Hyperthermie auf Blasentumore. *Urol int* **28**: 314-321, 1973.
- 18) Magin RL, Sikic BI, et al: Enhancement of bleomycin activity against Lewis lung tumors in mice by local hyperthermia. *Cancer Research* **39**: 3792-3795, 1979.
- 19) Marmor JB, Kozak D, et al: Effect of systemically administered bleomycin or adriamycin with local hyperthermia on primary tumor and pulmonary metastases. *Cancer Treat Rep* **63**: 1279-1290, 1979.
- 20) Marmor JB: Interaction of hyperthermia and chemotherapy in animals. *Cancer Research* **39**: 2269-2276, 1979.
- 21) Miyaki M, Ono T, et al: Binding of bleomycin to DNA in bleomycin-sensitive and -resistant rat ascites hepatoma cells. *Cancer Research* **35**: 2015-2019, 1975.
- 22) Mizuno S: Chemotherapeutic agents and hyperthermic effects. In *Cancer-Hyperthermia* edited by Egawa J, Tokyo, Shinohara shuppan: 183-199, 1982. (Japanese)
- 23) Mizuno S, Ishida A: Potentiation of bleomycin cytotoxicity toward cultured mouse cells by hyperthermia and ethanol. *Gann* **72**: 395-402, 1981.
- 24) Nakayama K, et al: Clinical experience in bleomycin therapy for esophageal cancer. *JJ Can Therapy Assoc* **4**: 45-46, 1969. (Japanese)
- 25) Okazaki S: Sequential morphological studies of the esophageal carcinoma of rats induced by N-Meythyl-N-Amylnitrosamine. *Nippon Shokakibyo Gakkai Zasshi* **78**: 1-7, 1981. (Japanese)
- 26) Okubo H, Okamoto Y: Microbioassay of antibiotic level in body fluid and tissue. *Clinic J Jap* **31**: 441-447, 1973. (Japanese)
- 27) Shibata G: Studies on supplementary chemotherapy combined with surgical treatment of carcinoma of the esophagus. *Arch Jpn Chir* **44**: 169-198, 1975.
- 28) Sugaar S, Leveen HH: A histopathologic study on the effects of radiofrequency thermotherapy on malignant tumors of the lung. *Cancer* **42**: 767-783, 1979.
- 29) Sugimoto T, Takayama M, et al: Hyperthermo-radiotherapy of bladder cancer. In *Cancer-Hyperthermia* edited by Egawa J, Tokyo, Shinohara shuppan, 309-316, 1982. (Japanese)
- 30) Umezawa H, Ichikawa T: Outline of Bleomycin, Tokyo, Nihonkayaku CO. LTD: 11 (Japanese)
- 31) Umezawa H, Maeda K: New antibiotics, Bleomycin A and B. *J Antibiot* **19**: 200-218, 1970.
- 32) Umezawa H, Takeuchi T, et al: Studies on squamous cell carcinoma. *J Antibiot* **25**: 409-420, 1972.
- 33) Westermarck F: Über die Behandlung des ulcerierenden Cervixcarcinomas mittels konstanter Wärme. *Zbl Gynaek*: 1335-1339, 1898.
- 34) Yamaoka K, Tanigawara U: Introduction to pharmacokinetics using microcomputer, Tokyo, Nankodo, 1983. (Japanese)
- 35) Yasumoto T: Studies on surgical adjuvant chemotherapy for cancer of the esophagus—especially the administration of bleomycin into the lumen of the esophagus using double balloon catheter and iontophoresis. *Arch Jpn Chir* **47**: 135-155, 1978.

和文抄録

食道癌に対する Hyperthermic Chemotherapy に 関する研究—特に BLM 加温水灌流による BLM の食道内腔内投与

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食道癌に対する集学的治療の1つとして温熱化学療法を行うことを目的として動物実験を行った。

1) 温熱は温水灌流で得ることとし、これを行うための装置を作製した。4-way double balloon catheter の2つの balloon によって閉鎖腔を作り、この間に温水を灌流した。温度測定は balloon 間の outlet 側に thermistor の sensor をつけて行った。正常犬において灌流を行い、内腔内の液温および食道各層の温度を経時的に測定した。温度分布は表面的であったが、灌流液の温度を調節することによって食道壁の温度を43°C に保つことができた。1時間の灌流後の組織標本では非可逆的な変化は認められなかった。

2) Bleomycin (BLM) 溶液を20, 40, 80°C でそれぞれ0, 30, 60, 120分間加温し、加温後の BLM 濃度を Bioassay, 帯培養法 (大久保) で測定した。これらの加温温度および加温時間による BLM 濃度には差は認められず、BLM はこれらの加温では不活性化を受けなかった。

3) 正常犬を用いて2-way catheter によって温水灌流による食道の加温を行い、同時に BLM を投与し、BLM 効果の温熱による増強を食道粘膜中の BLM 量を測定することによって検討した。BLM 量は Bioassay で測定した。BLM の投与は全身投与 (静注) と局所投与 (食道内腔内投与) の2つの方法で行い、投与方法による違いも検討した。静注では温熱群 (43°C) でも、対照群 (37°C) でも、BLM 量に差は検出されなかったが、内腔内投与では温熱群において対照群よ

り BLM 量が多く認められた ($p < 0.001$)。奇静脈血を経時的に採取し、血中の BLM 量を測定した。静注後は指数関数的に減少していたが、それぞれのイヌによりパターンの違いが認められ、排泄に差があることが示された。静注ではこの排泄の違いが BLM 量に対して影響を及ぼす主因子であり、温熱効果は小さいものと考えられた。内腔内投与では奇静脈血中の BLM は検出されず、副作用は少ないと考えられた。同時に食道の血流量を水素ガスクリアランス式組織血流計で測定した。温熱群において血流量の増加が認められたが ($p < 0.001$)、血流量と粘膜中の BLM 量との間には相関は認められなかった。

4) N-Methyl-N-Amyl-Nitrosamine を飲料水にまぜて投与し発生させたラット食道癌に温水灌流による温熱化学療法を行い、組織学的効果を検討した。直後では組織学的変化はみられなかったが、4日後では腫瘍が存在していたと思われる部に選択的に凝固壊死の像が認められた。またその周囲の血管に壊死の像が認められ、温熱の癌細胞に対する直接効果だけでなく、血管系を介した抗腫瘍効果の存在が示されたものと思われる。

以上の実験結果より、温熱化学療法施行時には BLM は内腔内投与を行った方がより有効であると考えられた。この際の効果増強の機序として BLM の組織による不活性化が温熱によって抑制されることが考えられた。温熱化学療法は食道癌に対する集学的治療の1つとして有用であると思われる。