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EFFECT OF GLUTATHIONE ON RADIOACTIVITY AND ANTIBACTERIAL ACTIVITY OF ^{14}C-BLEOMYCIN IN ORGANS

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ABSTRACT

I performed an experiment on the relationship between ^{14}C-Bleomycin and Glutathione in vivo, especially on the relationship of radioactivity and antibacterial activity, and the results will be reported here.

1) When Bleomycin and Glutathione were administered simultaneously and animals were killed after 30 minutes, decrease in Bleomycin concentration in every organ was observed. The decrease rate in tumor was especially great (radioactivity: 80.3%, antibacterial activity: 78.4%).

2) When Glutathione is administered 15 minutes after administration of Bleomycin and the animals were sacrificed after 30 minutes, the decrease rate was highest in the skin followed by that in tumor and extremely low in the lung.

3) When Glutathion was given 30 minutes after Bleomycin administration and the animals sacrificed after 60 minutes, the decrease rate of intraorgan Bleomycin concentration was in the order of lung > tumor > skin.

INTRODUCTION

In chemotherapy for malignant tumor, it is ideal that the selected drung has a high sensitivity for tumor cells and infiltrates specifically into tumor tissues in a high concentration, displaying antitumor action and at the same time, the systemically leaked out drug should be rapidly detoxicated and eliminated, reducing the accompanying side effects as much as possible. For this purpose, various methods of administration or inactivation have been tried.

As we have already recognized the usefulness of Glutathione (hereafter GSH in short) in prevention of side effects accompanied by chemotherapy, we have adopted combined administration as a rule. However, in case of combinaiton of Bleomycin (hereafter BLM in short) with GSH, the risk is supposed for anti-tumor action of BLM to be decreased by GSH.

Though there are various discussions on this problem, we have obtained the result suggestive of this from fundamental experiments on the behavior of BLM and GSH in vitro and in vivo[1,2,3,4], and we emphasized that period of administration of the drugs and dosage should be very carefully decided in case of combined administration of BLM and GSH clinically.

EXPERIMENTAL METHOD

Experimental method is the same as that reported in the 4th report, namely, 0.1 ml of

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Table 1. Experimental Method

Group \ Time	0	15'	30'	60'
BLM	BLM →		sacrifice	
BLM + GSH	BLM GSH →		sacrifice	
BLM + GSH	BLM →	GSH →	sacrifice	
BLM	BLM →			sacrifice
BLM + GSH	BLM →		GSH →	sacrifice

*^{14}C-BLM 50mg/kg
GSH 100mg/kg

0.3% acetone solution of 20-methylcholanthrene was applied twice a week to the skin of 200 ddN female mice to produce squamous cell carcinoma. Experiment was started from the 20th week when solid tumor had fully grown up.

Combination of the experiments was as Table 1, where ^{14}C-BLM (product of NIPPON KAYAKU CO., Ltd.) was diluted with non-labelled BLM, and 50 mg/kg of it (1.01×10^7 dpm per a mouse) and 100 mg/kg of GSH were intraperitoneally administered within the respective times. As samples for measurement, the skin, lung and tumor were resected and divided into two pieces from animals sacrificed 30 and 60 minutes after BLM administration.

Regarding radioactivity (abbreviated hereafter as R.A.), after weighing the samples, 2 ml of soluene TM 100 (prepared by Packard Co., Ltd.) was added and dissolved at about 50°C, then 30% H_2O_2 was dropped to decolorize and 15 ml of dioxane series scintillator (7 g of 2.5-diphenyloxazole, 0.3 g of 1, 4-bis-2 (4-methyl-5-phenyloxazolyl)-benzene and 100 g of naphthalene are dissolved into dioxane to make one[1]) was added, and the radioactivity was measured by means of a liquid scintillation counter (product of Packard Co., Ltd. Model

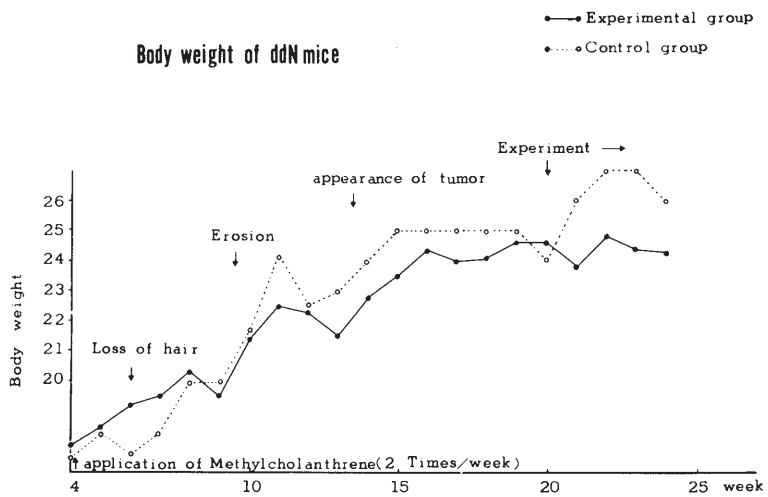

Fig. 1

3380). Correction of quenching was carried out according to the extra standard line source method.

Antibacterial activity (abbreviated hereafter as A.A.) was determined by biological assay (B. Subtilis PCI 219 strain, thin layer disk method) after weighing one half of the sample. In connection with ^{14}C-BLM, one group consisted each of 2 mice. Fig. 1 indicates variations in body weight and change in skin. From around the 2nd week after initiation of drug application, local depilation began, and in the 9th week erosion of skin became observed and the tumor produced in approximately the 13-14th week. Experiment was begun from the 20th week when the tumor had fully grown.

EXPERIMENTAL RESULT

1. Concentration in organs in groups sacrificed 30 and 60 minutes after single administration of BLM. (Tables 2 and 3).

Measurement of R.A. and A.A. in each organ of groups sacrificed 30 and 60 minutes after a single administration of 50 mg/kg of BLM showed rapid decrease in BLM concentration with lapse of time.

Table 2. Radio & Antibacterial Activities of ^{14}C-Bleomycin in Organs following intraperitoneal Administration

Animals were sacrificed after 30 min., 60 min. from BLM 50 mg/kg inj.

activity / group	Radioactivity dpm/mg			Antibacterial activity mcg/mg		
	lung	skin	Tumor	lung	skin	Tumor
BLM only (sacrificed after 30 min.)	225	2331	269	7.4	68.0	5.1
BLM only (sacrificed after 60 min.)	136	347	92.5	4.2	18.9	1.7
increase & decrease ratio (%)	39.6	85.1	65.6	43.2	72.3	66.7

Table 3. Changes in Concentration of active Bleomycin in Organs

group	mcg/g by radioactivity			mcg/g by antibacterial activity			% of active BLM		
	lung	skin	tumor	lung	skin	tumor	lung	skin	tumor
BLM only (sacrificed after 30 min.)	27.9	288.5	33.3	7.4	68.0	5.1	26.6	23.6	15.3
BLM only (sacrificed after 60 min.)	16.8	43.0	29.4	4.2	18.9	1.7	25.0	43.9	5.8

The decrease ratios of R.A. and A.A. were extremely parallel and a difference between organs was observed. The decrease ratio of the group sacrificed after 60 minutes compared with the group sacrificed after 30 minutes were decreases of 39.6% in lung, 85.1% in skin and 65.6% in tumor for R.A. and 43.2% in lung, 72.3% in skin and 66.7% in tumor for A.A.. Thus, the decrease ratios according to organs were in the order of skin > tumor > lung, being highest in skin and lowest in lung.

Transfer of BLM in organs was confirmed by R.A.. These BLM was considered as the active type.

In the lung, little difference was observed in organ active BLM percentage with lapse of time, i.e. 30 minutes after BLM administration, it was 26.6% and after 60 minutes 25%, while in the skin it was 23.6% after 30 minutes and 43.9% after 60 minutes, and in tumor, was 15.3% after 30 minutes and after 60 minutes had rapidly decreased to 5.8%.

2. Organ concentration of the group sacrificed 30 minutes after simultaneous administration of BLM and GSH. (Tables 4 and 5)

Comparison of organ concentration of the group sacrificed 30 minutes after simultaneous administration of BlM and GSH with the group administered only BLM indicated marked decrease in both R.A. and A.A. in the organs of the group administered GSH simultaneously. The decrease ratios in BLM single administration group were 51.1% in lung, 61.7% in skin and 80.3% in tumor, for R.A. and for A.A., the decrease ratios were 50% in lung, 89.3% in skin and 78.4% in tumor, the decrease in skin and tumor being especially.

Table 4. Radio & Antibacterial Activities of ^{14}C-Bleomycin in Organs (BLM 50 mg/kg & GSH 100 mg/kg admin. simultaneously)

Animals were sacrificed after 30 min. after administration

activity / group	Radioactivity dpm/mg			Antibacterial activity mcg/g		
	lung	skin	tumor	lung	skin	tumor
BLM only	225	2331	269	7.4	68.0	5.1
BLM +GSH (simultaneously)	110	892	53	3.7	7.3	1.1
increase & decrease ratio (%)	51.1	61.7	80.3	50.0	89.3	78.4

Tables 5. Changes in Concentration of active Bleomycin in Organs (BLM 50 mg/kg & GSH 100 mg/kg admin. simultaneously)

Animals were sacrificed after 30 min. after admin.

Group	mcg/g by Radioactivity			mcg/g by antibacterial activity			% of active BLM		
	lung	skin	Tumor	lung	skin	Tumor	lung	skin	Tumor
BLM only	27.9	288.5	33.3	7.4	68.0	5.1	26.5	23.6	15.3
BLM + GSH (simultaneously)	13.6	110.4	6.5	3.7	7.3	1.1	27.2	6.6	16.9

Active type BLM percentage was 26.5% by administration of BLM only and 27.2% by combined administration of BLM and GSH in lung, 15.3% by BLM only and 16.9% by combination of BLM and GSH. Thus no marked difference was observed in active type BLM. Hòwever, in the skin, it was 23.6% by BLM only and 6.6% by combination of BLM and GSH, the decrease being 1/3.5.

3. Organ concentration of the group sacrificed 30 minutes after administration of BLM and 15 minutes after administration of GSH. (Tables 6 and 7)

Each organ BLM concentration of the group sacrificed 30 minutes after BLM administration and 15 minutes after GSH administration were compared with those of the group given only BLM. The decrease ratio of the GSH combined group to the BLM only group showed for R.A. no change in lung and 64.3% in skin and 20.4% in tumor. Thus, the

Table 6. Radio & Antibacterial Activities of ^{14}C-Bleomycin in Organs (GSH 100 mg/kg admin. 15 min. after BLM 50 mg/kg inj.)

Animals were sacrificed after 30 min. from BLM inj.

activity / Group	Radioactivity dpm/mg			Antibacterial activity		
	lung	skin	tumor	lung	skin	tumor
BLM only	225	2331	269	7.4	68.0	5.1
BLM + GSH (after 15 min.)	220	832	214	7.0	30.2	2.3
increase & decrease ratio %	2.2	64.3	20.4	5.4	55.6	54.9

Table 7. Changes in Concentration of active Bleomycin in Organs (GSH 100 mg/kg admin. 15 min. after BLM inj.)

Animals were sacrificed after 30 min. from BLM inj.

Group	mcg/g gy Radioactivity			mcg/g by antibacterial activity			% of active BLM		
	lung	skin	tumor	lung	skin	tumor	lung	skin	tumor
BLM only	27.9	288.5	33.3	7.4	68.0	5.1	26.5	23.6	15.3
BLM + GSH (after 15 min.)	27.2	102.8	26.3	7.0	30.2	2.3	25.7	29.4	8.7

decrease ratio in skin was high, while that in lung was very low. As for A.A., the decrease ratios were 5.4% in lung, 55.6% in skin and 54.9% in tumor. Thus, A.A. was decreased to about 1/2 in skin and tumor of the GSH combined group. Active BLM was 26.5% by BLM single administration and 25.7% by GSH combined administration in the lung, and 23.6% by BLM only and 29.4% by GSH combination in skin, showing no marked difference. On the contrary, in tumor it was 15.3% by single BLM administration and 8.7% by GSH combination. Active BLM by GSH combination was observed 20.4% for R.A. and 54.9% for A.A.. Active BLM was decrease to about 1/2 at the time of GSH combination.

4. Organ concentration of the group where GSH was administered 30 minutes after BLM administration and sacrificed 30 minutes after GSH medication. (Tables 8 and 9)

Organ concentrations were compared between the groups where GSH was administered 30 minutes after BLM administration and sacrificed 60 minutes after BLM administration and the group administered only BLM.

Table 8. Radio & Antibacterial Activities of ^{14}C-Bleomycin in Organs (GSH admin. 30 min. after BLM inj.)

Animals were sacrificed after 60 min. from BLM inj.

Group	Radioactivity dpm/mg			Antibacterial activity mcg/g		
	lung	skin	tumor	lung	skin	tumor
BLM only	136	347	92.5	4.2	18.9	1.7
BLM + GSH (after 30 min.)	47.4	497.5	33.0	1.5	4.3	0.8
increase & decrease ratio %	65.1	+43.2	64.3	64.3	77.2	52.9

Table 9. Changes in Concentration of active Bleomycin in Organs
(GSH 100 mg/kg admin. 30 min. after BLM inj.)

Animals were sacrificed after 60 min. from BLM inj.

Group	mcg/g by radioactivity			mcg/g by antibacterial activity			% of active BLM		
	lung	skin	tumor	lung	skin	tumor	lung	skin	tumor
BLM only	16.8	43.0	29.4	4.2	18.9	1.7	25.0	43.8	5.8
BLM + GSH (100 mg/kg) (after 30 min.)	5.9	61.4	6.9	1.5	4.3	0.8	25.4	7.0	11.6

R.A. was decreased by 65.1% in lung and 64.3% in tumor by combined administration of GSH as compared with that of non-combination case. In contrast, in the skin an increase by 43.2% was observed. On the other hand, by examination on A.A., BLM concentration was decreased in every organ by combination of GSH than with BLM only. Namely, the decrease ratios were 64.3% in lung, 77.2% in skin and 52.9% in tumor, all decrease ratio being greater than 50%.

Active BLM percentages in organ in this case were 25% with BLM only and 25.4% with combination of GSH in lung, and showed little difference. In the skin it was 43.8% with BLM only and 7% with combination of GSH, namely a decrease to about 1/6. In tumor it was 5.8% with BLM only and 11.6% with GSH combination, and indicated that in contrast to decrease in both R.A. and A.A. to approximately 1/4–1/3, active type BLM percentage was increased, showing low inactivation rate inside tumor.

5. Comparison of organ BLM concentration between the groups where GSH was administered simultaneously or 15 minutes after BLM administration and the group administered BLM only. (Tables 10 and 11, Fig. 2)

Here, organ concentrations were compared between the group where GSH was given simultaneously or 15 minutes after BLM administration and the group given BLM only, and the decrease ratio compared with the group given BLM only was obtained.

As for R.A., there was a 51.1% decrease in cases of simultaneous administration of GSH, and no change in combined administration of GSH after 15 minutes in the lung, 61.7% decrease in simultaneous GSH administration and 64.3% decrease in combined administration of GSH after 15 minutes in the skin, and in tumor, 80.3% decrease in simultaneous

Table 10. Radio & Antibacterial Activities of ^{14}C-Bleomycin in Organs
(GSH & BLM admin. simultaneously or 15 min. after BLM inj.)

Animals were sacrificed after 30 min. from BLM inj.

activity / Group	Radioactivity dpm/mg			Antibacterial activity mcg/g		
	lung	skin	tumor	lung	skin	tumor
BLM only	225	2331	269	7.4	68	5.1
BLM + GSH 100 mg/kg (after 15 min.)	220 (2.2%)	832 (64.3%)	214 (20.4%)	7.0 (5.4%)	30.2 (55.6%)	2.3 (54.9%)
BLM + GSH 100 mg/kg (simultaneously)	110 (51.1%)	892 (61.7%)	53 (80.3%)	3.7 (50%)	7.3 (89.3%)	1.1 (78.4%)

( ) decrease ratio

Table 11. Changes in Concentration of active Bleomycin in Organs
(BLM only or simultaneoulsy with GSH or before 15 min. after GSH inj.)

Group	mcg/g by Radioactivity			mcg/g by Antibacterial activity			% of active BLM		
	lung	skin	tumor	lung	skin	tumor	lung	skin	tumor
BLM only	27.9	288.5	33.3	7.4	68.0	5.1	26.5	23.6	15.3
BLM + GSH 100 mg/kg (after 15 min.)	27.2	102.8	26.3	7.0	30.2	2.3	25.7	29.4	8.7
BLM + GSH 100 mg/kg (simultaneously)	13.6	110.4	6.5	3.7	7.3	1.1	27.2	6.6	16.9

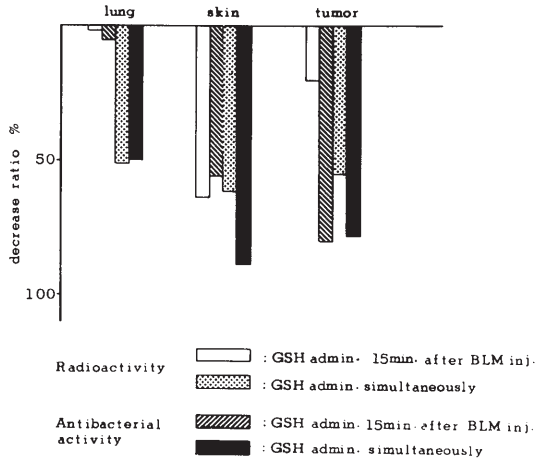

Fig. 2. Decrease ratio of Bleomycin in tumor bearing mice
(BLM & GSH admin. simultaneously or 15 min. after BLM inj.)

combined administration of GSH and 20.4% decrease in combined administration of GSH after 15 minutes. Thus excepting for the skin, in both lung and tumor, decrease in BLM concentration was marked in cases of simultaneous combined administration of GSH.

Regarding A.A., in the lung 50% decrease was observed in case of simultaneous combined administration of GSH, and a 5.4% decrease in case of combined medication of GSH after 15 minutes, in the skin, there was a 89.3% decrease in simultaneous combined administration of GSH and 55.6% decrease in combined administration of GSH after 15 minutes, and in tumor, 78.4% decrease in simultaneous GSH administration and 54.9% decrease in combined administration of GSH after 15 minutes. Comparison of active BLM percentages showed no marked difference due to combined administration of GSH, since it was 26.5% in case of BLM only, 27.2% in simultaneous GSH administration and 25.7% in combined administration after 15 minutes.

In the skin, it was 23.6% in BLM only, 6.6% in case of simultaneous GSH administration and 6.6% in GSH combined administration 15 minutes after, and in tumor, it was 15.3% in BLM only, 16.9% in simultaneous GSH administration, and 8.7% in GSH combined administration after 15 minutes.

Examination of R.A. and A.A. showed decrease in BLM concentration in each organ by combined administration of GSH, and the decrease was especially marked when BLM and GSH were administered simultaneously.

DISCUSSION

There are various disputes on the reaction between BLM and GSH, especially on their behavior in vivo, and in case of combined administration of GSH as a preventive measure for the side effects of BLM clinically, problems on anti-tumor activity of BLM occur. In this respect, we have reported results[1,2,3,4,10] suggestive of inactivation of BLM at a certain dose relation of both drugs when BLM and GSH were administered in various combinations of concentration and time of administration in animals having cancer. However, in this experiment, the point that anti-tumor activity was measured and inferred by antibacterial activity was considered to be problematic.

As micro-measurements of anticarcinogenic agents, there is a biological method[5] as application of antibacterial activity of anticarcinogenic drug and physico-chemical methods such as the radioisotopic method, ultraviolet absorption method, colorimetry, titration, etc. Biological assay has been extensively adopted because of its (sensitivity), sharpness without being interfered by various components in the living body so that we have also adopted this method conventionally. Therefore, we decided to pursue the behavior of BLM and GSH within the body of animals having cancer, from both aspects of radioactivity and antibacterial activity, using ^{14}C-labelled BLM. As a result, it was observed that both were in an extremely parallel relationship.

There have been several reports[5~9] on the concentrations of BLM and GSH in organs and tissues and Fujita[5] reported that in dd mice, BLM was detected in high concentration in the kidney and to a medium degree in the lung, liver, skin and spleen 30 minutes after administration into the tail vein and that elimination from the kidney was relatively rapid among anticarcinogenic agents, while accumulation of minute amounts was observed in the lung, kidney, spleen and skin, 30 minutes after administration of 15 mg of BLM to patients with cancer of the penis and uterus, and at a relatively high level of 0.5–0.8 mcg/ml in cancer tissue.[6] Umezawa[7] measured intraorgan radioactivity and antibacterial activity one hour after subcutaneous injection of ^{3}H-BLMA_2 to mice and supposed weaker inactivation of BLM in lung and skin from the finding that radioactivity indicating presence of BLM was observed in organs except skin and lung, and no antibacterial activity was observed. Ichikawa also demonstrated in the experiment of squamous cell carcinoma in mouse skin, that BLM was difficult to be inactivated inside squamous cell carcinoma from the fact that 15 mcg/g of BLM was present inside cancer tissue and 69.5% of it was active type.

On the other hand, as for GSH distribution inside tissues and organs, there are the experiments by Shiobara[8], Fukuda[9] etc., using ^{35}S-GSH which show marked elevation in concentration in the kidney just as in case of BLM and relatively low distribution in liver, skin, spleen and lung, though distribution of minute amounts was still observed after 24 hours. Considering the above results, it seems that distribution of BLM and GSH has organ specificity and that similarity is observed in their elimination, accumulation, ect.[2]

In our experiment, intraorgan BLM concentration is rapidly lowered with time in case of only BLM administration, and comparison of active type BLM percentage reveals difference between lung and tumor, indicating a difference to exist between organs. This is related with side effect observed clinically, and is interpreted to be an interesting feature. In case of combination with GSH, decrease in intraorgan BLM concentration is lowered in case of simultaneous administration of GSH and BLM than in case where GSH was administered 15 minutes after BLM administration. It is considered proper to understand this decrease in concentration as a result of inactivation of BLM and GSH within the living body

by some reaction. It is supposed to be a reaction in the blood because decrease in concentration is maximal by simultaneous administration but reaction in each organ or cell cannot be denied, as also the possibility for BLM which has lost once its antibacterial activity by combination with GSH to display again antibacterial activity inside the living body as has been pointed out. Thus, it is considered that pursuit on this point will become a subject of investigation in the future.

CONCLUSION

50 mg/kg of ^{14}C-Bleomycin ($1.0_1 \times 10^7$ dpm per a mouse) and 100 mg/kg of Glutathione were administered intraperitoneally in various combinations of administration time to ddN mice having cancer (skin solid squamous cell carcinoma), and Bleomycin concetrations in skin, lung, and tumor were measured by radioactivity and antibacterial activity and from the percentage of active type Bleomycin in each organ, the bahavior of Bleomycin and Glutathione inside the living body was examined.

1) When Bleomycin and Glutathione were administered simultaneously and the animals killed after 30 minutes, decrease in Bleomycin concentration in each organ was observed, and the decrease ratio in tumor was especially great (radioactivity: 80.3%, antibacterial activity: 78.4%). Active Bleomycin percentage in lung and tumor showed no marked difference between single Bleomycin administration and combined administration with Glutathione, but in skin it decreased by a combination with Glutathione to approximately 1/3.5 of that in case of only Bleomycin administration.

2) When Glutathione is administered 15 minutes after administration of Bleomycin and the animals sacrificed after 30 minutes, decrease ratio was highest in skin followed by in tumor and extremely light in lung. In this case, percentage of active type Bleomycin showed no marked difference between combined administration with Glutathione and no combination in the lung and skin, but in tumor, it was decreased to about 1/2 by combination with Glutathione.

3) When Glutathione was given 30 minutes after Bleomycin administration and animals sacrificed after 60 minutes, the decrease ratio of intraorgan Bleomycin concentration was in the order of lung > tumor > skin and active Bleomycin percentage showed little difference in lung and was decreased to about 1/6 in skin but in tumor inactivation rate was lowered.

4) Bleomycin is inactivated by Glutathione given at a certain period of administration. Accordingly, when both drugs are administered clinically, care should be taken on settlement of dosage and time of administration.

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