Effect of deoxidized Glutathione (Tathion) on Excretion of ^{203}Hg-MHP in Kidney

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腎臓における ^{203}Hg-MHP の排泄に及ぼす還元型 Glutathione (Tathion) の効果について

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R.I投与患者の被曝線量を軽減させる対策として,次の3つの方法がある．すなわち,1)短半減期かつ低エネルギーのR.I使用．2)高感度 Scannerの使用．3)投与したR.Iの体外排泄を促進せしめる様な薬剤の使用．

著者らは，^{203}Hg-MHP による腎被曝線量を可及的小ならしめるべく3)の方法で検討した．

すなわち，重金属の解毒作用を有する還元型Glutathione(Tathion)の効果について動物実験と臨床実験と合せて検討した．

動物実験ではTathion 2 mg/g腹腔内注射でBALに匹敵する程，優れた効果を示した．

臨床実験では2 mg/g投与は不可能なので^{203}Hg-MHP 投与後，Tathion 7,000mg＋BAL 200mg投与して ^{203}Hg-MHP の尿中排泄率を検討した．

その結果，対照群（11例）と投与群（10例）での尿中排泄率は，後者がわずかに大きかつた．そこで正常腎機能を有する症例についてさらに検討したところ，週平均尿中累積排泄率は，対照群10%，投与群24.5%で後者は前者の約2倍強の排泄をみせ，尿中排泄率が腎機能に影響されることがわかつた．

以上の事から^{203}Hg-MHP投与の際,腎被曝線量の軽減という見地から使用する還元型Glutathionは，^{203}Hg-MHP の体外排泄に優れた効果を持つているが，臨床的にはその投与量及び方法に問題がある．併し，^{203}Hg の代りに ^{197}Hg-MHP,高感度 Scaner 等を組合せて使用すれば，このような還元型 Glutathion の使用も充分腎被曝線量の軽減に役立つものと思われる．

Introduction

Recently radiological diagnosis and treatment have been widely available and, consequently, exposure of patients to radioactive substances for their medical treatment has been becoming a great issue. Since radionuclear medicine has been rapidly developed and followed by an increased usage of radioi-

sotope (RI), the problem on the radioexposure of patients should be seriously considered.

This problem was already picked up as a topic in a Symposium and discussed in the General Assembly of Medicine (held at Nagoya) last year.

There exist three adequate steps to reduce the amount of exposure of the patients to radioisotope (RI) as follows:

(1) Usage of RI with low energy and short half-life.

(2) Usage of high sensitive scanner.

(3) Usage of drugs to stimulate the excretion of administered RI.

(1) and (2) steps still require some time to be taken up in developing the investigation and in solving the financial problems.

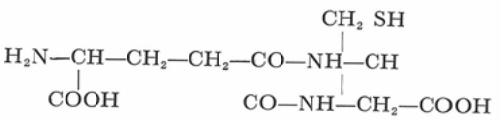
Physical half-life of ^{203}Hg-1-acetomercuric-2-hydroxypropane (MHP) is 46.9 days and its biologic half-life is 140 days. Its critical organ is considered to be kidney and RI is accumulated in the kidney for a long period.

According to the various reports, the renal dose per 100 μCi of ^{203}Hg-MHP is around 70 to 90 rad, which is not small at all, comparing with other RI (Table 1).

Table 1. Report of various authors on the renal dose

No.	authors	^{203}Hg	^{197}Hg
I	Wagne, et al. (1964)	about 70 rad per 100 μCi (kidney of one side)	about 7 rad per 100 μCi (kidney of one side)
II	Krost, et al. (1965)	about 30.4 rad per 25 μCi (kidney of both sides)	about2.5 rad per 50 μCi (kidney of both sides)
III	Fisher, et al. (1965)		13 rad per 400 μCi (kidney of one side)
IV	Croll, et al. (1965)	about 76 rad per 100 μCi (kidney of one side)	
V	Tatsuno, et al. (1967)	91.6 rad per 100 μCi (kidney of one side)	

Fig. 1. Deoxidized Glutathione ("Tathion" as the tradename)



γ-L-glutamyl-L-cysteinyl-glycine

The authors have been trying thereupon to minimize the renal dose as much as possible by administering the drugs to stimulate the excretion of ^{203}Hg-MHP.

Generally, BAL is widely used in detoxication of so-called mercurial substance. However, it has a disadvantage that toxicity is quite high in itself.

Deoxidized glutathione (Fig. 1) is capable to detoxicate the SH-poison as BAL is. The ability is still less due to the chemical form of monothiol. Deoxidized glutathione exists in living body and its toxicity is so low that there might be a possibility to reduce the renal dose by the administration in larger dosage. From this point, the present study was carried out and the results are described here

in detail.

Deoxidized glutathione used in this study was furnished by Yamanouchi Pharmaceutical Co., "Tathion"as the tradename.

(I) Basic experiment on mice

Female ICR-JCL strain mice weighing about 30 g, 10 weeks after the birth, were selected in this study and received ^{203}Hg-MHP injection intraperitoneally. Radioactive distribution in the body was investigated 3 days and 5 days after the injection. After the injection of ^{203}Hg-MHP, the effect of BAL and Tathion on the excretion of radioactive Hg from the kidney as a critical organ was further evaluated.

Concerning the radioactive distribution in the body, relative specific activity (R.S.A.) according to Watanabe[1] was assessed in this experiment. (Fig. 2)

Fig. 2. Relative Specific Activity (R.S.A.)

$$\text{R.S.A.} = \frac{\dfrac{\mu\text{Ci of the tissue}}{\text{wet weight of the tissue (g)}}}{\dfrac{\mu\text{Ci administered}}{\text{body weight (g)}}} (\times 100)$$

I. Watanabe: Nippon Acta Radiologica, 19, 1303, 1959.

Table 2. Distribution of ^{204}Hg in mouse after intraperitoneal injection of ^{204}Hg-MHP (R.S.A.)

Organ \ Days	3	5
Thyroid Gland	1.3	0.8
Heart	0.6	1.2
Lung	0.6	1.3
Thymus	0.6	1.9
Liver	51.5	21.4
Kidney	66.4	49.3
Stomach	1.5	0.5
Spleen	0.9	1.1
Pancrease	3.7	1.4
Intestine	11.7	4.8

(Method)

15 μCi of ^{203}Hg-MHP (0.08 mCi/mg) was administered intraperitoneally to each mouse and radioactive bodily distribution was investigated 3 days and 5 days after the injection. Each experimental group is consisted of 30 mice and the group receiving ^{203}Hg-MHP alone is considered as a control. BAL administered group was formed in such a way that BAL (0.015 mg/g) was given in the dorsal muscle immediately, 2 hours and 6 hours after the injection of ^{203}Hg-MHP[2]. Tathion was administered in a different dose of 0.02 mg/g, 1 mg/g, and 2 mg/g intraperitoneally immediately after the injection of ^{203}Hg-MHP.

Animals were sacrificed 3 days and 5 days after the administration of ^{303}Hg-MHP and ten tissues were removed such as thyroid, heart, lung, thymus, liver kidney, stomach, spleen, pancreas and intestine. Radioactivity was assayed in the form of fresh tissue by Shimadzu Well-type scintillation counter.

(Results)

1. Radioactive distribution in the body.

Bodily distribution after the intraperitoneal injection of ^{203}Hg-MHP is illustrated in Table 2 in the course of time. 3 days after the injection, R.S.A. revealed the highest value, 66.4 in the kidney, 51.5 in the liver, 11.7 in the intestinal and other tissues were low and negligible. 5 days after the injection, R.S.A. value in the kidney decreased to 49.3, but still remained highest. R.S.A. in the liver decreased to the

below half and that in the intestine also fell to negligible value. These results indicated that critical organ was still the kidney.

2. Dosage of Tathion.

R.S.A. in the kidney obtained from each group 5 days after the injection was illustrated in Table 3.

Table 3. R. S. A. of kidney 5 days after administration

Group	Control	BAL	Tathion-group		
			2 mg/g	1 mg/g	0.02 mg/g
R. S. A	49.3	2.2	6.6	34.9	45.3

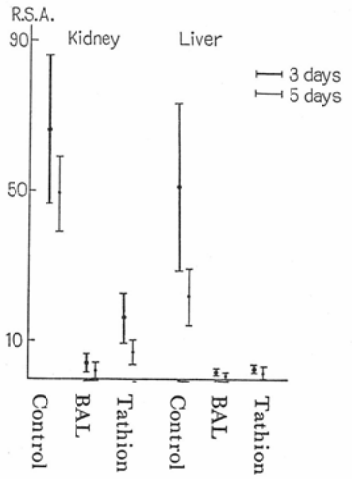
It is 49.3 from the control group and 45.3 from 0.02 mg/g Tathion administered group which was similar to the control value. The more dosage of Tathion was increased, the lower R.S.A. was obtained. Namely R.S.A. was 6.6 in 2 mg/g administered group which was fairly close to the value obtained in BAL administered group.

These results indicated that the effect of Tathion on the renal excretion of ^{203}Hg-MHP appeared to be matched for that of BAL, depending upon the dosage.

3. Radioactive distribution in the liver and the kidney about the control, BAL administered and Tathion 2 mg/g administered group.

R.S.A. in the kidney and liver 3 days and 5 days after injection of ^{203}Hg-MHP was expressed in Fig. 3. It showed high level in the control kidney and still showed only slightly lower level 5 days after the injection. It is quite low level in BAL administered group and it is also low in Tathion 2 mg/g administered group. R.S.A. decreased even in the control liver as the days advanced. It was quite close to 0 in other two groups, and no appreciable amount of radioactivity seemed to be left in the liver.

Fig. 3. R.S.A. of kidney and liver 3 and 5 days after administration



These results indicated that the effect of Tathion in a dose of 2 mg/g on ^{203}Hg-MHP excretion appeared to be appreciable and it was as effective as BAL in the liver and the effectiveness seemed to be close to that of BAL in the kidney.

(II) Clinical application

From the previous data, the effect of Tathion on the renal excretion of ^{203}Hg-MHP was elucidated and still the dosage is to be determined. No effectiveness was obtained in a small dose and large dose appeared to be required.

2 mg/g administration to animals were almost equal to 120 g/60 kg of human adults, which seemed to be too large to give. The authors arbitrarilly selected 1000 mg of daily Tathion administration for the period of 7 days, resulting in 7000 mg as a total in a drip infusion. Besides, 100 mg of BAL was given twice intramuscularly 1 hour and 6 hours after the intravenous injection of ^{203}Hg-MHP to reinforce the effect of Tathion.

After the injection of ^{203}Hg-MHP, urine volume was measured daily for 7 days and urinary excretory rate was calculated from the factor based upon the volume to compare with that of control group. The state of renal function is possibly related to the ^{203}Hg-MHP excretion and therefore the urinary protein was checked. P.S.P. and Fishberg's concentration test were performed on cases as many as possible.

(Results)

Total numbers of cases were 21, in which 11 cases were control, and 10 were treated group. (Table 4, 5)

Table 4. Control group

No.	Name Sex age	Disease	Kidney function tests			Urinary excretionrate %	
			Urine allbumin	P.S.P. 15min	Fishberg's test	Average /day	Total /week
1	M. M. ♀ 50	Breast Cancer	(−)	27%	1017	2.7	19.1
2	M. O. ♀ 24	Acute myelocytic leukemia	(+)			1.3	8.9
3	Y. K. ♂ 23	Hepatitis chr.	(−)	28%	1028	1.1	8.0
4	S. O. ♀ 68	Livercirrhosis	(−)	33%	1038	1.2	8.7
5	H. H. ♂ 61	Stomach Ca.	(+)			1.6	11.2
6	T. T. ♀ 49	Hypoplastic anemia	(−)			1.8	12.9
7	K. I. ♂ 67	Bronchogenic Ca.	(±)	12%	1015	0.8	5.7
8	N. S. ♀ 73	Hodgkin's Disease	(−)			2.0	14.3
9	K. N. ♀ 48	Hypertension	(±)	20%	1014	0.6	3.9
10	A. H. ♀ 27	Anemia	(−)			1.4	9.6
11	H. N. ♂ 20	Hepatitis chr.				0.9	6.1

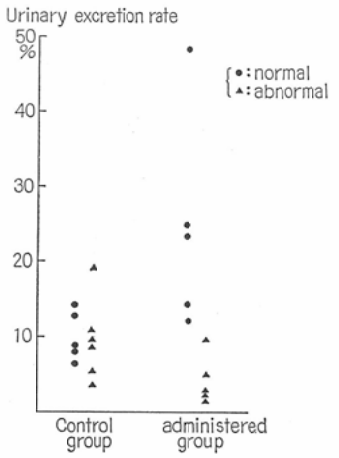
They were 13 males and 8 females, and also they can be divided by ages such as 6 cases under 30, 8 cases between 30 and 60, 7 cases over 60, indicating slight inclination of cases to old age. Mean daily excretory rate was 1.4% and mean weekly accumulated excretory rate was 9.9% in the control group. They were 2.6% and 14.4% respectively in the treated group which were slightly elevated.

Urinary excretion of ^{203}Hg-MHP seemed to be highly affected by the state of renal function. Excretory rate was plotted against the presence or absence of renal dysfunction in Fig. 4. Mean weekly accumulated excretory rate of 5 control and 5 treated cases with satisfactory renal function was 10% and

Table 5. Administered group

No.	Name sex age	Disease	Kidney function tests			Urinary excretion rate %	
			Urine allbumin	P.S.P. 15min.	Fishberg's test	Average /day	Total /week
1	H. K. ♂ 31	Seminoma	(−)			3.6	24.9
2	A. K. ♂ 31	Hepatitis chr.	(−)	45%	1020	6.9	48.3
3	K. T. ♂ 39	Idiopatic portal hypertension	(−)			2.0	14.3
4	M. T. ♂ 77	Gastric Ul.	(−)	6%	1020	0.7	5.1
5	K. M. ♂ 67	Esophageal Ca.	(+)	30%	1019	1.4	9.8
6	H. S. ♀ 26	Hepatitis chr.	(−)			1.7	12.1
7	H. K. ♂ 29	Duodenal Ul.	(−)	38%	1028	3.3	23.1
8	F. T. ♂ 76	Hepatitis chr.	(−)	20%	1010	0.3	1.7
9	M. F. ♂ 49	Hepatitis chr.	(−)	13%	1018	0.3	2.0
10	Y. N. ♂ 57	Bronchogenic Ca.	(+)			0.4	3.0

Fig. 4. Urinary excretion rate/week in two groups with normal and abnormal renal function



24.5% respectively. It was more than doubled in the treated group.

Discussion

The amount of exposure to the radioisotope has caused serious discussions and three adequate steps are considered to be taken up to minimize the exposure as already mentioned, namely, (1) to use RI with low energy and short half-life, (2) to use high sensitive scanner allowing to administer small dose of RI and (3) to use the drugs to stimulate the excretion of administered RI.

The excretion of ^{203}Hg-MHP which is used at present in assessing the function of spleen is quite delayed and remains in the kidney, as a critical organ.

Various reports[3)4)5)6)7)] on the renal dose are summarized in Table 1. The values were estimated by using Quimby's equation[8)], but were not in good agreemen due to the different factors. The renal dose appeared to be minimal in ^{197}Hg, but that of ^{203}Hg-MHP was about 10 times as much as in

^{197}Hg, which was not small at all.

The authors tried to minimize the renal dose to ^{203}Hg-MHP by taking the third step which was previously mentioned.

SH toxin of heavy metals such as mercury is considered to be derived from the combination with SH radical of deoxidized glutathione (GSH) to form mercaptide blocking the enzymatic action of SH radical, consequently rendering the impairment of cell metabolism and function and leaving the hazardous action on the living body.

Each reaction is reversible and the reactions favors dithiol. BAL is also a kind of dithiol which is frequently used for the detoxication of mercury. Dithiol exerts the effect on detoxication in such a way that it reacts with mercury which has been bound to SH radical and forms a ring derivative with the low dissociation constant.

GSH is a sort of tripeptide which is composed of glutamic acid, cystine and glucine. This substance also possesses the detoxicating activity on SH poison as BAL does. However, it is a monothiol which is quite different form BAL, dithiol. Its detoxicating action is considered to fairly weak. It forms mercaptide with mercury. But its binding force is so weak and dissociation is so strong that dissociated mercury would be bound to other SH radical, resulting in potentiation of blocking the enzymatic action[2)9)].

GSH exists in living body originally and its toxicity is markedly weak. From this point the idea emerged that detoxicating effect as strong as BAL might be obtained depending upon the dosage. Thus, the effect of GSH was investigated.

The dosage of Tathion (GSH) exerting the detoxicating effect was evaluated in comparison to that of BAL. The amount of BAL and the way of its administration were followed according to Tomii[9)]. This was based upon the following results. The amount of renal GSH was markedly decreased after the mercury was administered. Such mercury administered mice were subjected to receive BAL (15 mg/kg) injection hypodermically on the back three times as a total, immediately after, 2 and 6 hours after mercury administration, and the renal GSH content was found to be elevated.

According to the other reports[10)11)], mercury was found to tend to be accumulated in the kidney more than any other tissues and similarly ^{203}Hg-MHP was considered to have its critical organ as the kidney[2)]. The authors' experiment also revealed that the critical organ of ^{203}Hg-MHP was the kidney.

R.S.A. in the kidney of BAL administered group was close to 0 in 5 days after the administration and BAL was found very effective in detoxication by this way of administration.

Concerning the dosage of Tathion, small dosage was the least effective in the author's experiment as was expected, but moderate effectiveness was obtained as the dosage increased. There appeared to be a limit of increasing. LD_{50} of Tathion given intraperitoneally was 4 mg/g. Tathion was administered in a dose of 2 mg/g which was just a half of LD_{50} and was observed to possess the detoxicating activity as effective as BAL.

However, clinical application of this dosage has some problems to be clarified. 2700 mg of BAL should be theoretically used for obtaining the marked effectiveness to the adult with 60 kg of body weight. Likewese, Tathion should be administered in a dose of 2 mg/g, that is, 120,000 mg (120 g) as a total. These amounts were too much to be used clinically. BAL in such a big dosage was toxic and dangerous. Tathion should be used in a dosage of 1,200 ampules of 100 mg, which was commercially available. It appeared to be unreasonable to use such a large amount from the point of toxicity as well as financial

situation.

From these considerations, urinary ^{203}Hg-MHP excretory rate was investigated after the administration of 7,000 mg of Tathion with the aid of 200 mg BAL.

According to the results of Wagner[3], daily urinary excretory rate was about 0.5% and this value was fairly constant.

The authors determined the excretory rate from 11 controls and 10 treated group. Daily and weekly excretory rate was 1.4% and 9.9% respectively in the control group. They were 2.6% and 14.4% which figure were slightly elevated in the treated group. The status of renal function would be thought to be related to urinary ^{203}Hg-MHP excretory rate. Therefore, the cases with normal renal functions were selected to perform this kind of study. Weekly mean accumulated excretory rate in the control and treated group was 10% and 24.5% respectively. The figure of the treated group was more than twice as high as that of control group. This was indicating that excretion of ^{203}Hg-MHP was closely related to the status of renal function.

With regard to decreasing radioactive exposure of the kidney to ^{203}Hg-MHP, the administration of Tathion could be said to be favorable to some extent. Tathion possessed fairly marked effect on the excretion of ^{202}Hg-MHP from the kidney, but dosage and the way of administration was still to be elucidated. Therefore it seemed to be natural that sufficient reduction could not be obtained clinically for the renal dose because of using the dosage 1/15 as much as that used on mice.

The application of Tathion to the reduction of renal dose close to 0 could be well provided in combination of forementioned three steps. Renal dose to ^{197}Hg-MHP per 100 μCi is almost 1/10 of ^{203}Hg, that is, about 7 rad, and high sensitive scanner could diminish the dosage. Furthermore, the application of Tathion could minimize the renal dose.

In conclusion, deoxidized glutathione, Tathion, was valuable enough to be administered clinically.

Summary

The effect of deoxidized glutathione was studied as one of means to minimize the renal dose to ^{203}Hg-MHP.

Basic experiment on mice revealed that deoxidized glutathione, Tathion, exerted marked effect as much as BAL, in a dose of 2 mg/g intraperitoneal injection.

Clinical application of 2 mg/g Tathion appeared to be impossible due to its large amount. Therefore, 7,000 mg Tathion with 200 mg BAL was applied to determine the renal excretory rate of ^{203}Hg-MHP after the administration of ^{203}Hg-MHP. The renal excretory rate was found to be slightly greater in the administered group than in the control group. It was also investigated on the cases with normal renal function, and weekly mean urinary accumulated excretory rate was observed to be 10% in control and 24.5% in the administered group, which was almost more than doubled. Urinary excretory rate was found to be affected by renal function.

From forementional evidences, it could be concluded that deoxidized glutathione possessed the beneficial effect on the excretion of ^{203}Hg-MHP from the kidney to reduce the renal dose. However, in the clinical cases, dosage and the way of administration was still to be determined. It could be also assumed that deoxidized glutathione would be useful for the reduction of renal dose by using ^{197}Hg-MHP instead of ^{203}Hg, and with high sinsitive scanner.

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