Studies on Radiorestorative Chemicals and Their Clinical Application*

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放射線障害回復剤とその臨床的応用に関する研究

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われわれは，10余年前より，Cysteine, Cysteamine, AET, MEA, Serotonin 等多数の放射線防護物質について，その防護効果と作用機序に関しての研究を行なつてきた．同時に，これら防護物質を臨床的に放射線治療患者に使用する試みを行なつてきたが，これらの薬剤の強い毒性のために，臨床的応用がかならずしも成功しなかつたことと，また実際に臨床的に使用する場合には，放射線照射前に薬剤を与えた場合のみに防護効果があらわれる上記“放射線防禦剤”よりは，照射後に投与した時にも防護効果をあらわす“放射線障害回復剤”の方が重要であるという観点から，防護物質に関するわれわれの研究方向は，主として回復促進剤の研究にむけられ，Uridine monophosphate (UMP), Vitamin B_6 ならびに Taurine が，放射線照射後の投与により，放射線照射マウスの生存率を高めるといつた障害回復効果を有することを見出した．

これら薬剤の回復促進効果およびそれらの作用機序について検討し，放射線誘発白血球減少症に対する臨床的応用について記述した．

Since 1951, I, Dr. Fukuda, have investigated the effects of radiation on the liver function as a part of studies on radiation hazard induced by an atomic bomb. As a moment that we have examined the therapeutic effect of liver protective substances such as glucose and l-methionine on radiation damage to the liver and found the amount of reduced glutathione in blood and the liver being decreased after exposure, we commenced to study widely radiation protection and restoration in human beings. With support of a grand from the Scientific Research Funds of the Education Ministry, I have organized a research group with investigators in this field in Japan between 1959 and 1965 (4). A great number of chemicals were tested regarding their protective effects on radiation injury and cysteine, glutathione, cysteamine, AET, MET and serotonin were proved to be effective. However, our efforts have been directed to the studies on radiation restoration from the following reasons.

1. Since in most cases radiation injury in man is occurred unexpectedly, radiorestorative chemicals which are effective after exposure are clinically more important than radioprotective chemicals which exert effects only when administered shortly before irradiation.

2. Most of these radioprotectants are effective in the very closed dose ranges between LD_{50} of these

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substances and the effective dose as a protectant. Therefore their application to clinical uses are dangerous due to their toxicity.

Uridine Monophosphate

In screening radioprotective and restorative substances, we have found that ATP is effective even when administered after exposure, although its effect is not prominent. From the view point that since radiation inhibits the synthesis of nucleic acids, nucleosides and nucleotides may be effective as a material of nucleic acids in restoration of radiation injury, we have examined uridine monophosphate (UMP) and cytidine monophosphate(CMP)with experimental animals in 1964 and found that not only prophylactic but also therapeutic effect upon irradiated mice was observed by an administration of UMP (5).

Table 1. Effects of UMP and CMP on the survival rate of irradiated mice

	dose (R)	% Survival rate	P-value
2 mg UMP 15 min. before irradiation	600	48.0	0.15–0.1
5 mg UMP 15 min. before irradiation	600	62.0	0.01–0.005
2 mg CMP 15 min. before irradiation	600	40.0	0.45–0.4
5 mg CMP 15 min. before irradiation	600	50.0	0.15–0.1
Control groups	600	38.0	
5 mg UMP 15 min. before irradiation	750	30.0	<0.0005
5 mg UMP 5 min. after irradiation	750	20.0	<0.0025
5 mg CMP 15 min. before irradiation	750	0	
5 mg CMP 5min. after irradiation	750	0	
Control groups	750	0	

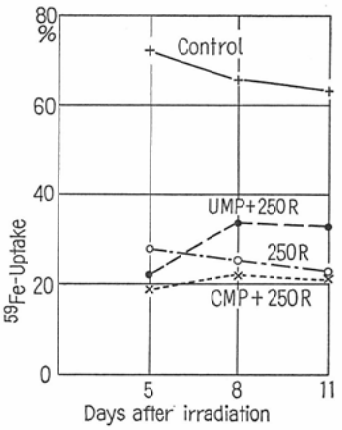


Fig. 1

The ^{59}Fe-uptake of rats to which UMP was given before irradiation was higher than that of irradiated control rats. On the other hand, the ^{59}Fe-uptake of rats to which CMP was delivered prior to exposure was not increased.

At that time, a member of our group Dr. Sugahara investigated the difference in the effectiveness of 3'– and 5' nucleotides of RNA and found that 3'-nucleotide has radiorestorative effect (8).

Vitamin B_6

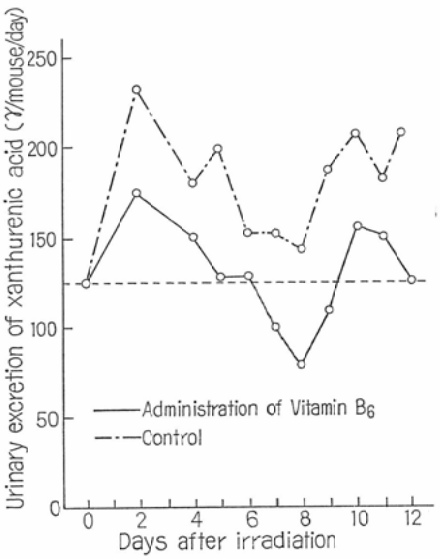
Since 1962 Dr. Abe has researched radiation injury in mammals from the view point of radiation induced disturbance of biochemical processes, especially of tryptophan and cysteine metabolisms. He has demonstrated that amino acid metabolisms in man and animals are strongly disturbed by irradiation (1, 2, 3, 6) and radiation-induced damage to pyridoxal phosphate plays a role in these metabolic disturbances (1, 2).

One of the actions of pyridoxine as a coenzyme is the decarboxylation of a particular amino acid, being involved in many enzymatic reactions concerned with the synthesis and breakdown of protein and amino acids. As one of the biochemical responses to exposure of animals, it has been shown that xanthurenic acid (XA) increases in urine after irradiation and this phenomenon results from the disturbance of pyridoxal phosphate which catalyzes the decarboxylation of 3-hydroxy-kynurenine to 3-hydroxy-anthuranilic acid. Therefore, this pathway is blocked by irradiation and XA is excreted as a shunt product. These considerations raise the question whether the administration of pyridoxine after irradiation normalizes the disturbances of tryptophan metabolism.

As Fig. 2 shows, urinary XA excretion increases markedly in nonmedicated irradiated controls, while the amount is fairly normalized by the administration of pyridoxine after exposure.

The mortality rate of mice to which pyridoxine was given 10 times at 2 day intervals after irradiation decreases significantly. Unexpectedly however, a single dose of pyridoxine exerts no therapeutic effect. Nor does pyridoxal-5-phosphate. (Tab.2).

From these experiments it is considered to be basically important for the understanding of the funda-



Urinary excretion of xanthurenic acid after irradiation with 550R. 6mg of pyridoxine was given i.p. on the 1st, 3rd and 5th post exposure days.

Fig. 2

Table 2. Effect of B_6 and pyridoxal-5-phosphate on the mortality rate of irradiated mice

Treatment	Number of mice used	% mortality	P-value
B_6 6 mg 5 min after irradiation	40	37.5	0.2 –0.25
B_6 6 mg 10 times at 2 day intervals after irradiation	47	25.5	0.01–0.0125
Pyridoxal-5-phosphate 3 mg 5 min. after irradiation	40	37.5	0.2 –0.25
Non-treated, irradiated controls	39	48.8	

mental concept of radiation injury and for the establishment of its treatment to determine which part of the biochemical changes have an intimate relationship with radiation injury.

Taurine

The increase in urinary excretion of taurine (2, 3) is one of the early manifestation of exposure of animals to whole-body irradiation. The origin of taurine excreted after exposure is not clear. However, several works indicate that radiation-induced excretion of taurine is accounted for by damage to lymphoid tissue and leucocytes which contain substantial concentration of taurine. From Soupart's works (8), the concentration of free taurine in leukocytes and thrombocytes is several hundred times as high as in plasma and amounts to about 25% of the total volume of intracellular taurine. The evidence that leukocytes contain such a high concentration of this compound leads to an assumption that it might be important as a structural component of leukocytes. Therefore the possibility exists that radiation-induced loss of taurine from leukocytes may lead to structural damage, though the possibility that the damage precedes and may be regarded as the cause of this phenomenon has not been eliminated. Then we would like to show you our works on restorative effect of taurine in radiation injury.

Table 3. Effect of taurine on the fraction of mice surviving for 30 days postexposure (Hannover strain)

Time of taurine (1 mg) injection	Fraction of mice surviving for 30 days		P value
	%		
Nontreated, irradiated controls (590 R)	35.6	21/59	
Day 1 before irradiation with 590 R	39.3	11/28	$P>0.5$
Day 1 after irradiation with 590 R	48.3	14/29	$P>0.2$
Day 2 after irradiation with 590 R	69.0	20/29	$P<0.01$
Day 3 after irradiation with 590 R	48.2	13/27	$P>0.2$
Day 4 after irradiation with 590 R	72.4	21/29	$P<0.01$
Day 5 after irradiation with 590 R	68.9	20/29	$P<0.01$
Day 7 after irradiation with 590 R	67.9	19/28	$P<0.01$
Day 9 after irradiation with 590 R	72.4	21/29	$P<0.01$
Days 3, 4, 5, 6, 7 and 8 after irradiation with 590 R	66.0	33/50	$P<0.01$

(Melching H.J., Abe, M. and Streffer, C. 1964 (6).)

One miligram of taurine was injected intraperitoneally on the first day prior to exposure or on the 1st, 2nd, 3rd, 4th, 5th, 7th or 9th postirradiation day. One mg of taurine was given daily from the third to eighth postirradiation day.

In this experiment it was demonstrated that the fraction of mice surviving for 30 days postexposure increased when taurine was given after irradiation.

Then, we would like to demonstrate the results of clinical application of taurine to the treatment of leukopenia induced by radiotherapy.

Taurine was given to patients during radiotherapy, beginning on the day when the leukocyte count was depressed below 4,000 per cmm. A dose of 750 mg of taurine was given orally 4 times a day. During taurine administration, radiotherapy was continued with no change of dose. The examination of blood cell count was carried out once a week until the completion of radiotherapy, after which no further examination was made, because spontaneous repair in the hematopoietic system may occur soon after radiotherapy stops. The number of patients treated with taurine was 81. The control group includes 41 patients to whom 20% glucose 20 ml, Vitamin B_1 10 mg, B_2 10 mg, and C 100 mg were injected during radiotherapy beginning also on the day when the leukocyte count was depressed below 4,000

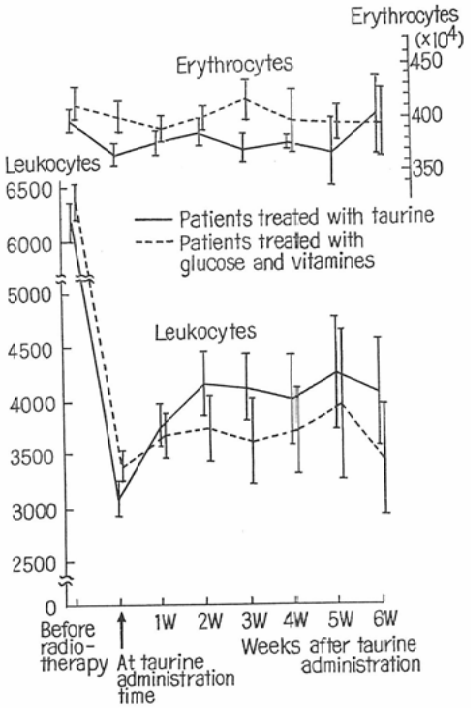


Fig. 3

As Figure 3 shows, the leukocyte count increased soon after an administration of taurine and reached maximum value 2 weeks later. Thereafter the leukocyte level did not decrease, although irradiation was continued with no change of dose. In the control group, no increase in leukocyte count was found, al-

though no further decrease in leukocytes was observed.

Conclusion

We demonstrated radiorestorative substances which have been investigated in our clinic. We think these substances will contribute to the treatment of radiation injury which will be called in problem with an increase in the practical use of atomic energy in the various fields and we believe that we could throw a light on this research field through our works which have been performed from a stand point of radiation-induced metabolic disturbances in mammals.

Summary

Since more than 10 years ago, a large number of radioprotective substances such as cysteine, cysteamine, AET, MEA and serotonin have been examined in our clinic as regards their efficacy and the mechanisms of their action. The clinical use of these substances in the radiotherapeutic field was also investigated. However the results were discouraging chiefly because of their toxisity. From the clinical point of view, substances which are effective even if they are given after irradiation—"radiorestorative chemicals"—are more important than the "radioprotective substances" which are effective only when administered shortly before exposure. Therefore our efforts have been directed to research radiorestorative substances and we found that uridine monophosphate, vitamin B_6 and taurine have radiorestorative effects in the sense of an increase in the survival rate of irradiated mice when they are given after irradiation.

The efficacy of these chemicals and the mechanisms was discussed. Clinical application of these substances to radiation-induced leukopenia also was reported.

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