特 別 掲 載

On the modificatiou of the acute lethality in mice following whole body X-irradiation by several vasoconstricting agents

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放射線によるマウス致死作用に対する各種血管収縮剤の防護効果

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X線全身照射によるマウスの致死作用に対する種々の血管収縮剤の防護効果を比較検討した．その結果次のような実験成績を得た．

最も防護効果の著明な薬剤は phenylephrine, naphazoline, tetrahydrozoline 及び norepinephrine であつた．cocain 及び epinephrine にもかなりの防護効果を認め得た．coffein 及び phenylethylamine にはわずかしか防護効果を認め得なかつた．ephedrine はほとんど無効であつた．このような血管収縮剤の放射線防護効果には薬剤の適量があり，その適量よりも多過ぎても少な過ぎても効果が劣る．phenylephrine 投与による放射線防護効果については，照射前5～10分投与が最も効果的であり，30分前投与でも防護効果は減弱し，60分前投与では防護効果はほとんど消失した．

これら血管収縮剤の放射線障害防護機転として血管収縮による組織の酸素欠乏について考察を加えた．

Introduction

In the present days stress is given to the importance of studies on the protection and treatment of radiation injury. Regarding the mechanism of radiation injury a chemical theory[3)5)] has come to be favorably maintained. The theory asserts that radiation gives rise to the production of free radicals in the presence of oxygen, leading to the decomposition of certain substances in the living body. On the basis of this chemical theory attempts were made to protect radiation effect by inhibiting the chemidal reactions of free radicals. As a result, cystein[14)20)21)22)], gluthalion[4)], cystemaine[1)2)], cystamine[1)2)], AET[6)] and MEG were found to possess protective effect against radiation. These agents belong to sulphhydrile compound. Bacq[1)] et al report that amines which does not belong to the sulphhydrile compound and shows a sympathomimetic action as norepinephrine also have protective effect against radiation. The radio-protective effect is observed in epinephrine and pitressin by Gray et al[10)], in serotonin by Langendorf et al[15)], and in methoxamine by Smith et al[24)]. It is to be noted that these agents are possessed with vaso-constricting action. Consequently, in the present study several vasoconstricting compounds were examined as to their radio-protective effect in reference to vasoconstriction.

Method

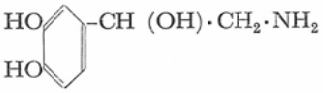
Male mice of Na2 strain weighing approximately 20 g were randomly grouped for irradiation. Five

minutes after intraperitoneal injection of the tested pharmacological agents in varying dosage the animals were exposed to 700 R whole body X-irradiation. Factors of irradiation were: 200 KV. 20 mA, filter Cu 0.5 mm+Al 0.5 mm, HVL 1.08 mmCu, TSD:50 cm, and 30R/min. dose rate measured in air. Then mice were simultaneously irradiated with an irradiation device contrived by the authors. The device was equipped with a slowly rotating disc on which ten mice were fixed with their heads placed on the center so as to give the animals an equal dosage of X-rays. The animals survival and weight were followed for 30 days. Experimental animals were housed about 10 per cage in ar-conditioned room. Laboratory chow and drinking water were supplied at livitum. The pharmacological agents tested were epinephrine (Daiichi), norepinephrine (Sankyo), ephedrine (Dainippon), phenylethylamine, phenylephrine (Kowa), Naphazoline (Cyba), tetrohydrozoline (Pfizer), cocain and caffein in varying dosage.

Experiment 1.

The lethality of this strain of mice following X-irradiation of varying does is shown in Fig. 1. A dose level of 700R is considered to be $LD_{100/30}$.

Experiment 2.



epinephrine

The effect of epinephrine in varying dosage on the survival rate of mice after 700R whole body X-irradiation is shown in Fig. 2. There is observed only minimal radio-protective effect.

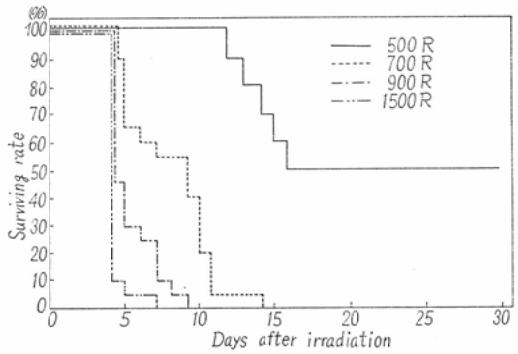


Fig. 1. Surviving rate of mice following whole body irradiation in varying dose. Na 2 strain (19 g ±1)

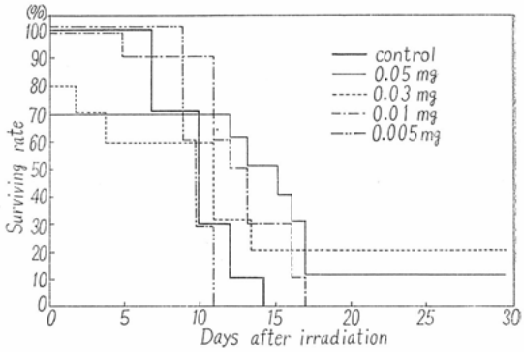
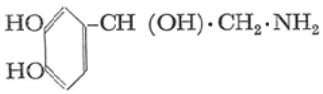


Fig. 2. Effect of intraperitoneal injection of epinephrine in varying dosage on the surviving rate of mice following 700R whole body irradiation.

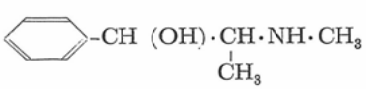
Experiment 3.



norepinephrine

The effect of norepinephrine in varying dosage in the survival rate of mice after 700R whole body X-irradiation is shown in Fig. 3. A dose level of 0.1 mg and 0.05 mg administered 5 minutes before X-irradiation produced marked protective effect.

Experiment 4.



ephedrine

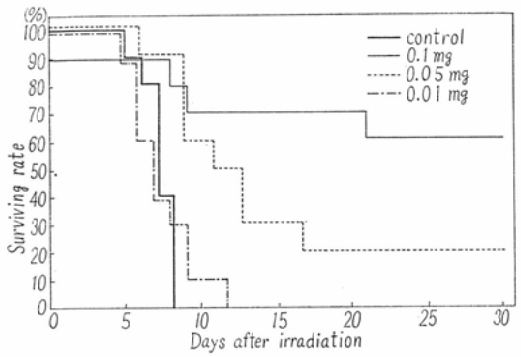


Fig 3. Effect of intraperitoneal injection of norepinephrine in varying dosage on the surviving rate of mice following whole body irradiation.

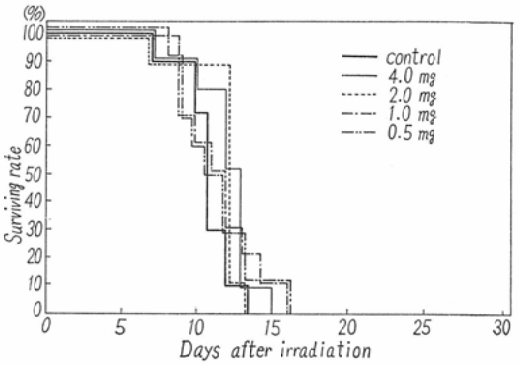
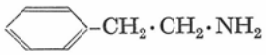
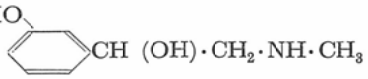


Fig 4. Effect of intraperitoneal injection of ephedrine in varying dosage on the surviving rate of mice following whole body irradiation.

The effect of ephedrine in a varying dosage in the survival rate of mice after 700R whole body irradiation is shown in Fig. 4. There is observed no protective effect of this agent.

Experiment 5.
phenylethylamine 

The effect of phenylethylamine in a varying dosage in the survival rate of mice after 700R whole body irradiation is shown in Fig. 5. There is observed minimal protective effect of this agent.

Experiment 6.
phenylephrine 

The effect of phenylephrine in a varying dosage in the survival rate of mice after 700R of whole body irradiation is shown in Fig. 6.

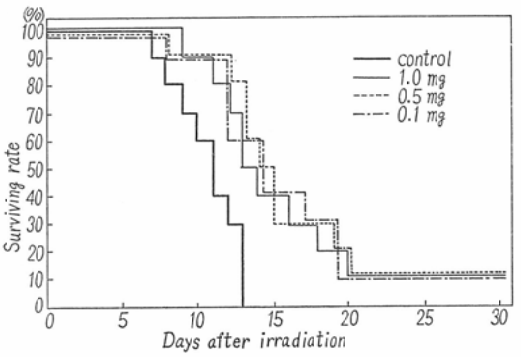


Fig. 5. Effect of intraperitoneal injection of phenylethylamine in varying dosage on the surviving rate of mice following whole body irradiation.

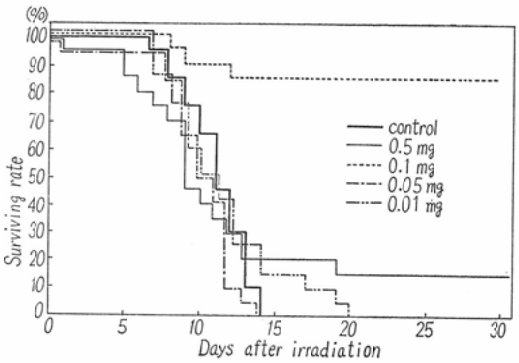


Fig. 6. Effect of intraperitoneal injection of phenylephrine in varying dosage on the surviving rate of mice following whole body irradiation.

A dose level of 0.01 mg and 0.05 mg injected 5 minutes before exposure produced no marked protection. A dose level of 0.1 mg administered at the same interval prior to exposure produced a marked

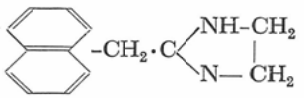
protective effect. However, a dose level of 0.5 mg produced a little protection.

Experiment 7.

In this experiment phenylephrine was administered in different intervals before and after exposure. Influence of length of time between injection and irradiation on the survival rate of mice after whole body X-irradiation is shown in Fig. 7.

Phenylephrine given 5 minutes before irradiation afforded a marked protection with a survival rate of 90%. Similarly, phenylephrine injected 15 minutes before irradiation decreased the mortality to 50%. With injection 30 minutes prior to irradiation the protection was markedly diminished. Phenylephrine given 60 minutes before irradiation affored no marked protection. Phenylephrine given immediately after irradiation produced no marked protection

Exderiment 8.



The effect of naphazoline in a varying dosage on the survival rate of mice after 700R whole body X-irradiation is shown in Fig. 8.

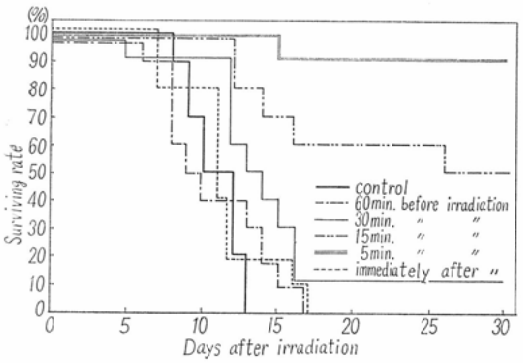


Fig. 7. Influence of length of time between injection of phenylephrine and irradiation on the survival rate of mice following whole body irradiation.

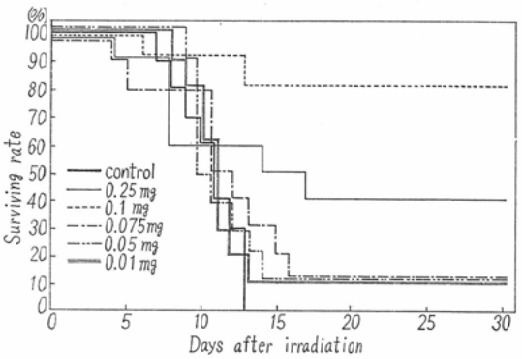
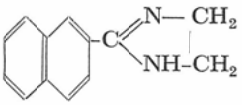


Fig. 8. Effect of intraperitoneal injection of naphazoline in varying dosage on the surviving rate of mice following whole body irradiation.

A dose level of 0.1 mg injected 5 minutes before irradiaion produced a marked protective effect. A dose level of 0.01 mg, 0.05 mg and 0.75 mg produced a minimal protective effect. However, a dose level of 0.25 mg produced a diminished protective effect. A dose level of 0.5 mg proved fatal due to the toxicity of the agent.

Experiment 9.

tetrahydrozoline

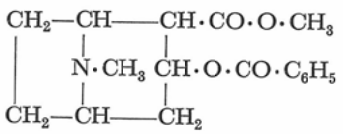


The effect of tetrohydrozoline in a varying dosage in survival rate of mice after 700R whole body irradiation is shown in Fig. 9.

A dose level of 0.5 mg produced the most marked protective effect. A dose level of 0.2 mg and 1.0 mg produced a less marked protective effect.

Experiment 10.

cocain



The effect of cocain in a varying dosage in the survival rate of mice after 700R whole body X-irradiation is showh in Fig. 10.

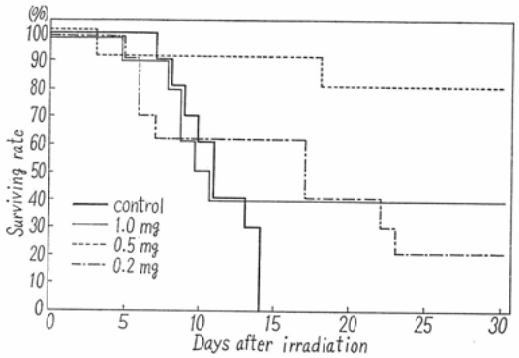


Fig. 9. Effect of intraperitoneal injection of tetrahydrozoline in varying dosage on the surviving rate of mice following whole body irradiation.

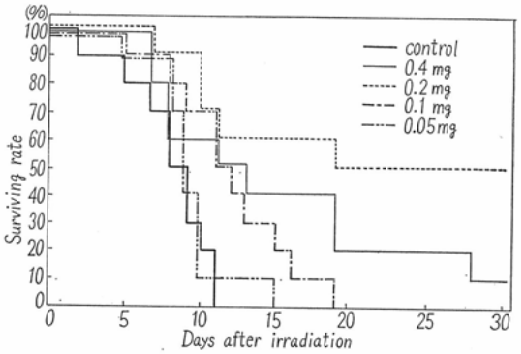
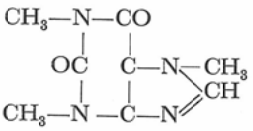


Fig. 10. Effect of intraperitoneal injection of cocain in varying dosage on the surviving rate of mice following whole body irradiation.

A dose level of 0.2 mg produced considerable protective effect.

Experiment 11.

caffeine



The effect of caffeine in a varying dosage in the surivival rate of mice after 700R of whole body irradiation is shown in Fig. 11.

Caffein produced only minimal protective effect.

Experiment 12.

An assumption that a pharmacological action of sympathomimetic agents may result in radio-protective effect led to a subsequent study of the effect of these agents when administered in combination with pharmacological antagonists. Consequently the radio-protective effect following combined administration of phenylephrine and benzylimidazoline was studied.

The effect of benzylimidazoline alone in varying dosage in the survival rate of mice after 700R whole body irradiation is shown in Fig. 12.

No marked protective effect was produced by benzylimidazoline.

The effect of combined administration of phenylephrine and benzylimidazoline in varying dosage in the survival rate of whole body irradiated mice is shown in Fig. 13.

A combined administration of phenylephrine and benzylimidazoline resulted in a decrease in the radioprotective effect of phenylephrine. The more the administered dose of benzylimidazoline, the more decrease in the radio-protective effect. However, combined administration of phenylephrine 0.1 mg and benzylimidazoline 0.8 mg proved to be fatal for some of mice due to toxity of these agents.

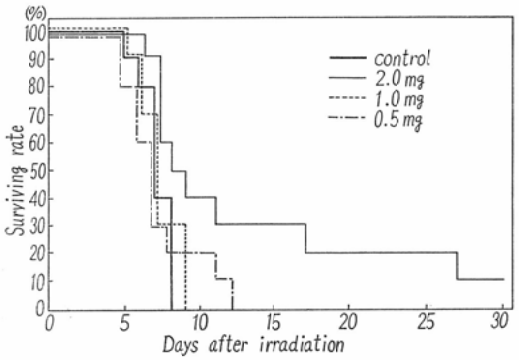


Fig. 11. Effect of intraperitoneal injection of caffeine in varying dosage on the surviving rate of mice following whole body irradiation.

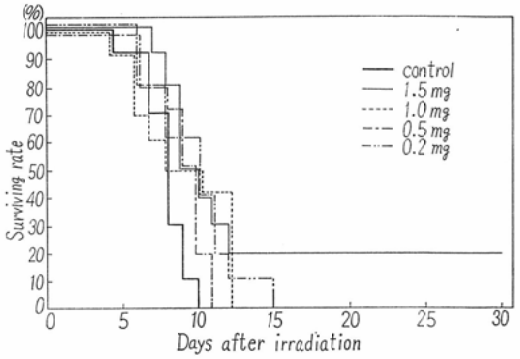


Fig. 12. Effect of intrapeitoneal injection of benzylimidazoline in varying dosage on the surviving rate of mice following whole body irradiation.

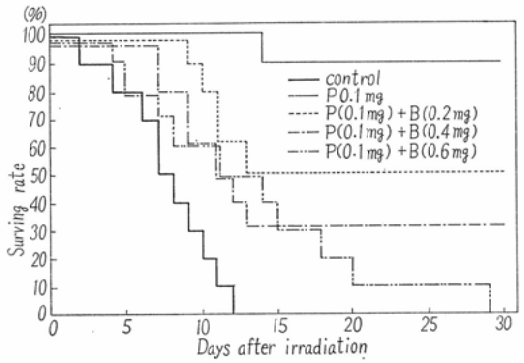


Fig. 13. Effect of combined administration of phenylephrine (0.1 mg) and benzylimidazoline in varying dosage on the surviving rate of mice following whole body irradiation.

Discussion

Gray[10] and Hara[11] report that epinephrine is effective in protecting mice exposed to lethal whole body X-irradiation. However, of the vasoconstricting agents studied in the present author's experiments epinephrine was not among the markedly effective. The author's experiment shows a considerably marked radio-protective effect of norepinephrine as reported by Hara but no effect of ephedrine reflecting the results of Langendorf[14] and Bacq[1]. The present authors have proved the first to observe a marked radio-protective effect of phenylephrine, naphazoline and tetrahydrozoline against lethal whole body X-irradiation in mice. These agents are also powerful sympathomimetic vasoconstricting agent. The author also tried phenylethylamine as an example of the sympathomimetic agent which has a very simple chemical formula, but observed almost no effect, although Bacq[1][2] reports a certain degree of effect on this agent.

The experimental results so far made reveal that radioprotective effect is most markedly evidenced by those lasting powerful vasoconstrictor agents which little stimulate the heart such as phenylephrine, naphazoline and tetrahydrozoline. Epinephrine showed less radio-protective effect than norepinephrine which affect the heart in a less degree. Ephedrine which shows a comparatively weak action on the

blood vessels and the heart has proved to be non-effective against radiation hazards. Phenylethylamine which is very weak vasoconstrictor shows a very slight protective effect. Cocain which is a considerably strong vasoconstrictor is a fairly good protective agent. Caffeine is a vasoconstrictor which works centrally. But it also being a vasodilator by a direct effect onvascular musculature shows very little protective effect. The present experimental resuts of vasoconstrictors suggest that there is a co-relationship between the degree of vasoconstriction and radioprotection.

A review of the radio-protective effect of vasoconstricting agents in reference to their dosage has disclosed that the highest radioprotective effect is maintained by their optimal dosage, higher level than which results in a corresponding decrease of radio-protective effect possibly due to the toxicity of overdosage. This result supports an assumption that the radio-protective effect of constrictor agents does not depend so much on a direct chemical action of free radicals as on a pharmacological action represented by vasoconstriction. Because any chemical action of free radicals would have reasonably been increased as a dose of the compounds increased.

In studying the radio-protective effect of vasoconstricting agents in reference to the lapse of time between administration and X-irradiation, pheny.lephrine which proved to be most effective of all the agents studied, had its radioprotective effect increasingly decreased as the lapse of time (between administration and X-irradiation) was prolonged. Namely it was found that the greatest protection was obtained when the time of pharmacological action coincided with the time of irradiation. Apparently there is correlation between the degree and duration of vasoconstricting property and the protection against radiation. Hence a suggestion that the longer the response of the constricting agent the better its radio-protective effect. An assumption that a pharmacological action of sympathominetic agents may result in radioprotective effect led to a subsequent study of the effect of these agents when administered in combination with pharmacological antagonists. A combined administration of phenylephrine and benzly-imidazoline resulted in a decrease in the radio-protective effect of the former agent. It is taken into consideration that the benzylimidazoline may decrease the sympathomimetic action of phenylephrine but it may with its toxicity increase fatality.

As a mechanism of the development of radio-protective action, it is apparently considered that thevasoconstricting agents antagonizes postirradiation hypotension. However, such consideration does not seem to reasonable. Because, sympathomimetic agents are effective only when administered before irradiation, and the hypotension due to X-irradiation show its maximum fall 3–4 hours after irradiation. Another mechanism may be referable to hypoxia. In mammals large doese of the sympathomimetic agents generally constrict arterioles of the skin, and other organs, and blood is shunted into brain, heart and lungs[9]. As a result it is assumed that hypoxia in certain critical tissues thus developed causes reduction in tissue susceptibility of X-irradiation. Meer and Bekkum[17][18] showed that the protection afforded to mice by a number of biologically-active amines is related to their ability to cause hypoxia in the spleen (and possibly also in other blood-forming organs). Regarding a decrease in radiation susceptibility due to hypoxia a number of reports have been made represented by Hollaender et al[12] on microorganismus, Mottram[19] on plants, Dowdy et al[7], Evans, Rambach et al[8], Stender and Hornykiewytsch[26], and Langendorf et al[16] on mammals. Stearner et al[25] report that epinephrine as well as low oxygen tension markedly reduce radiation mortality in young chicks, while combined treatment had a synergistic, protective effect. Gray et al[10] recognized radio-protective effect of pitressin and epinephrine in rats. They consi-

der the mechanism of radio-protective action to be due to a temporaly tissue hypoxia caused by vasoconstriction. Kahn[18] observed radioprotective effect in morphine possibly due to a mechanism of hypoxia caused by depression of the respiratory center.

The above author's results as well as the presnet experimental results have led to an assumption that hypoxia due to vasoconstriction following administration of sympathomimetic agents plays an important part in the mechanism of preventing radiation damage. Another possible mechanism, that these compounds compete with tissue constituents for radiation induced radicals, seems to be hardby indicated by the fact that these compounds are no more active at a excessive dose as described above. Radiation lethality and its protection are extremely complicated problem. It must be admitted that various mechanisms are present in the radioprotective action.

Summary

Experimental studies were made on the protective effect of several vasoconstricting agents for mice exposed to lethal whole body X-irradiation with the following results:

1. Protective effect:

a) Markedly effective were phenylephrine, naphazoline, tetrahydrozoline and norepinephrine.

b) Considerably effective were cocain and epinephrine.

c) Slightly effective were caffeine and phenylethylamine.

d) Non-effective was ephedrine.

2. The radio-protective effect of these vasoconstricting agents was the highest at an optimal dosage, higher level or lower level of which revealed proportionally less effect.

3. Phenylephrine was most effective when administered intraperitoneally 5–10 minutes before exposure to X-rays. It showed a marked decrease in efficacy against X-radiation 30 minutes after administration, loosing its effect 60 minutes after administration. No radio-protective effect was observed when the agent was administered immediately after irradiation.

4. As a possible mechanism of the radioprotective effect of these vasoconstricting agents, the tissue hypoxia due to vasoconstriction is discussed.

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