Influence of Methylene Blue on Liver Glycogen, Blood Glucose, and Electrolytes in Serum of Rats Exposed to X-rays

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Methyleue blue가 X線에 照射된 흰쥐의 肝臟 glycogen, 血糖 및 血液內 電解質에 미치는 影響

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摘 要

成熟한 albino 雄性 흰쥐를 對照群과 實驗群으로 나누어 對照群에는 0.9% 生理食鹽水를, 實驗群에는 methylene blue (40 mg/kg, 食鹽水에 溶解, pH 7.0)를 各各 皮下注射處理하였다. 30分後에 兩群共히 每分當 33 r 으로 總線量 300 r 의 X-線을 一時 全身照射하였다. 照射後 24日間에 걸쳐 肝臟 glycogen 含量(Carroll *et al.* 方法), 血糖含量(Folin and Wu 方法) 및 K와 Na 含量(flame photometer)과 Cl 含量(automatic chloride titrator)을 各各 測定하여 methylene blue의 放射線에 對한 防禦效果를 硏究調査하였다. 一般的으로 肝臟 glycogen 含量은 兩群共히 X-線照射直後 若干 減少하나 1日區에서부터 急激한 增加를 나타내고 있으며 特히 實驗群인 methylene blue 處理群에서는 對照群인 生理食鹽水群에 比하여 增加의 遲滯性을 나타내었다. 血糖含量에 對해서는 兩群모두 X-線 照射直後 若干 減少하는 傾向을 보였으나 時日經過에 따라서 서서히 增加하며 特히 實驗群인 methylene blue 處理群은 對照群인 生理食鹽水群에 比하여 增加에 遲滯性을 나타내었다. 또한 血液內 電解質含量에 있어서 K, Na 및 Cl 含量은 一般的으로 兩群모두 時日經過에따라 큰 變化는 없었으나 特히 K와 Na의 含量은 初期狀態에서 若干의 變化가 있었고 Cl에서는 別變化를 觀察할 수 없는것으로보아 methylene blue가 血液內 電解質含量에는 別影響이 없었다. 以上의 結果로 미루어보아 炭水化物代謝에 있어서 methylene blue가 放射線에 對한 防禦效果가 있는것으로 思料된다.

INTRODUCTION

The ability of biologically active compounds to offer protection to the animal against X-irradiation has been studied extensively. The value of methylene blue as a protective agent against ionizing radiation in animals was first established by Chung and Nam (1967).

Sulfhydryl compounds have been known as protective agents against X-irradiation. Such compounds as cysteine (Patt *et al.*, 1949; Straub and Patt, 1953; Park and Nam, 1967), glutathione (Chapman *et al.*, 1959; Paterson *et al.*, 1951), cysteamine (Rugh and Wang, 1953; Becq *et al.*, 1953; Straub and Patt, 1953; Rugh and Clugston, 1956), S_2, β-aminoethylisothiouronium Br. HBr (AET) (Grouch and Overman, 1957), and others have exhibited a significant protective effect on animals irradiated with various ionizing radiations. Chung and Nam (1967), giving methylene blue to mice, found the protective effect of it against total body X-irradiation. Nam and Koh (1957) have reported that the effect of X-rays for animals is modified by methylene blue injected to it 30 minutes prior to X-irradiation. Kim and Nam (1957) indicated that the effects of 600 r X-rays for adult male and female mice were modified by the subcutaneous injection of methylene blue 30 minutes pre-irradiation and 3 minutes post-irradiation so that protective effects show on the survival rate and changes in weight of the mice.

It is well established that total body X-irradiation of rats results in an increase in the levels of liver glycogen (Ross and Ely, 1951; Mckee *et al.*, 1952; Supplee *et al.*, 1954; Nims and Sutton, 1954). On the other hand, Rother (1928) have reported that the levels of liver glycogen of rabbits were reduced markedly following total body X-irradiation. Although X-irradiation raises liver glycogen levels of rats and decreases that of rabbits, blood glucose levels in both animals were increased by exposure to X-radiation.

Warren (1950) and Dunham (1951) emphasized the importance of parenteral fluids for nutrition and for correcting changes in electrolytes and acid-base balance in discussions of the treatment of the acute radiation. A variety of alterations in plasma and serum levels of sodium, chloride, and potassium have been reported after acute whole-body irradiation injury (Bowers *et al.*, 1953). Moon *et al.* (1941) found, however, an increase in blood potassium content after abdominal irradiation of the dog at 1,400 to 1,800 r.

There have been few reports on the effect of methylene blue against total body X-irradiation on the carbohydrate and electrolytes in serum. We would like to present this data concerning the metyhlene blue protection giving the injection 30 minutes before X-irradiation and its effect on the levels of liver glycogen, blood glucose, and electrolytes in serum during 24 days following single total body exposure to X-rays.

MATERIAL AND METHODS

Male rats of Albino strain weighing 110-150 g were employed and adapted to environment and food. Methylene blue was administered in 0.9% solution of sodium chloride in the dose of 40 mg/kg (adjusted to pH 7.0) by subcutaneous injection with tuberculin syringes and 22 gauge needles at 30 minutes before the exposure to X-radiation. Each group in each test consisted of 9 rats. Irradiated controls received corresponding volumes of 0.9% sodium chloride solution subcutaneously. Radiation source was General Electric Maximar 250-III type of 220 KVP and 15 mA, with filter of 0.5 mm copper and 1 mm aluminum. All doses were delivered at 300 r (33 r per minute) to the rats at a target-to-skin distance of 50 cm. The control and experimental rats were restrained in acetate-plastic cage (18 x 18 x 7 cm) and slowly rotated under the beam to insure uniform exposure (Bond *et al.*, 1950).

X-irradiated rats of both control and experimental groups were sacrificed at 1st, 3rd, 5th, 8th, 12th, 18th, and 24th day after X-irradiation and blood samples were obtained with tubes from severed left saphenous vein. Before sacrifice, animals were starved for 7 hours. The serum was separated by centrifugiation at 3,000 rpm for 15 minutes and used immediately. The levels of glucose were measured at the wavelength of 420 mμ by the method of Folin and Wu (1920). After blood samples were obtained, the liver was removed and the anterior portion was excised and this portion was weighed with a Torsion balance. The levels of liver glycogen were measured at the wavelength of 640 mμ by the method of Carroll *et al.* (1956). In electrolyte measurement, the levels of chloride were measured in the serum by an automatic chloride titrator. The levels of potassium and sodium were measured in the serum by a flame-photometer KY-3 type made by Bario-Atomic Company.

RESULTS

Although the general condition seemed to have deteriorated during the experimental period, all rats survived without any mortality. The changes of liver glycogen levels under experimental conditions are shown in Table 1 and Fig. 1. The changes of blood glucose levels under experimental condition are shown in Table 1 and Fig. 2. The changes of chloride levels in serum are shown in Table 2 and Fig. 3; potassium in Table 2 and Fig. 4; and sodium in Table 2 and Fig. 5.

The levels of liver glycogen of normal rats was 131.32±3.91 mg%; in the case of normal serum, blood glucose 113.54±2.57 mg%; chloride 107.83 ±3.22 mEq/l; potassium 8.10±0.38; and sodium 168.02±3.61. Total body X-irradiation was followed by a rise in the levels of the liver glycogen and blood glucose of both control and experimental groups. In the levels of the electrolytes, however, no remarkable change appeared immediately after total body X-irradiation. The levels of liver glycogen dropped slightly within the first 24 hours. This drop was, however, followed by a sharp rise in the levels of liver glycogen during the next 48 hours and continued

Table 1 Effects of methylene blue administration on the liver glycogen and blood glucose levels of serum in rats receiving 220 Kv. roentgen irradiation for a dose of 300 r

Treatment	Days after irradiation	No. of Rats	Glycogen mg %	Glocose mg %
Sodium chloride 30 min. pre-irradiation	0	9	*131.32 ±3.91	113.54 ±2.57
	1	9	40.76 ±4.22	80.01 ±2.54
	3	9	167.71 ±4.18	118.30 ±2.08
	5	9	225.43 ±3.86	130.02 ±2.04
	8	8	300.28 ±4.28	137.24 ±2.43
	12	9	432.84 ±3.83	144.93 ±1.75
	18	9	578.21 ±4.74	155.04 ±1.86
	24	9	631.57 ±4.11	149.38 ±3.00
Methylene blue 30 min. pre-irradiation	0	9	131.32 ±3.91	113.54 ±2.57
	1	9	55.08 ±4.02	82.25 ±2.89
	3	9	101.62 ±4.28	131.46 ±2.32
	5	9	187.37 ±4.36	121.31 ±1.86
	8	9	207.42 ±4.57	134.83 ±2.07
	12	9	397.64 ±3.90	138.75 ±2.11
	18	9	559.34 ±3.97	137.92 ±2.57
	24	8	600.43 ±4.01	143.24 ±2.39

* Mean ± standard error

rising for 18 days in both groups. The rise in the levels of liver glycogen in the experimental group appeared to be delayed in comparison with the control group for the entire experimental procedure. It was significant that the delayed rise in levels of liver glycogen of experimental group showed remarkable increase on the 5 th and 18th day in comparison with rising of control group. However, the analysis of variance by t test (Croxton, 1959) revealed that the differences of the levels of blood glucose and liver glycogen in the experimental and control groups were highly significant ($P<0.01$).

The levels of blood glucose dropped slightly in the first 24 hours and then rose on the 3rd day in both experimental and control groups. The blood glucose levels of the experimental group showed delayed rise when compared with those of the control group. There was a

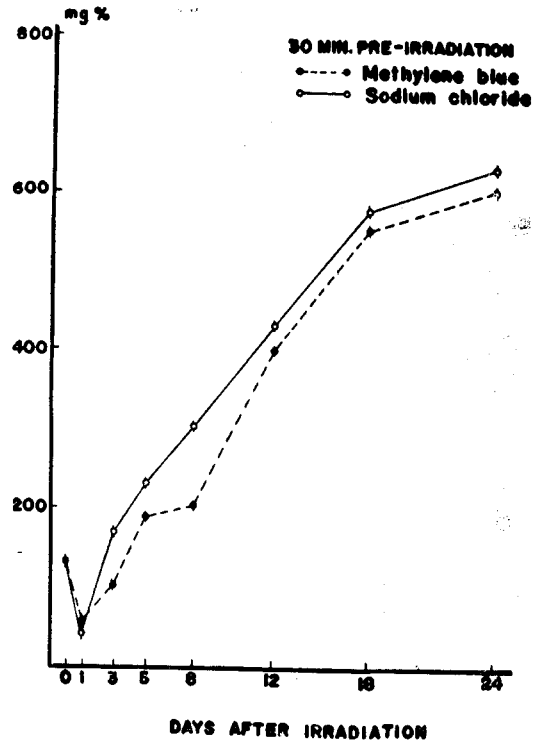

Fig. 1. Influence of methylene blue on the liver glycogen level of serum in rats given total body irradiation (220 Kv. X-rays) for a dose of 300 r. Vertical lines represent two standard errors of the mean. 8-9 rats were used for each measurement(The same is true for succeeding Figs.).

remarkable delay in rise on 5 th and 18 th day.

In the levels of electrolytes in serum (1) chloride appeared to decrease slightly from 1 st day to 12 th day after total body X-irradiation and from 12 th day, the levels of chloride came to normal values. However, there appeared to be no remarkable difference in both the experimental and control groups. (2) Potassium appeared to decreased slightly on the 1st day in both the experimental and the control groups. However, the experimental group was slightly lower than the control group. It rose to normal at 3 rd and 12 th day, the levels in the control group rose less than the experimental group. (3) Sodium was appeared slightly different on

Table 2 Effects of methylene blue administration on the electrolytes of serum in rats receiving 220 Kv. roentgen irradiation for a dose of 300 r

Treatment	Datesafter irradiation	No. of Rats	Chloride mEq/l	Potassium mEq/l	Sodium mEq/l
Sodium chloride 30 min. pre-irradiation	0	9	*107.83 ±3.22	8.10 ±0.38	168.02 ±3.61
	1	9	107.92 ±4.04	7.44 ±0.34	171.23 ±3.34
	3	9	102.87 ±3.84	7.32 ±0.33	176.01 ±2.41
	5	9	99.14 ±3.01	6.99 ±0.28	161.56 ±2.64
	8	8	97.63 ±3.24	6.93 ±0.17	169.84 ±2.79
	12	9	92.86 ±2.57	6.43 ±0.26	164.53 ±4.18
	18	9	96.94 ±3.84	7.36 ±0.31	168.06 ±2.92
	24	9	98.42 ±2.41	8.05 ±0.36	172.04 ±4.03
Methylene blue 30 min. pre-irradiation	0	9	107.83 ±3.22	8.10 ±0.38	168.02 ±3.61
	1	9	107.22 ±3.04	6.13 ±0.33	160.48 ±3.73
	3	9	103.14 ±4.21	7.62 ±0.40	170.17 ±1.79
	5	9	99.12 ±2.74	7.11 ±0.40	163.04 ±3.87
	8	9	93.83 ±2.62	7.35 ±0.37	170.52 ±3.65
	12	9	94.79 ±4.21	7.60 ±0.38	170.47 ±3.83
	18	9	97.52 ±3.69	7.28 ±0.28	165.82 ±2.75
	24	8	96.87 ±3.44	7.77 ±0.29	172.04 ±3.23

* Mean ± standard deviation.

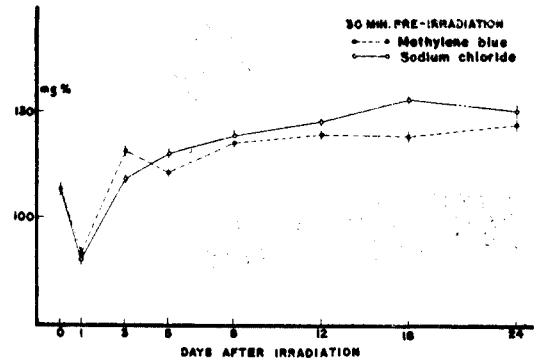

Fig. 2. Influence of methylene blue on the blood glucose level of serum in rats given total body irradiation (220 Kv. X-rays) for a dose of 300 r.

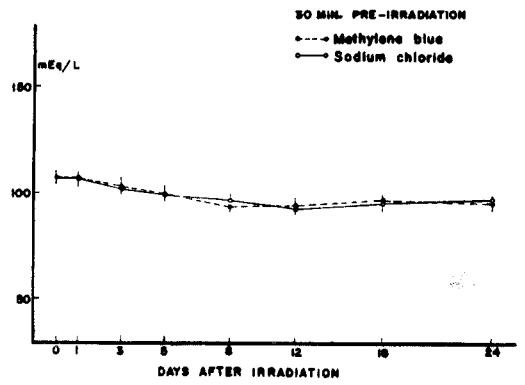

Fig. 3. Influence of methylene blue on the chloride level of serum in rats given total body irradiation (220 Kv. X-rays) for a dose of 300 r.

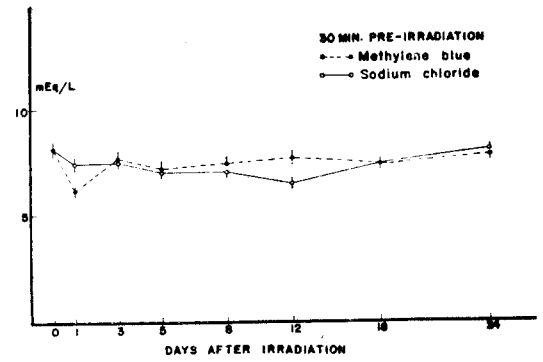

Fig. 4. Influence of methylene blue on the potassium level of serum in rats given total body irradiation (220 Kv. X-rays) for a dose of 300 r.

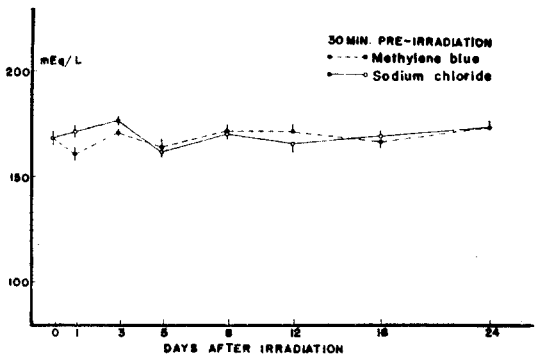

Fig. 5. Influence of methylene blue on the sodium level of serum in rats given total body irradiation (220 Kv. X-rays) for a dose of 300 r.

both groups. There was, however, no remarkable changes and was not significant.

DISCUSSION

Protective effect of methylene blue in total body X-irradiation was concerned with before or after the exposure. It revealed that the methylene blue must be given immediately before the exposure in the study of the time course of methylene blue protection against total body X-irradiation (Chung and Nam, 1957). This suggests that the reactions leading to morbidity are complete when the irradiation is terminated and that these reactions are apparently not reversible. There was shown that the methylene blue treated animal lost much less weight and were able to regain their lost weight more rapidly than the control animals (Kim and Nam, 1957). Chung and Nam (1957) have shown that methylene blue greatly delays the increase in the levels of the serum albumin-globulin ratio and the decrease in the levels of total serum protein when it is administered prior to X-irradiation in mice. Nam and Koh (1957) have reported that methylene blue administered to rats prior to X-irradiation, greatly delays the increase in the levels of total cholesterol of serum.

Kay and Entenman (1956) found that whole-body X-irradiation increases the percentage of liver glycogen in the fasting rat, and at 24 hours after the irradiation glycogen deposition was shown to increase with an increase in dose. Mckee (1952) has reported that the X-irradiated rats rapidly produced the liver glycogen with values up to 1.5%, during fasting the blood sugar of irradiated rats falls less than control animal and it is likely that this production of glycogen is a glyco-neogenesis stimulated by the pituitary and the adrenals.

The increase in the magnitude and duration (2-4 days) which was depended upon dosage, it was followed by a return to normal levels of the glucose (Kohn, 1951). Kay and Entenman (1956) have noted that whole-body X-irradiation increased the blood sugar levels in fasting rats. Although X-irradiation raises liver glycogen levels of rats and decreases that of rabbits, blood sugar levels in both animals are increased by exposure to X-radiation. Kohn (1951) found that in rats irradiated with 600-900 r, plasma glucose level rises within 2 to 3 days. Mckee (1952) has found, on the first day after irradiation, an increase in blood sugar level of rats exposed to 1,500 r.

Radiosodium excretion in the feces was increased at the height of the post-irradiation diarrhea, while urinary levels were decreased (Bowers and Scott, 1951). Kohn (1951) have reported increases in plasma or serum sodium of 3 to 8 mEq in rats three to four days after an acute LD_{50}. Soberman *et al.* (1951) were, however, unable to detect any significant changes in plasma sodium or potassium levels in the dog after an acute LD_{50} exposure. After abdominal irradiation of the dog at 1,400 to 2,800 r. Moon *et al.* (1941) found an increase in blood potassium content. In view of the selectivity of the tissue injury, the ability of the kidneys to excrete the potassium moving out of these tissues, and the absence of associated changes in acid-base balance in experiments (Bowers *et al.*, 1953), it is not surprising that there were no significant changes in plasma potassium levels and only minor deviations in plasma sodium.

Since methylene blue was effective in altering sensitivity to radiation, one may speculate that the methylene blue acts by protecting certain cellular components from oxidation by the products of irradiated water. One can theorize in accord with the views of Barron (Barron, 1946; Barron *et al.*, 1949; Barron and Dickman, 1949) that methylene blue in these experiments may protect the sensitivity of SH groups of certain enzymes. This action may decrease in the rise of liver glycogen and blood glucose levels which follows irradiation.

It was generally considered that the compounds were the effective agent in the protective substances against ionized products of irradiated water and oxygen content in the organism and assumed that many of the biological effects of X-irradiation can be attributed to the action of the decomposition products of water which result from the irradiation. It was, therefore, felt that the protective effect of methylene blue was related to its sulfhydryl group and caused radiation protection on this basis. In other words, sulfhydryl group was the most evident distinguishing feature between the substances which definitely protect and those which do not. The machanism of the action of methylene blue in producing the above

beneficial effects after exposure to a single dose of radiation remain obscure. It was hoped that some insight into the mechanism of the methylene blue protection might be forthcoming from a consideration of the influence of certain related substances on sensitivity to radiation.

SUMMARY

Male rats of the Albino strain received methylene blue in the dose of 40 mg/kg by subcutaneous injection and were subjected to total body X-irradiation, 300 roentgen, at 30 minutes after the injection. The protective effect of methylene blue against the single total body X-irradiation was studied for 24 days after X-irradiation with regard to the levels of liver glycogen, blood glucose, and electrolytes in serum.

1. Total body X-irradiation generally caused an increase in the levels of the liver glycogen and blood glucose in both methylene blue treated and control group during the entire experiment.

2. Methylene blue has been shown to delay slightly the increase of the levels of the liver glycogen and blood glucose when comparing with both groups which were given methylene blue and control saline injection before irradiation in the rats.

3. The delay in the increase in the levels of liver glycogen, in experimental group injected with methylene blue, significantly came in two phases. The first phase appeared at three days after the exposure, the second followed at eighth day. It appeared that the recovery phase was at nineteenth day.

4. During the experimental days the levels of the blood glucose increased generally, methylene blue, however, caused delay in two phases; the first at fifth day, the second at eighteenth day after the exposure to X-rays.

5. In electrolytes, there was not a significant difference. The levels of chloride were, however, slightly decreased in both groups, levels of potassium appeared different in two phases at first day and twelfth day, and the levels of sodium appeared to show irregular changes at the same levels, but there was no significant difference.

6. It may be considered that methylene blue greatly reduces the sensitivity of rats to X-rays, provided that methylene blue is given before the exposure.

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