Radioprotective Effect of Methylene Blue

2. Electron Microscopy of the Effect of Methylene Blue on the Liver and Heart of Rats following Gamma-Irradiation*

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Methylene Blue의 放射線防禦効果

2. Methylene Blue가 γ線에 照射한 흰쥐의 肝 및 心臟組織에 미치는 電子顯微鏡的 硏究

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摘 要

成體 albino 흰쥐를 實驗群과 對照群으로 나누어 對照群에는 0.9% 生理食鹽水를, 實驗群에는 methylene blue (38 mg/kg, pH 7.4)를 各各 腹腔에 注射한後 約 30分後에 總線量 360 rads의 ^{60}Co-γ線을 一時全身照射하였다. 照射後 64時間區와 212時間區로 나누어서 肝 및 心臟組織을 觀察하였다.

照射直後 肝 및 心臟組織을 6% glutaraldehyde (pH 7.4)와 1% osmium tetroxide (pH 7.4)로 冷室에서 二重固定하였으며 脫水한後 Epon 812로 胞埋하여 MT-2 Porter Blum ultramicrotome으로 超薄切斷하여 切片을 만들었다. 이를 uranyl acetate와 lead citrate로 二重染色한후 Hitachi HU-11E型 電子顯微鏡으로 觀察하였다.

64時間區의 methylene blue處理群과 對照群에서 肝組織은 顯著한 組織學的 變化의 差를 나타냈다. 다시 말하면 methylene blue處理群에 比하여 對照群에서는 mitochondria의 膨大, cristae의 切斷, endoplasmic reticulum의 破壞를 觀察할 수 있었으며 또한 endoplasmic reticulum에 glycogen粒子의 蓄積을 觀察할 수 있었다. 한편 methylene blue處理群에 比하여 對照群의 212時間區에서도 같은 變化樣相을 나타냈으나 endoplasmic reticulum에 많은 vacuole이 形成되었음을 觀察할수가 있었다. 그러나 methylene blue處理群은 正常群 (γ線의 照射와 methylene blue를 處理하지 않음)에 比하여 若干의 差가 觀察되었을 뿐 顯著한 組織學的變化는 나타나지 않았다.

心臟組織에서는 兩群 即 實驗群과 對照群과 正常群사이에 顯著한差는 別로 觀察할 수 없었으나 methylene blue群에 比하여 對照群에 若干의 變化가 觀察되었다. 다시말하면 methylene blue處理群에 比하여 64時間區의 對照群에서는 mitochondria의 若干의 膨大, cristae에 若干의 切斷을 觀察할 수 있었고 212時間區의 對照群에서는 sarcoplasmic reticulum에서 若干의 液胞와 glycogen粒子의 若干의 增加를 觀察할 수 있었다. 이로 미루어 보아 methylene blue가 放射線에 對하여 防禦効果가 있는것으로 思料된다.

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INTRODUCTION

In the previous papers (Chung and Nam, 1967; Kim and Nam, 1967; Chang and Nam, 1968; Nam and Koh, 1969; Nam and Chang, 1969), we have reported the radioprotective action of methylene blue against X-and gamma-irradiation injury. Methylene blue greatly reduced the sensitity of mice to sublethal dose of X-rays, provided that it was given prior to exposure. Smirnov (1960) reported that ionizing radiation had been observed to produce chromatolytic reaction, cytoplasmic vacuolization, and under appropriate conditions, general cellular degeneration or necrosis. Masorovsky *et al.* (1967) reported that mitochondria and other cell organelles underwent degenerative changes when they were irradiated with X-radiation. Andres (1963 b) observed mitochondria with ruptured cristae, and vacuolization in cytoplasm as well as extensive loss of matrix elements in degenerating neurons following proton irradiation *in vivo*. There has been no report on the alteration of fine structure of liver and heart organelles of methylene blue-treated rat before total body gamma-irradiation.

MATERIALS AND METHODS

Laboratory-conditioned male rats of albino strain weighing between 110-150 g were used and fed *ad libitum* on water and commercial diet. They were divided into control and treated groups. Purified methylene blue provided by the Wako Pure Chemical Industries, Ltd., Osaka, Japan.) was used in the treated group. Each rat of the control group received an intraperitoneal injection of 0.9% saline (7ml/kg), while that of the treated group was injected intraperitoneally with an equal volume of an aqueous solution of methylene blue (pH 7.4) at a dosage of 38mg/kg.

The rats in both the control and methylene blue treated groups were given 360 rads of whole body gamma-irradiation 30 minutes after the intraperitoneal injection.

The rats in both groups were irradiated with gamma-rays of ^{60}Co in less than 4 minutes. Whole-body irradiation was performed at the Radiology Research Institute, Seoul, Korea. The control and methylene blue-treated groups were reestrained and irradiated acutely in a wooden cage (18×18×7cm) slowly rotating under the beam to insure uniform exposure.

After irradiation, both groups were kept under the above mentioned condition up to the time of sacrifice at 64 and 212 hours. Rats were excised immediately after they were sacrificed by rupture of their spinal cord and the anterior portion of liver and left ventricle of heart were removed. Liver and heart tissues were cut less than 1 mm^3, were fixed at once in cold 6% phosphate buffered glutaraldehyde (pH 7.4) for 6 hours, and thereafter refixed in 1% phosphate buffered osmium tetroxide (pH 7.4) which contained 0.1 M sucrose, and were kept refrigerated for 1 to 2 hours at 4-0°C. After fixation they were dehydrated with graded ethanol and embedded in Epon 812. Ultra-thin sections showing silver-gold interference colors were cut on a MT-2 Porter Blum ultramicrotome with glass knives, mounted on copper grids, and stained with uranyl acetate and lead citrate. Grids were examined in a Hitachi HU-11E electron microscope at a magnification of 3,300 to 12,000 using 75 Kv accelerating potential. The electron microscope photograph was subsequently further enlarged by optical photography.

OBSERVATION

Living organisms irradiated with ionizing radiation, in general, appears severely altered in histological structure. In the present experiment the animals were sacrificed at two different time intervals following irradiation and severe damage was noted to the liver and heart. Degenerative cytoplasmic alterations were common, in both cases, in which included were damage of ribosome, endoplasmic and sarcoplasmic reticulums, formation of vacuoles, as well as significant damage in the mitochondria and increase of glycogen particles after exposure. On the other hand, the liver and heart tissues of methylene blue-treated rats appeared slightly altered in comparison with normal rats (Figs. 1 and 6). Control rats generally exhibited more severe alteration than methylene blue-treated rats.

46 hours after irradiation

One can easily observe severe alteration in the fine structure of the liver tissue of the saline treated rats (control) irradiated with gamma-rays(Fig. 2). Mitochondria

Fig. 1. Electron micrograph of normal liver cell of the rat, showing nucleus (N), mitochondria (M), lysosome (L) and endoplasmic reticulum (ER). ×6,600.

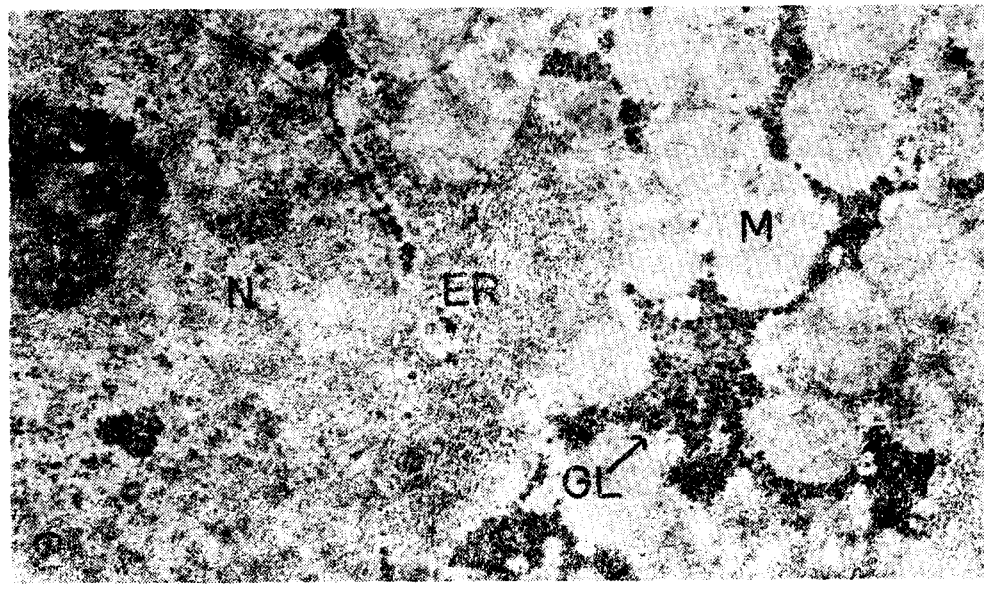

Fig. 2. Part of a liver cell of control rat at 64 hours after exposure (^{60}Co-360 rads), showing the appearance of fully accumulated glycogen particles (GL), altered mitochondria (M) and slightly degenerated endoplasmic reticulum (ER). ×23,000.

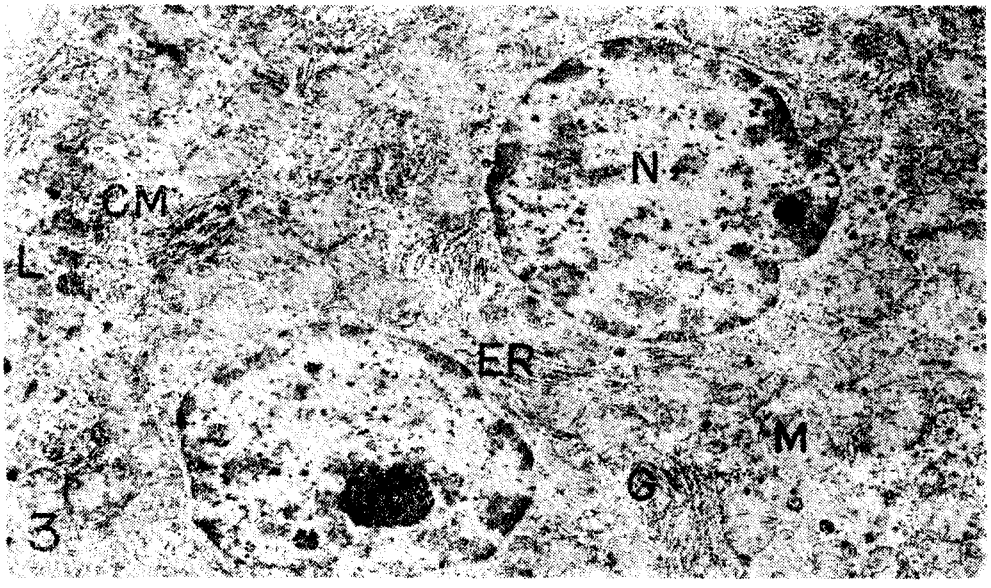

Fig. 3. Slight abnormal liver cell of methylene blue-treated rat at 64 hours after exposure (^{60}Co-360 rads); nucleus (N), mitochondria (M), endoplasmic reticulum (ER), Golgi (G), lysosome (L) and cell membrane (CM). ×12,000.

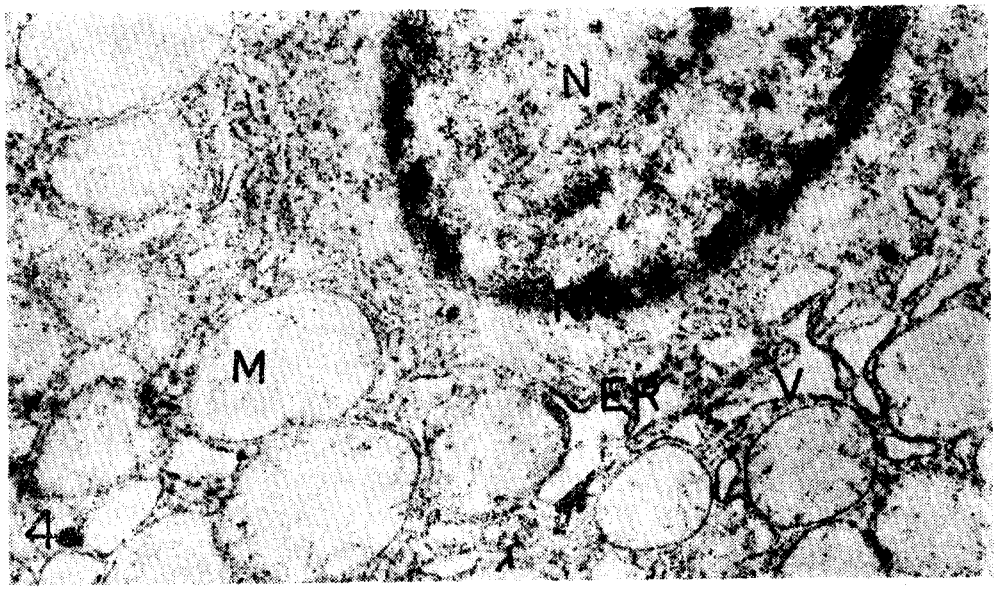

Fig. 4. Severe alterations in control saline-treated rat at 212 hours after exposure (^{60}Co-360 rads); mitochondria (M), vesicles in endoplasmic reticulum (V) and nuclear membrane (NM). ×22,000.

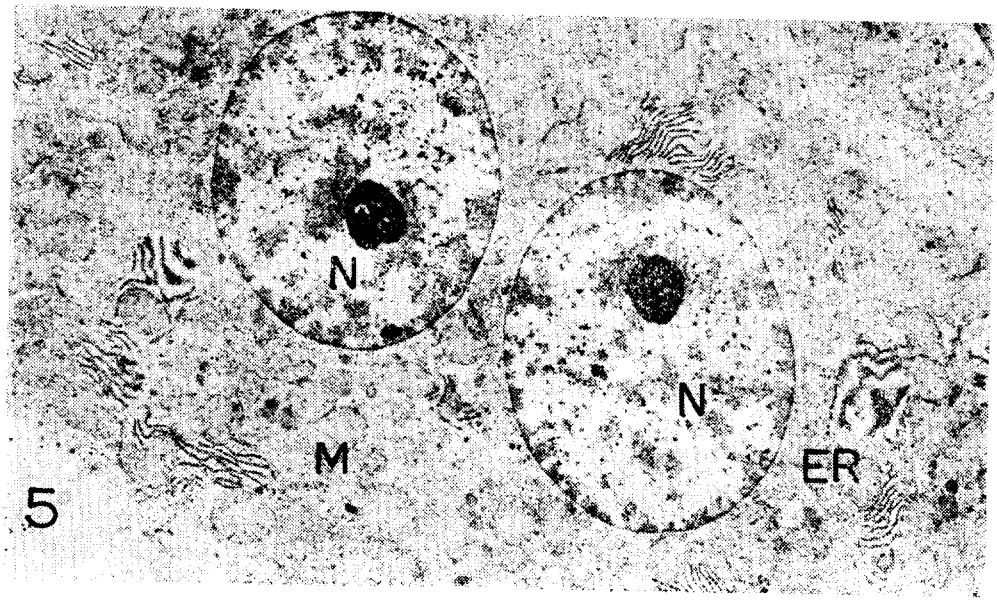

Fig. 5. Portions of liver cell of methylene blue-treated rat at 212 hours after exposure (^{60}Co-360 rads); dilated endoplasmic reticulum (ER). ×12,000.

Fig. 6. Longitudinal view of heart muscle of normal rat. Mitochondria (M). ×6,600.

Fig. 7. Heart muscle of control rat at 64 hours after exposure (^{60}Co 360 rads), showing abnormal cristae in several mitochondria (M) (arrow). ×18,000.

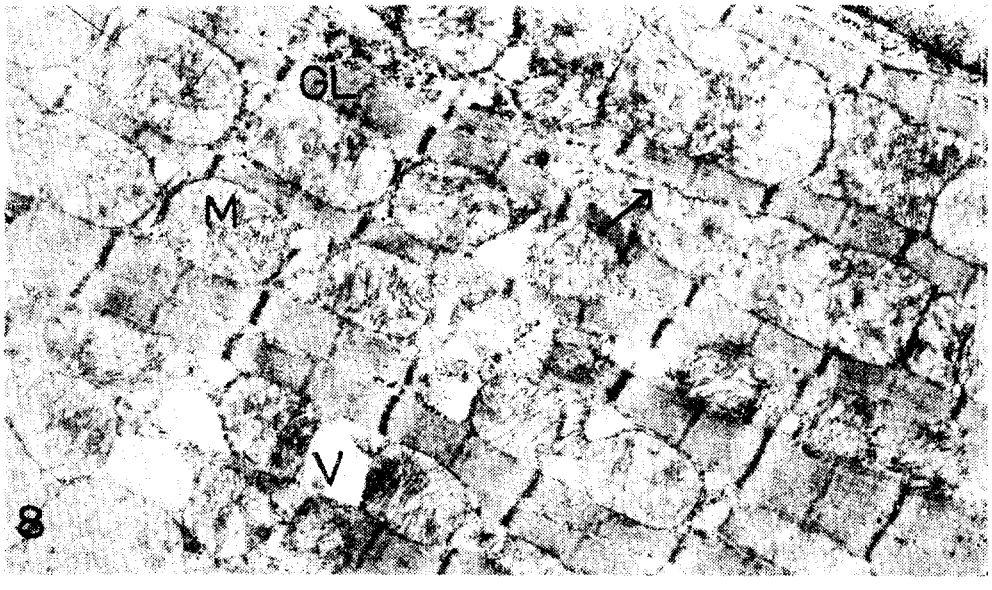

Fig. 8. Heart tissue of control rat at 212 hours after exposure (^{60}Co-360 rads), showing swollen, vacuolated mitochondria (M) (arrow), increased glycogen particles (GL) and dilation and vacuolization in the sarcoplasmic reticulum (V). ×15,000.

in control liver tissues were abnormal in size and shape, internal fine structural alteration chiefly involved the endoplasmic reticulum which was dispersed and variably vesiculated. The mitochondria were found enlarged and bizarre shaped and were prominently enveloped by endoplasmic cisternae in comparison with methylene blue-treated rats following gamma-irradiation. Most mitochondria were severely swollen with disruption shortening and irregulartities of thier broken cristae. The endoplasmic reticulum in control liver tissue was degenerated with breaks and frequently arranged in short units by the damage following irradiation. In the cytoplasm of the controls there appeared some vacuolization and significantly increased glycogen particles. Accumulations of glycogen particles were packed between mitochondria as glycogen aggregates freely dispersed in the cytoplasmic matrix, whereas liver cell organelles of methylene blue-treated rats appeared only slightly altered with no significant changes in the mitochondria and endoplasmic reticulum (Fig. 3).

On the other hand, the study showed that control rats exhibited slight changes in fine structure of heart tissue in comparison with methylene blue-treated rats. Most cell organelles in control rats appeared as their original structure in normal rat. Especially, mitochondria of the muscle tissue were only slightly dilated and appeared to be degenerated with several vacuole formations following gamma-irradiation (Fig 7).

212 hours after irradiation

In the liver tissue of control rats, the mitochondria appeared to be abnormal showing cytoplasmic alteration and greatly swollen, and the cristae were broken into small sections (Fig. 4). The cristae mitochondriales in control liver tissues were ruptured along with its membrane and mitochondria, as a whole, were largely vacuolized. Endoplasmic reticulum of the liver underwent degeneration following gamma-irradiation and this alteration stimulated the formation of vacuoles. Generally, the changes in cytoplasm of liver tissue consisted of various types of vacuolar abnormalities, whereas liver of methylene blue-treated rats appeared to have no such alteration in its cytoplasm (Fig. 5). It was shown that methylene blue-treated rats were protected against ionizing radiation. In heart tissue of the control rat, no significant changes were observed in comparison with methylene blue-treated rats. It was shown, however, that there are slight alteration and degeneration between control and normal groups. Heart tissue of the control group showed increase of glycogen particles, elongation of some cristae mitochondriales and dilated, vacuolized sarcoplasmic reticulum (Fig. 8).

DISCUSSION

For the protective action against total body X- or gamma-irradiation the effect of methylene blue in mice and rats has been shown in the provious studies. In the internal mitochondrial structure following irradiation, most of the mitochondria display a varying degree of alterations in size and shape. Masorovsky *et al.* (1967) reported the degeneration of mitochondria as well as cytoplasm after irradiation to dorsal root ganglion neurons.

Observation of its organelle alterations and degenerations in the liver and heart tissues shows the occurrence of physical damage following irradiation. However, direct physical injury to the liver and heart tissues and its processes is not a sole factor in irradiation tissue injury (Zeman *et al.*, 1962; Harris, 1966). This type of damage could be explained indirectly through the physiologic prccesses in the cells. Among these organelles which exhibit various changes following irradiation, it is shown that mitochondria, endoplasmic reticulum including ribosome and glycogen particles were severely degenerated.

Since the endoplasmic reticulum spaces might be connected directly to the exterior of the cell, it is possible that glucose coming from outside the cell could be converted into glucose-6-phosphate in the endoplasmic reticulum lumen and thus be prevented from passing through the membrane of endoplasmic reticulum. Thus, it could pass along all the ramifications of the endoplasmic reticulum canals and come in direct contact with the nuclear fold of the reticulum (Bourne, 1962).

The sarcoplsmic reticulum was not only the subcellular site in mammalian myocardium responsible for the control of calcium involved in excitation-contraction coupling, but is also a system which has the ability to reversibly bind the calcium responsible for the degree of muscle inotropism (Langer, 1965).

In the irradiated liver and heart, abnormal dilation of

cisternal element of endoplasmic and sarcoplasmic reticulum led to the formation of large vacuoles. Vacuole of this kind might also exhibit extensive dilation in mitochondria, which were empty without any cristae. The abnormal vacuoles formed were probably linked to metabolism and membrane permeability of the cells and mitochondria.

This appeared under the condition of unbalanced metabolism, various organelles in liver were degenerated and altered, and this might produce severe damage in the process of protein synthesis. It was also noted that vacuolization in mitochondria was rare in heart muscle after irradiation in comparison to that of the liver.

Andres (1963 a) observed mitochondria with ruptured cristae mitochondriales and vacuoles as well as extensive loss of matrix element after irradiation. Such alteration was probably associated with a severe disturbance in their protein synthetic and energy-yielding enzymatic systems (Andres, 1963a). It has been suggested that degeneration and alteration in mitochondria, which contain various inner materials is influenced by oxidative phosphorylation, reduction in ATP synthesis (Lehninger, 1965), enzyme systems of Krebs citric acid and fatty acid oxidation cycles (Bacq and Alexander, 1963).

Accumulation of glycogen particles may be an indicative of a situation in which oxidative phosphorylation has become elevated locally. In glycogen synthetase systems, the supply of high energy phosphate is needed for glycogen synthesis. It is exhibited that accumulation of glycogen particles may have severely decreased its activities in local catabolism and glyconeogenesis. However, no progressive accumulation of glycogen is shown in the heart tissue of methylene blue-treated and saline-treated-groups. Thus, it is observed that methylene blue exerts a protective action in liver and heart tissue damages following gamma-irradiation,

SUMMARY

Electron microscopic examination of the liver and heart tissues of methylene blue-treated rats before gamma-irradiation was observed in this study.

1. It was observed severe alteration and degeneration of organelles: accumulation of glycogen particles, severe swollen mitochondria, and broken endoplasmic reticulum in liver tissue of saline-treated rat(control) opposed by methylene blue-treated rat at 64 and 212 hours following gamma-irradiation.

2. Heart muscles of both methylene blue-treated and saline-treated rats showed no significant alterations, but it was observed that slightly elongated mitochondria with broken cristae and some of vacuoles as well as increased glycogen particles in sarcoplasmic reticulum at 212 hours following gamma-irradiation.

3. It may be considered that methylene blue greatly reduces the sensitivities of rats to gamma-irradiation.

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