Effects of Carbon Monoxide Inhalation on the Fine Structure of the Rat Heart Muscle

Tsuneo Suzuki

*Department of Forensic Medicine (Prof. S. Akaishi),
Tohoku University School of Medicine, Sendai*

The effects of carbon monoxide inhalation on the ultrastructure of the heart muscle of rat were investigated. Rats were exposed to 1% carbon monoxide for 10 minutes and sacrificed immediately, 10 and 30 minutes, and 1, 5 and 24 hours after the cessation of carbon monoxide inhalation, and ultrastructural changes of the hearts were observed.

The cardiac ultrastructural changes consisted of intracellular edema, swelling of mitochondria and sarcoplasmic reticulum, disruption and reduction of cristae, disappearance of mitochondria, appearance of lipofuscin pigment granules and lysosomes, increase of glycogen granules and fat droplets. The ultrastructural reactions to carbon monoxide were evident in 10 minutes after carbon monoxide inhalation for 10 minutes, and became most prominent in 30 minutes to 1 hour. In 24 hours after the inhalation, the hearts of most rats appeared essentially normal.

These findings suggest that the effects of carbon monoxide on the heart result not only from hypoxemia but also from the direct toxic effects on the specific respiratory enzymes, and the duration of carbon monoxide inhalation is more important than the level of blood CO-Hb concentration in producing these changes.

In carbon monoxide poisoning, cerebral lesions have been frequently described, but the cardiac changes are not recognized in general. Cardiac involvement in carbon monoxide poisoning was first noted by Klebs.¹ He reported acceleration, irregularity and retardation of the heart beat, and he observed punctiform and diffuse hemorrhage in the pericardium and endocardium including the tips of the papillary muscle, and fatty dystrophy of the myocardium. Later, Herzog² reported necrosis and fatty dystrophy in the papillary muscle and elsewhere in the left chamber, and Gey³ found hemorrhage in apex of the papillary muscle, fatty dystrophy of the heart muscle, and occasional circumscribed necrosis. On the other hand, Stearns *et al.*⁴ observed the electrocardiographic changes in carbon monoxide poisoning and reported that the alterations of the T waves and the S-T segments occurred most frequently, but carbon monoxide asphyxia, even though it was severe enough to cause death, might produce only insignificant electrocardiographic changes. In the experimental carbon monoxide poisoning, Ehrich *et al.*⁵ reported inversion of T waves, heart block, and elevation of the S-T segments, and they noted degenerative change of individual muscle fibers, as well as necrosis, and hemorrhages of the myocardium.

Recently, Hayes and Hall⁶ and Anderson *et al.*⁷ emphasized the significance and potentially serious effects of carbon monoxide poisoning on the myocardium, and they presented some observations which seemed to indicate that the heart was frequently involved in carbon monoxide poisoning and that the cardiac changes were severer than previously assumed. The work of Hiden and Schulz⁸ suggested that the effects of carbon monoxide on the heart resulted not from hypoxemia alone, but from the direct poisoning of specific respiratory enzymes of the cell by carbon monoxide.

In order to investigate these possibilities, the present work was carried out on rats poisoned by inhalation of carbon monoxide.

MATERIALS AND METHODS

Thirty-seven Wistar breed rats, weighing 200 to 250 g each, were divided into two groups, the experimental and control ones. The experimental group consisted of thirty rats. They were placed, two or three at a time, in a closed large glass vessel and exposed to 1 vol.% carbon monoxide for 10 minutes, and sacrificed immediately, 10 and 30 minutes, 1, 5 and 24 hours thereafter. The seven control rats were placed, two or three at a time, in the same closed glass vessel as used in the experimental group and let inhale room air for 10 minutes and sacrificed immediately, and 1 and 5 hours thereafter.

The spectrophotometric determinations of blood carboxyhemoglobin were made on each rat at the time of sacrifice or death.

The heart was exposed, and a small piece of the papillary muscle of the left chamber was excised while the heart was beating, and fixed in cold 1% osmium tetroxide in barbiturate acetate buffer, dehydrated in a graded series of alcohol and embedded in epoxy resin. Sections were cut on a Hitachi UM-3 ultratome with glass knives and mounted on 150 mesh copper grids with collodion film and carbon coating. The grids were stained with lead citrate and uranyl acetate, and examined with a Hitachi HS-7 electron microscope. Micrographs were taken at magnifications of 4,000 to 10,000, and photographically enlarged to a desired size.

The remains of the heart were fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for examination with the light microscope.

RESULTS

Gross observations

Most experimental rats showed a rapid respiratory stimulation and fell to the ground several minutes after the start of carbon monoxide inhalation. This was followed by agitation, convulsion and very weak respiration. Three rats died within 10 minutes after the start of exposure to carbon monoxide, and 5 rats died in 30 minutes to 3 hours after the carbon monoxide inhalation for 10 minutes. The other rats recovered from these symptoms within one to two hours after the carbon monoxide inhalation. On the other hand, the control rats did not show any abnormal signs, while they were in the closed large glass vessel and even after they were released from it.

CO-Hb concentration

CO-Hb concentration of the poisoned rats reached 65.5~68.0% immediately after carbon monoxide inhalation for 10 minutes. Thereafter, it descended

Fig. 1.

promptly to 45.5~51.0% in 10 minutes, 18.5~22.0% in 30 minutes, and 10.1~13.2% in 1 hour after carbon monoxide inhalation. The level of CO-Hb concentration of the rats which died during and after the carbon monoxide inhalation was almost the same as that of the rats sacrificed at the corresponding times. In five and twenty-four hours after the carbon monoxide inhalation, the CO-Hb level was estimated at almost zero, as that of control rats (Fig. 1).

Light microscopic observations

Light microscopic findings of the hearts of poisoned rats were essentially normal except that some sections showed slight hyperemia and punctiform hemorrhage. All the sections from control rats did not show any abnormal findings.

Electron microscopic observations

1) *Control rats.* The fine structure of the heart muscle was similar to that previously reported. There was no change attributable to confinement in the large closed vessel for 10 minutes (Fig. 2).

2) *Carbon monoxide poisoned rats.* The hearts of the rats killed immediately after carbon monoxide inhalation for 10 minutes did not show any abnormal findings. In the rats killed 10 minutes after carbon monoxide inhalation, intracellular edema was often quite severe and extensive. In some muscle fibers, swelling of sarcoplasmic reticulum, moderate or severe swelling of mitochondria and diminution of density of mitochondrial matrix were noted. The cristae were generally sparse, and in some sections disorganization and disruption of the cristae were observed. Some mitochondria lost their external membranes. The myofibrils stretched slightly or moderately with lengthening of the I bands. Glycogen particles and fat droplets were moderately increased (Figs. 3 and 4).

In the rats sacrificed 30 minutes and 1 hour after carbon monoxide inhalation,

the most striking findings were disappearance of the external membranes and disruption of cristae of mitochondria (Figs. 5 and 6). Beneath the sarcolemma, especially in the part adjacent to the capillary endothelial cells, disappearance of mitochondria was prominent (Figs. 7 and 8). Among the disrupted mitochondria, there were aggregates of dense granules of varying size with or without fat droplets. The granules had not a constant or characteristic structure, and no limiting membrane was seen around any single granule. From these findings, the aggregates were thought to be lipofuscin pigment granules (Figs. 5 and 9).⁹ In some of other sections, several homogeneous dense bodies surrounded by a limiting membrane were noted. Some of them contained granular material. They seemed to be lysosomes (Fig. 6).⁹ In these groups, moderate lengthening of sarcomeres without disruption of myofibrils was observed, but intracellular edema was slight (Figs. 7 and 8).

In the rats sacrificed 5 hours after carbon monoxide inhalation, there were many mitochondria of which cristae were sparse but not disorganized among the disrupted mitochondria. In some muscle fibers, moderate increase in the density of the mitochondrial matrix and densely arranged cristae were noted. The vesicles of sarcoplasmic reticulum were slightly swollen. Glycogen granules and fat droplets increased moderately. Some fat droplets showed shrinkage. The contractile elements were almost normal. In some fibers, partial disappearance of the nuclear membrane was observed (Figs. 9 and 10).

In the rats sacrificed 24 hours after carbon monoxide inhalation, most hearts appeared to be essentially normal. Disruption of mitochondria or disorganization of cristae were scarcely noted. Neither lipofuscin pigment granule nor lysosome was observed. In a few fibers, there were many mitochondria with branching cristae, and slight intracellular edema was noted. Several fat droplets were seen adjacent to the mitochondria and most of them showed shrinkage (Fig. 11).

DISCUSSION

The mechanism of the deleterious effect of carbon monoxide is generally sought in anoxia. According to previous reports, the light microscopic and the electrocardiographic changes of the hearts in carbon monoxide poisoning closely resemble those observed in anoxia due to other causes. The anoxia in carbon monoxide poisoning arises in two ways: Firstly, the combination of carbon monoxide with hemoglobin renders the oxygen carriage incapable. This combination is facilitated by the great affinity of carbon monoxide for hemoglobin which exceeds 200 times that of oxygen. Secondly, an alteration occurs in the oxygen dissociation curve of the uncomplexed hemoglobin, which is brought about by the presence of CO-Hb. On the other hand, Roughton and Root¹⁰ suggested an initial, temporary, extra-circulatory and reversible combination of carbon monoxide with other iron-containing pigments. Apart from combination with hemoglobin, carbon monoxide combines also with myohemoglobin and other hem compounds such as the respiratory enzymes, cytochrome oxidase and so on. These compounds are said to dissociate so readily that they are probably not of physiological importance, but this raises the

toxicity of carbon monoxide through a route other than oxygen lack, that is, direct interference with the enzymatic oxidative processes of the tissues.

In the present work, the experimental animals were let inhale 1% carbon monoxide in a closed glass vessel for 10 minutes. The effects of decrease of oxygen in the vessel was negligible, because the control rats confined in it for 10 minutes did not show any abnormal signs, and there was no ultrastructural change in the heart muscle.

The main electron microscopic changes of the heart muscle in the present study were intracellular edema, swelling of mitochondria and the vesicular transformation of sarcoplasmic reticulum, diminution of mitochondrial matrix density, reduction and disruption of cristae, disappearance of mitochondria especially beneath the sarcolemma, appearance of lipofuscin pigment granules and lysosomes, and increase of glycogen granules and fat droplets.

Some investigators¹¹⁻¹³ have obtained the above findings except disappearance of mitochondria and appearance of lipofuscin pigment granules and lysosomes in the anoxic or ischemic heart muscle. These reports suggest that the changes observed in the present experiment were caused by anoxic effects of carbon monoxide. Of the above findings, the disappearance of mitochondria was noted in the heart muscle of rats in cyanide poisoning and in alcoholic cardiomyopathy.^{14,15} In these cases the disappearance of mitochondria seemed to have resulted from disruption caused by direct toxic actions of cyanide and alcohol. In carbon monoxide poisoning, direct toxic effects of the poison would have also caused severe disruption of the whole mitochondria, and it was suggested that the oxidative enzymes in the heart muscle were severely inactivated. The partial disappearance of the nuclear membrane observed in 5 hours after carbon monoxide inhalation seemed also to be due to partial disruption of the nuclear envelope by the direct toxic action of carbon monoxide.

Increase of fat droplets and glycogen granules may be attributed to interference of metabolism by anoxia and extensive mitochondrial disruption or disappearance. The shrinkage of fat droplets seen 5 and 24 hours after carbon monoxide inhalation was probably related with edema around the fat droplets, and this finding suggested recovery of fat metabolism. The lipofuscin pigment granules such as those found in 1 and 5 hours after carbon monoxide inhalation had been also observed by Malkoff and Strehler¹⁶ in the aged human hearts and by the present author¹⁷ in the rat hearts several hours after death. These granules were considered to develop from autolysis of fat.⁹ The process seemed to be concerned with severe anoxia in carbon monoxide poisoning.

It is accepted in general that lysosomes provide the enzymes for some materials taken into the cell, but the lysosomal enzymes are released and digest the cell on starvation or cell death.⁹ They have been scarcely observed in normal heart muscle, but they are often found in the heart muscle with extensive disruption of cell organellae.¹⁵ In carbon monoxide poisoning, the appearance of lysosomes also seemed to be due to extensive disruption of cell organellae.

In common with the effects of hypoxemia on the heart muscle, the above-mentioned myocardial ultrastructural changes in carbon monoxide poisoning suggest that carbon monoxide has really a direct toxic effect on the specific respiratory enzymes of the mammalian heart muscle, and these ultrastructural changes may be a major factor contributing to functional disturbances of the heart in carbon monoxide poisoning. The lengthening of sarcomeres observed in 10 minutes to 1 hour after carbon monoxide inhalation seemed to be a finding showing disturbance of contractility of the heart muscle.

The changes were most prominent and extensive in the rats killed 30 minutes and 1 hour after carbon monoxide inhalation. On the other hand, CO-Hb level was 65.5~68.0% immediately after carbon monoxide inhalation, but they decreased soon after the cessation of inhalation, and became almost normal within two to five hours. These results revealed that the severity of the ultrastructural changes were most prominent after the CO-Hb concentration had fallen below the toxic level. Carbon monoxide, however, was detected in blood for one to two hours and the heart continued to be affected for this period. The severity of the ultrastructural changes seemed to be concerned with the duration of affection of carbon monoxide to the heart rather than the concentration of CO-Hb itself.

According to Anderson *et al.*,⁷ the electrocardiographic abnormalities in carbon monoxide poisoning frequently occur after acute episode has subsided and the CO-Hb concentration has been well below toxic level. Takahashi¹⁸ observed also that there was no intimate correlation between the degree of electrocardiographic changes and the CO-Hb levels of the blood. From these reports and the results of the present study, the functional cardiac disturbances in carbon monoxide poisoning seems to be due to direct damage to the heart muscle and not due to hypoxia *per se*.

In five hours after carbon monoxide inhalation, the ultrastructure of the hearts showed that some recovery processes such as shrinkage of fat droplets and increased opacity of mitochondrial matrix started in the damaged muscle fibers. In 24 hours after inhalation, most muscle fibers appeared essentially normal except for some fibers in which there were a number of shrunk fat droplets and many mitochondria with branching cristae. The branching of cristae seemed to indicate regeneration of damaged cristae. These results show that the ultrastructural changes caused by carbon monoxide become less severe with disappearance of CO-Hb and almost recover in 24 hours after carbon monoxide inhalation.

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Legends for Figs. 2-11

ss, mitochondrion	s, sarcomere
cr, crista	n, nucleus
sr, sarcoplasmic reticulum	nm, nuclear membrane
sl, sarcolemma	f, fat droplet
mf, myofibril	g, glycogen granule
z, Z band	lg, lipofuscin pigment granule
m, M band	ly, lysosome
a, A band	e, edema
i, I band	ce, capillary endothelial cell
	r, red blood cell

Fig. 2. Longitudinal section of rat heart muscle 30 minutes after the room air inhalation in the closed vessel (control). No change attributable to confinement in the closed vessel. $\times 20,000$.

Fig. 3. Longitudinal section of rat heart muscle 10 minutes after carbon monoxide inhalation. Intracellular edema and swelling of sarcoplasmic reticulum are prominent. Mitochondria show moderate swelling, diminution of matrix density and reduction of cristae. Myofibrils are moderately stretched. Some fat droplets are noted. $\times 18,000$.

Fig. 4. Longitudinal section of rat heart muscle 10 minutes after carbon monoxide inhalation. Moderate swelling of mitochondria, reduction and disorganization of cristae, and diminution of matrix density of mitochondria are noted. Numerous glycogen particles are seen adjacent to the mitochondria. $\times 30,000$.

Fig. 5. Longitudinal section of rat heart muscle 30 minutes after carbon monoxide inhalation. Disappearance of external membranes of the mitochondria and disruption of cristae are prominent. Among these mitochondria, an aggregate of dense granules with a fat droplet is noted (lipofuscin pigment granule). $\times 20,000$.

Fig. 6. Longitudinal section of rat heart muscle 1 hour after carbon monoxide inhalation. Dense bodies containing granular material surrounded by a limiting membrane (lysosomes) are noted among the disrupted mitochondria. $\times 17,000$.

Fig. 7. Longitudinal section of rat heart muscle 1 hour after carbon monoxide inhalation. Note extensive disappearance of mitochondria beneath the sarcolemma adjacent to the capillary endothelial cell. $\times 9,000$.

Fig. 8. Longitudinal section of rat heart muscle 1 hour after carbon monoxide inhalation. Extensive disappearance of mitochondria beneath the sarcolemma and between the myofibrils is noted. The length of sarcomere increases moderately. $\times 9,000$.

Fig. 9. Longitudinal section of rat heart muscle 5 hours after carbon monoxide inhalation. Many small mitochondria with sparse cristae are observed. The sarcoplasmic reticulum are moderately swollen. Partial disappearance of nuclear membrane is noted. Fat droplets and lipofuscin pigment granules are also observed. Glycogen granules increase slightly. $\times 22,000$.

Fig. 10. Longitudinal section of rat heart muscle 5 hours after carbon monoxide inhalation. In some mitochondria, the matrix density is moderately high and the cristae are densely arranged. But there are many disrupted mitochondria. A shrunk fat droplet is observed. $\times 22,000$.

Fig. 11. Oblique section of rat heart muscle 24 hours after carbon monoxide inhalation. Disruption of mitochondria and disorganization of cristae are scarcely observed. There are many mitochondria with branching cristae. Several shrunk fat droplets and slight intracellular edema are noted. $\times 24,000$.