Effect of Perfluorochemical (PFC) Emulsion on Acute Carbon Monoxide Poisoning in Rats

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ABSTRACT: The effect of perfluorochemical (PFC) emulsion (25 w/v per cent of PFC concentration) on carbon monoxide poisoning was studied in rats exposed to 97 per cent O_2 and three per cent CO. There was no significant difference in conversion rate of hemoglobin to carboxyhemoglobin (COHb) between PFC emulsion and saline groups. However, after re-exposure to pure oxygen, PFC emulsion group circulation reconverted to oxyhemoglobin (oxyHb) at a significantly faster rate than circulation in the control group. Survival time of rats was considerably affected by infusion of PFC emulsion and prolonged in proportion to increased injection dosage. Significant elevations in plasma glucose, lactate and aldolase levels and lactate/pyruvate ratio were found in the saline group and these levels remained well within normal range in the PFC emulsion group. These results indicate that PFC emulsion can function as an oxygen carrier in the presence of carbon monoxide and can deliver sufficient oxygen to peripheral tissues.

KEY WORDS: perfluorochemical emulsion, artificial blood substitute, acute carbon monoxide poisoning, oxygen transport.

INTRODUCTION

Carbon monoxide (CO) combines rapidly and tightly with red cell hemoglobin and disrupts the oxygen transport system. The affinity of CO for hemoglobin is 230–270 times greater than that of O_2. Oxygen combined with hemoglobin is rapidly displaced by CO, resulting in a reduction of O_2 carrying capacity.[7] Since CO causes tissue hypoxia resulting in serious manifestations, including death, the primary treatment for CO poisoning is to supply enough O_2 and the more O_2 is administered, the more effective the treatment will be.[8,18]

Due to its high oxygen solubility, perfluorochemical (PFC) emulsion functions as an oxygen carrying agent. Pure oxygen at atmospheric pressure can result in dissolved oxygen content as high as seven per cent in 25 w/v per cent PFC emulsion at 37 °C, whereas it is only 2.1 per cent in plasma.

Since A-V O_2 difference in the whole body is usually around five vol. per cent, theoretically, in pure oxygen breathing, infusion of a PFC emulsion containing 25 w/v per cent PFC and in volume identical to circulating blood volume, can supply a sufficient amount of oxygen, although hemoglobin in the circulation may be completely inactivated by CO.

In experimental animals, the injection or total exchange of blood with PFC emulsion significantly increased CO tolerance levels.[6,17] Sloviter et al.[17] found that normal mice given PFC emulsion intravenously continued to survive as long as two hours in an atmosphere of 96 per cent O_2 and four per cent CO, while control animals without PFC emulsion

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died within 10 min, and Geyer[6] reported that rats totally exchange-transfused with perfluorotributylamine emulsion stabilized with pluronic F68 survived for 24 hours or longer in an atmosphere of 90 per cent O_2 and 10 per cent CO. This suggests the possibility of clinical application of PFC emulsion in carbon monoxide poisoning. The present study was undertaken to evaluate the effects of PFC emulsion on acute carbon monoxide poisoning in animals.

Materials and Methods

1. Materials

PFCs utilized in this study were perfluorodecalin (FDC, Imperial Smelting Co., U.K.) and perfluorotripropylamine (FTPA, Dainippon Ink Co., Ltd., Japan). A mixture of FDC and FTPA at a ratio of 7:3 by weight was emulsified with both Pluronic F68 (Asahi-denka Co., Ltd., Japan) and a small amount of yolk phospholipids (Vitrum AB, Sweden) by the method of Yokoyama et al.[19]

Just prior to injection, the emulsion was made physiologically isotonic with a proper amount of sodium chloride and the emulsion composition was 25 w/v per cent PFCs (17.5 per cent FDC and 7.5 per cent FTPA), 3.4 w/v per cent Pluronic F68, 0.4 w/v per cent yolk phospholipids and 0.9 w/v pre cent sodium chloride.

2. CO Exposure Experiments

The gas consisting of 97 per cent O_2 and three per cent CO was prepared in a mixing chamber by mixing pure O_2 and CO, introduced into a CO exposure chamber (10 l glass vessel), and the mixed gas flow rate was maintained at a constant rate of 4 l/min during the experiment.

Wistar male rats weighing 150–180 g were injected intravenously at an injection rate of 2.5 ml/kg/min with PFC emulsion at dosage levels ranging from 20–80 ml/kg body weight, corresponding to 5–20 g/kg PFC. As a controls, rats were injected with 80 ml/kg of saline. Five to ten rats injected with either the emulsion or saline were placed in the CO exposure chamber which was equilibrated with 97 per cent O_2 and three per cent CO at atmospheric pressure and survival times were determined. In addition, rats were sacrificed at various CO exposure intervals and changes in blood CO and O_2 levels, plasma carbohydrate contents (glucose, lactate and pyruvate) and lactic dehydrogenase (LDH) and plasma aldolase activity were determined.

3. Determination of COHb, O_2 and CO Content in Blood

As soon as possible after sacrifice, heparinized arterial blood of rats exposed to CO was drawn anaerobically from the left ventricle with a 5 ml glass syringe and gently transferred to a 10 ml glass test tube containing 0.5 ml liquid paraffin to prevent dissociation of CO from hemoglobin.

Blood COHb content was determined by the spectrophotometric method of Yuzuriha,[21] O_2 content in blood or PFC emulsion measured by a Lexington O_2 Con (Lexington Instruments, USA), and CO content in blood or PFC emulsion determined by the gas chromatographic method of Blackmore.[1]

4. Carbohydrate Analysis

Plasma glucose levels were determined by the glucose oxidase method[9] and a commercial enzymatic glucose reagent kit (International Reagents Co., Japan) was used in this study. The plasma lactate and pyruvate levels were determined by the enzymatic method using lactic dehydrogenase.[10] A commercial lactate-UV-test kit (Boehringer,

Mannheim, W. Germany) was used for determining lactate and a commercial pyruvate-UV-test kit (Boehringer, Mannheim, W. Germany) for pyruvate.

5. Determination of Lactic Dehydrogenase (LDH) and Aldolase Activity

Plasma LDH was determined by the method of Raabo[13] using a commercial test kit (Tetra-Form LDH Set, International Reagents Co., Japan) and plasma aldolase activity was determined by the method of Sibley and Lehninger[16] using a commercial aldolase test kit (Sigma Chemical Co., USA).

6. Histological Studies

Rats intravenously injected with either 40 ml/kg PFC emulsion or the same volume of saline were exposed for 30 min to 97 per cent O_2 and three per cent CO. The rats were subsequently anesthetized with sodium thiopental and the brain, lungs, heart, liver and kidneys were removed for histologic study. The tissues were either fixed for electron microscopy by immersion in 2.5 per cent glutaraldehyde followed by postfixation in osmium, or in 10 per cent formalin for conventional light microscopy which was performed with hematoxylin-eosin stain. For electron microscopy, the tissues were routinely processed and embedded in epoxy resin. The electron microscope used in this study was JEOL Electron Microscope Model JEM-T7 (Japan Electron Optics Laboratory Co., Ltd., Japan).

Results

In a preliminary experiment, the tolerance levels to CO in rats injected with either the PFC emulsion or saline were studied to select suitable experimental conditions for various CO concentrations.

Rats injected with either 40 ml/kg body weight of PFC emulsion or the same volume of saline were allowed to breathe O_2 and CO mixtures at mixing ratios of 99:1–90:10 by volume and survival times were determined (Table 1). The animals given PFC emulsion had a very high CO tolerance as compared with the saline group. Breathing 97 per cent O_2 and three per cent CO, half the rats in the PFC group survived for more than 10 hr, while rats in the saline group died within 30 min. Survival time was prolonged in both the PFC and saline groups by decreasing carbon monoxide concentration. Almost all saline treated animals which were exposed to higher concentrations of CO (e.g. five per cent and 10 per cent CO) died within 10 min, and the biochemical changes effected by CO exposure could not be clarified due to the high acute toxicity of CO. Therefore, we used the mixture of 97 per cent O_2 and three per cent CO as the exposure gas.

Fig. 1 shows the effects of PFC emulsion on the conversion and reconversion rate of

Table 1. Half lethal time of rats exposed to various concentrations of carbon monoxide (n=10)

Exposure gas	Half lethal time	
	Saline group*	PFC emulsion group*
99% O_2- 1% CO	86 min.	22 hrs.
97% O_2- 3% CO	30 min.	10 hrs.
95% O_2- 5% CO	9 min.	6 hrs.
90% O_2-10% CO	6 min.	3 hrs.

*Rats were injected with either 40 ml/kg of the PFC emulsion or same volumes of saline.

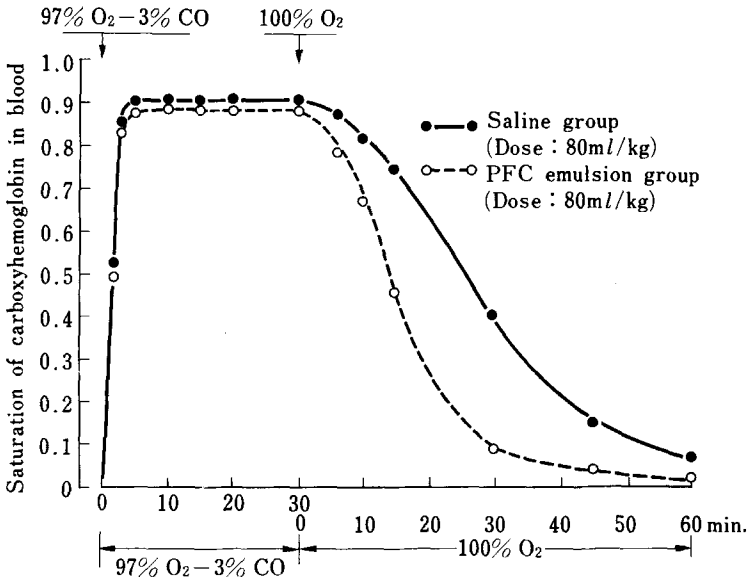

Fig. 1. Effect of PFC emulsion on conversion and reconversion of oxyhemoglobin to and from carboxyhemoglobin in rats exposed to carbon monoxide

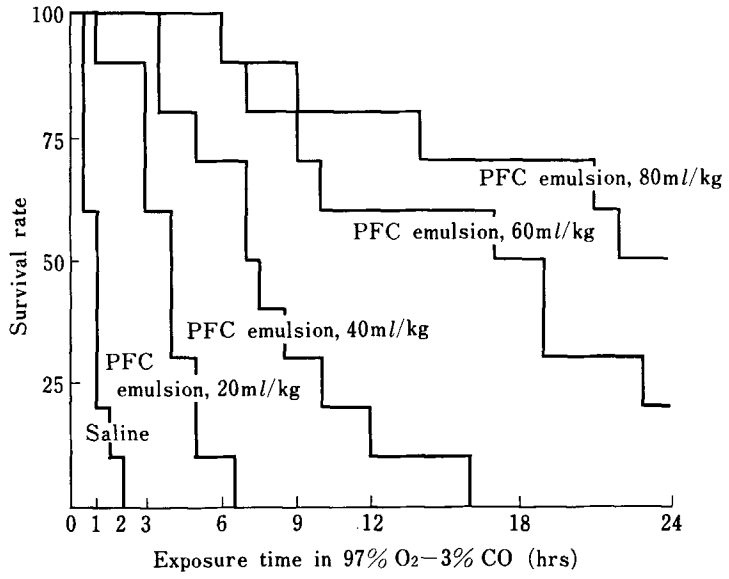

Fig. 2. Effect of PFC emulsion dosage on tolerance levels of carbon monoxide in rats kept in an atmosphere of 97% O_2-3% CO

oxyHb to and from COHb in rats exposed to CO. The saturation rate of COHb was very rapid in both groups and within five mins. the COHb content reached maximum values as great as 90 per cent, which were subsequently maintained at that level throughout exposure. No difference in the conversion rate of oxyHb was found between the two groups.

However, after re-exposure to pure O_2, COHb in rats receiving PFC emulsion was reconverted to oxyHb at a significantly faster rate than in the saline group.

In order to evaluate the effect of PFC emulsion on CO tolerance levels, rats were intravenously injected with either the emulsion or saline and exposed to 97 per cent O_2 and three per cent CO, and survival time was determined (Fig. 2). Rats injected with emulsion survived considerably longer than rats from the control group under conditions in which O_2 transport by erythrocytes was almost completely blocked and survival time was prolonged in proportion to increased injection dosage. The half lethal time was calculated to be 30 min. in the saline group and 4, 8, 10 and 20 hr. in the groups administered 20, 40, 60 and 80 ml/kg of emulsion, respectively. The progressive prolongation of survival time by infusion of PFC emulsion was due to the increase of delivered O_2, the amounts of which were calculated from A-V O_2 differences (Fig. 3). The arterial O_2 content in the group administered 80 ml/kg emulsion was about five vol. per cent which was three times higher than in the saline group. The increase in the arterial O_2 content from 2.9 to 6.1 vol. per cent was attributed to 3.2 vol. per cent O_2 dissolved in the PFC particles.

Subsequently, the effect of PFC emulsion on plasma glucose, lactate, pyruvate, LDH, and aldolase levels in rats exposed to CO was examined. Variations in plasma glucose, lactate and pyruvate levels and lactate/pyruvate (L/P) ratio in the emulsion and saline groups are shown in Table 2. Within 15 min after exposure to CO, saline group glucose levels rapidly reached values 1.5 times above normal and glucose remained at the high levels until death. On the other hand, glucose levels in the PFC emulsion groups tended to decrease with lapse of time and a similar phenomenon was observed in lactate levels. Exposure to CO effected a significant increase in lactate levels of the saline groups and the maximal value was found to be about three times above normal. In the group receiving 80 ml/kg PFC emulsion, lactate levels remained within normal range during six hours after exposure, however, the levels were found to increase slightly in the low doasge group.

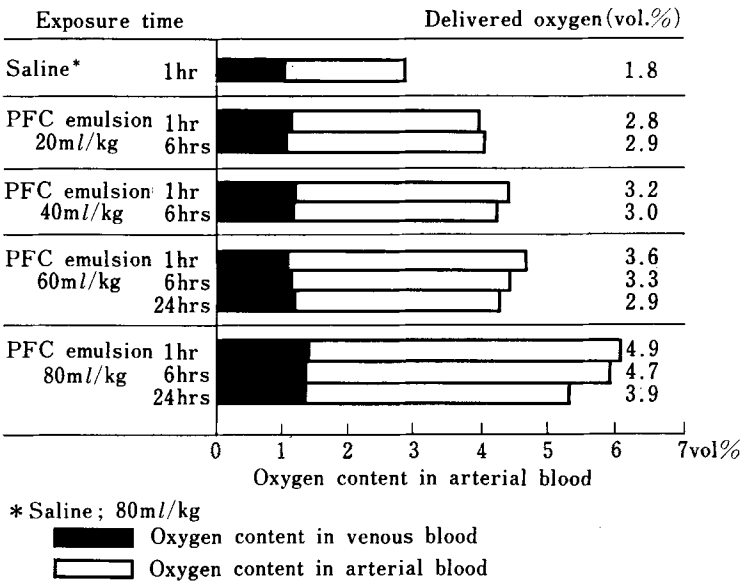

Fig. 3. Oxygen content in arterial and venous blood and subsequently calculated delivered oxygen in rats kept in an atmosphere of 97% O_2-3% CO

Table 2. Changes of plasma levels of glucose, lactate, pyruvate and lactate/pyruvate ratio in rats exposed to 97% O_2-3% CO. (n=4–5)

	Exposure time	Glucose (mg%)	Lactate (mg%)	Pyruvate (mg%)	L/P Ratio
Normal	0	245±36*	77±21	1.34±0.29	55.3± 5.9
Saline 80 ml/kg	30 min.	410±49	205±20	1.90±0.37	111.3±16.5
	60 min.	400±39	227±29	1.76±0.53	127.9±14.3
PFC emulsion 40 ml/kg	30 min.	255±33	107±23	1.64±0.22	66.5±12.4
	60 min.	173±20	98±17	1.76±0.53	57.3± 9.8
	120 min.	185±29	93±21	1.50±0.19	59.0±11.0
	180 min.	129±34	118±19	1.59±0.20	73.1±18.4
PFC emulsion 80 ml/kg	30 min.	226±30	74±15	1.38±0.18	56.0± 9.4
	60 min.	195±21	70±16	1.43±0.12	48.2±10.7
	120 min.	158±23	73±10	1.61±0.23	49.9±13.2
	180 min.	72±26	87±17	1.52±0.25	58.6±15.1

*Mean±S.E.

Table 3. Changes of plasma LDH and aldolase activities in rats exposed to 97% O_2-3% CO

	Exposure time	LDH*	Aldolase**
Normal	0	239±36	3.1±2.2
Saline 80 ml /kg	30 min.	274±25	6.0±1.2
	60 min.	306±29	13.9±1.7
PFC emulsion 40 ml/kg	30 min.	220±41	4.1±0.9
	60 min.	202±19	5.3±1.6
	90 min.	265±23	6.1±1.8
PFC emulsion 80 ml/kg	30 min.	247±53	3.0±0.4
	60 min.	258±31	3.9±1.9
	90 min.	244±26	4.2±1.3

* LDH: Wacker unit.
** Aldolase: micromole FDP split/ml/hr

A slight increase in plasma pyruvate was observed in all groups. No significant difference was found between the PFC emulsion and control groups. In the control group, the L/P ratio rapidly increased up to 130 with in one hour after exposure as compared to 52 for normal L/P ratio, while in both PFC emulsion groups (40 and 80 ml/kg), no significant increase in L/P ratio was found during six hours.

Table 3 shows variations in plasma LDH and aldolase activities in rats injected with either the PFC emulsion or saline. A slight tendency toward increased LDH activities was found in all groups, but these increases did not differ significantly from normal values. On the other hand, aldolase activity in rats receiving PFC emulsion increased slightly with passage of time but no significant difference was found as compared to normal levels. In the control group the activity was doubled by exposure for 30 min and increased five times by exposure for 60 min.

Light microscopic findings revealed that significant changes had occurred only in the

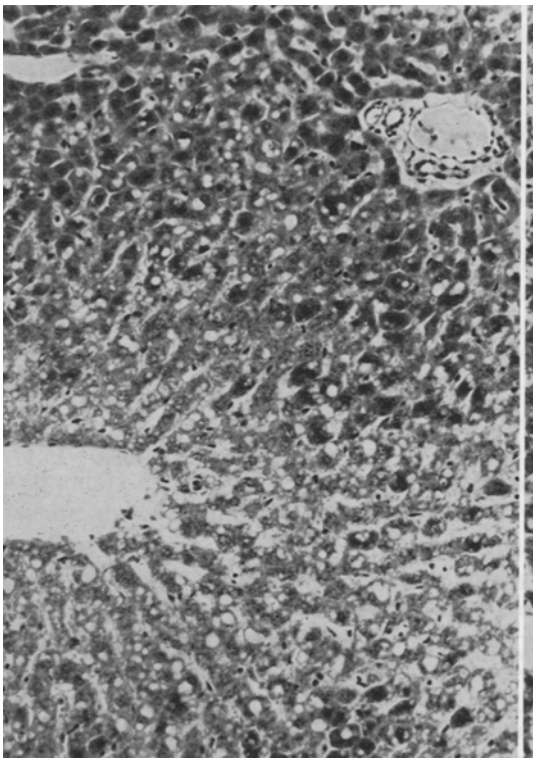

Fig. 4. Liver of rat in saline group (hematoxylin-eosin)

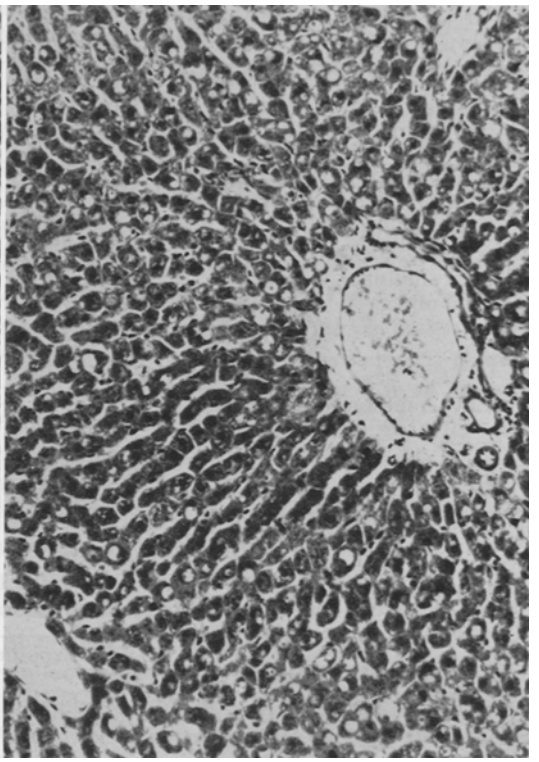

Fig. 5. Liver of rat in PFC emulsion group (hematoxylin-eosin)

livers of control rats. No significant changes in brain, heart and kidneys was found in either saline or PFC emulsion groups. In the lungs, slight edematous changes were recognized in the perivascular interstitial tissues; the alveolar walls were normal. In the saline group, hepatocytes were coarsely vacuolated and basophilic granules were observed in the cells located near the center of lobules (Fig. 4). In addition, swelling of the sinusoid was observed. Similar but less pronounced changes were found in the PFC emulsion group (Fig. 5) and only a few necrotic and/or vacuolated cells were noted near the center of lobules.

Saline group electron micrographs of the liver showed dense osmiophilic mitochondria and disappearance of the mitochondrial granules. In the hepatocytes, many vacuoles of low density caused by autolysis were noted. The endoplasmic reticulum was rough and organellae were damaged (Fig. 6). In the PFC emulsion group, similar but far less extensive changes were noted (Fig. 7). No vacuoles caused by autolysis were found but occasional vescicles containing small vacuoles surrounded by membranes were noted. These vacuoles were PFC particles and were commonly isolated from organelles with electron dense membranes. Some disappearance of granules and increased density of mitochondria was present, as well as slight alterations of the endoplasmic reticulum.

Discussion

In 1966 Clark[3] demonstrated that rats were able to survive by breathing O_2-saturated fluorocarbon liquids. Animals not only survived during the breathing periods but also maintained good health after having been returned to air. In 1968 Geyer[4] reported that rats whose blood had been replaced by fluorocabon emulsion survived for eight hours when kept in an atmosphere of pure O_2. These findings pointed out both the O_2 carrying capacity and low toxicity of these emulsion compounds.

Fluorocarbon emulsions have stimulated great interest as a possible whole blood substitute in transfusion.[5,11,12,20] Oxygen is highly soluble in PFCs and the amount of carried O_2 depends on its partial pressure. Liquid PFCs dissolve 40 per cent or more oxygen in an atomosphere of pure O_2, whereas normal saline or blood plasma dissolves only three per cent or less.[12,20] PFCs also highly dissolve CO (38 vol. per cent at 37 °C) as well as O_2 and their solubility increases linearly with CO partial pressure according to Henry's law.

Carbon monoxide competes with O_2 for binding sites on the hemoglobin molecules, and a very small concentration of CO causes high COHb due to the high affinity of CO to hemoglobin. In addition, high blood concentrations of COHb increase the affinity of O_2 for hemoglobin, resulting in the impediment of O_2 supply from blood to tissues. Actually, only 0.2 per cent of CO in atmosphere resulted in an equilibrium of 80 per cent or higher COHb in blood.[18]

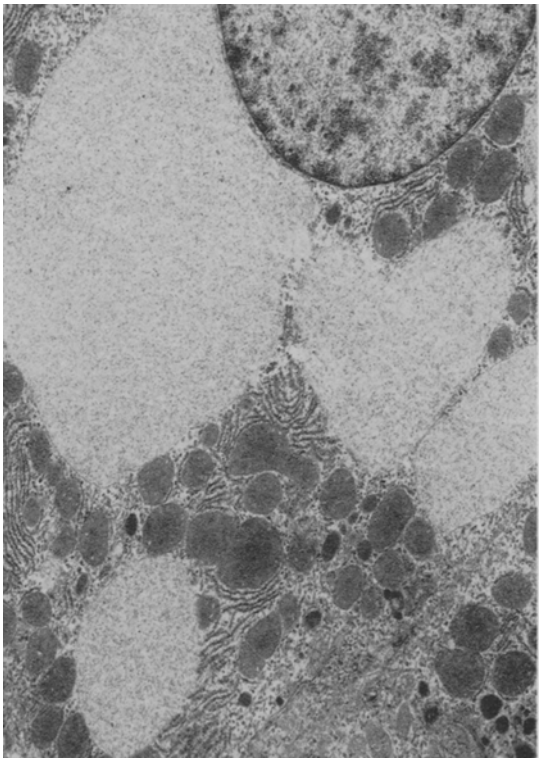

Fig. 6. Electron micrograph of hepatocyte in saline group

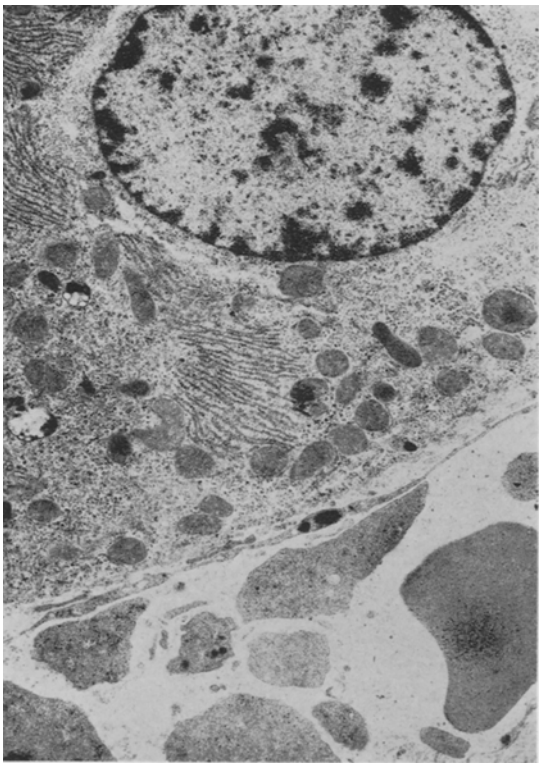

Fig. 7. Electron micrograph of hepatocyte in PFC emulsion group

PFCs can function as oxygen carrying agents even in the presence of CO, as their carrying capacity is governed only by O_2 partial pressure. The infusion of PFC emulsion significantly prolonged survival time of rats exposed to CO. The O_2 content in arterial blood of rats injected with 80 ml/kg of PFC emulsion containing 25 w/v per cent of PFCs was approximately six vol. per cent, while in the saline groups it was only three vol. per cent, when rats were exposed to 97 per cent O_2 and three per cent CO. The amount of O_2 delivered to peripheral tissues in the PFC emulsion group (calculated from A-V O_2 difference) was around five vol. per cent. Since A-V O_2 difference is usually five to six vol. per cent, PFC emulsion infusion of 80 ml/kg can supply the necessary amount of O_2 even when animals are exposed to 97 per cent O_2 and three per cent CO. The PFC emulsion also significantly accelerated the reconversion rate of COHb to oxyHb after reexposure to pure oxygen. Because COHb concentrations are not dependent on the concentration of CO alone but on the ratio of CO to O_2, this rapid elimination of COHb by infusion of PFC emulsion is interpreted in terms of high O_2 supply capacity of PFC.

Carbon monoxide effect impediments in the metabolic system, especially affecting carbohydrate metabolism. It has been reported that significant increases in plasma glucose and lactate levels were found in animals exposed to CO.[14,15] The increase in blood lactate level is a feature of hypoxia and the levels of excess lactate correspond to the degree of hypoxia. The L/P blood ratio is also an indicator of oxygen debt, reflecting the tissue redox state. The markedly increased plasma lactate level and L/P ratio during exposure to CO in saline group indicate that the injected saline is unable to transport

sufficient O_2 to tissues, as reflected by the survival rate in this group. On the other hand, since only a slight increase of lactate and L/P ratio was found in animals receiving PFC emulsion, PFC emulsion administration may possibly alleviate tissue hypoxia by its high O_2 carrying capacity.

Plasma aldolase levels in rats injected with saline were markedly elevated by exposure to CO, resulting in the impediment of carbohydrate metabolism in muscle.[2] The activity in plasma of rats with the PFC emulsion, however, was maintained within normal range, suggesting that infusion of PFC emulsion controlled the abnormality of carbohydrate metabolic system in muscle.

Light and electron microscopic histological studies also indicated the effectiveness of PFC emulsion in carbon monoxide poisoning. In the saline group, severe alterations with many necrotic cells were found in hepatocytes. Many vacuoles caused by autolysis and the disappearance of the mitochondrial granules were observed. On the other hand, similar but far extensive changes were found in the PFC emulsion group. Only a few necrotic cells with vacuoles were noted near the center of lobules. These findings indicate that tissue hypoxia caused by CO was significantly alleviated by the infusion of PFC emulsion.

The present results indicate that the infusion of PFC emulsion markedly increases the tolerance level to CO in animals and alleviates damage caused by hypoxia, suggesting possible clinical application of PFC emulsion in acute carbon monoxide poisoning.

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