Acute hypoglycemia in rats after injection of tin-protoporphyrin and photoirradiation

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ABSTRACT

The combination of injection of tin-protoporphyrin IX and photoirradiation produced an acute decrease in serum glucose levels in rats, as well as increased serum lactate dehydrogenase activity, and increased serum levels of creatinine and potassium. A reduction in the serum levels of glucose was particularly noticeable. The severe hypoglycemia may be one of the main causes of death in such rats.

Rats injected with tin-protoporphyrin (SnPP) react with neurological signs, changes in blood pressure and heart rate (2), and eventual death (3). Under photoirradiation, SnPP-injected suckling rats suddenly become agitated, tumbling on the floor, and squeaking. Then they become inactive and finally die. It has been assumed that these responses are caused by the photosensitizing effects of SnPP (4). Significant increases in lipid peroxide in rat blood has been detected both *in vivo* (2) and *in vitro* (5). In this context, the present study was designed to evaluate the biological effects of photoirradiation on SnPP-treated suckling rats.

SnPP was purchased from Porphyrin Products (Logan, UT, U.S.A.). All solutions were prepared under dim light just before use. Rats were injected subcutaneously with SnPP (10 nmol/g body weight) and immediately placed in a light box for photoirradiation as described previously (3). Control rats were injected with physiological saline. Biochemical analysis of serum levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactate dehydrogenase, alkaline phosphatase, total protein, albumin, urea nitrogen, creatinine, total bilirubin, Na⁺, K⁺, Cl⁻, Ca²⁺ and Pi was carried out using 20-day-old rats. Blood Na⁺, K⁺, Ca²⁺ and Cl⁻ levels were measured by using electrodes. Pi, alkaline phosphatase, GOT,

GPT, and lactate dehydrogenase levels were measured by methods of Fiske-Subbarow, King-King, Karmen, Orłowski, and Wroblewski, respectively. Total protein, albumin, urea nitrogen, creatinine and total bilirubin levels were measured by the methods of Lowry, Dumas, DAMO, Jaffe and Malloy-Evelin, respectively. Blood glucose levels were measured by hexokinase method using 10- and 20-day-old rats.

Results of the biochemical analysis of the serum from SnPP-treated, photoirradiated rats are shown in Table 1, in comparison with those from control rats. There were no significant differences between irradiated and non-irradiated control rats and between these rats and SnPP-treated but non-irradiated rats. In SnPP-treated, photoirradiated rats, increases in lactate dehydrogenase activity and in serum levels of creatinine and K⁺ ions were significant ($P < 0.005$). Alkaline phosphatase activity in these rats was also significantly reduced ($P < 0.005$) and a reduction in serum levels of glucose was particularly noticeable in these rats. Of these changes, hypoglycemia seems to be the most likely cause of death in these rats. To analyze the development of hypoglycemia, 10-day-old rats were injected subcutaneously with 10 nmol of SnPP/g body weight and photoirradiated. The rats showed neurological signs as soon as photoirradiation was started. The

Table 1 *Biochemical Analysis of Serum*

Photoirradiation	Control		SnPP	
	(-) (n = 3)	(+) (n = 3)	(-) (n = 3)	(+) (n = 6)
GOT (U/l) [#]	80 ± 12.8	65 ± 3.1	79 ± 7.1	79 ± 5.1
GPT (U/l) ^{##}	12 ± 2.1	9 ± 2.1	13 ± 0.5	12 ± 2.1
Lactate dehydrogenase (U/l)	668 ± 115	620 ± 94	642 ± 88	1,045 ± 101*
Alkaline phosphatase (U/l)	630 ± 54	687 ± 21	688 ± 52	479 ± 25*
Total bilirubin (mg/dl)	1.6 ± 0.45	1.2 ± 0.48	2.5 ± 0.48	1.4 ± 0.27
Total protein (g/dl)	3.4 ± 0.16	3.1 ± 0.16	3.4 ± 0.25	3.7 ± 0.13
Albumin (g/dl)	2.4 ± 0.08	2.2 ± 0.29	2.5 ± 0.33	1.9 ± 0.05
Blood urea nitrogen (mg/dl)	21.5 ± 2.5	24.3 ± 2.1	19.3 ± 2.1	22.8 ± 2.3
Creatinine (mg/dl)	0.27 ± 0.05	0.30 ± 0.0	0.30 ± 0.05	0.50 ± 0.04*
Glucose (mg/dl)	184 ± 1.41	159 ± 17.0	184 ± 4.2	55.8 ± 14.4*
Na ⁺ (mEq/dl)	135 ± 0.0	135 ± 0.8	136 ± 0.5	136 ± 1.3
K ⁺ (mEq/dl)	4.9 ± 0.2	4.9 ± 0.1	5.2 ± 0.1	6.5 ± 0.3*
Cl ⁻ (mEq/dl)	95 ± 0.8	95 ± 0.0	95 ± 0.8	94 ± 0.7
Ca ²⁺ (mg/dl)	10.8 ± 0.1	11.3 ± 0.1	10.5 ± 0.2	9.5 ± 1.0
Pi (mg/dl)	11.5 ± 0.1	26.2 ± 10.7	31.6 ± 5.8	15.0 ± 0.5

Ten nmol of SnPP/g body weight were administered subcutaneously to 20-day-old rats with subsequent photoirradiation (60 W/m²) for 3 h. Control animals received physiological saline. For details, see the text. Each value represents the average ± SD. [#]GOT, glutamate oxaloacetate transaminase; ^{##}GPT, glutamate pyruvate transaminase; *, *P* < 0.005 compared with other three groups

Table 2 *Serum Glucose Levels before and after Photoirradiation*

	Number of rats used	Serum glucose level (mg/dl)
Before photoirradiation	3	171.8 ± 12.0
1-h Photoirradiation	4	163.4 ± 42.3
2-h Photoirradiation	4	84.9 ± 27.1
3-h Photoirradiation	5	4.4 ± 2.5

Ten-day-old rats were injected subcutaneously with 10 nmol of SnPP/g body weight. Photoirradiation was performed at an energy level of 60 W/m². Each value represents the average ± SD.

frequency of neurological signs rapidly increased and reached a maximum at 100 min, then animals became less and less active. As shown in Table 2, serum glucose levels fell to about a half the pre-irradiation levels in as little as 2 h after the start of irradiation. After 3 h, the mean serum level of glucose was extremely low and all the treated rats died within next 30 min.

In experimental animals, the combination of administration of SnPP and photoirradiation results in neurological signs, growth retardation, and/or death (2, 3, 5). It is known that SnPP acts as a photosensitizer and a photodynamic mechanism

has been implicated in the peroxidation of cell membrane lipids (7). The protective effects of L-ascorbic acid against the phototoxicity (1) support this possibility. Hyperpotassemia and the increased activity of serum lactate dehydrogenase (Table 1) are thought to be due to hemolysis (2). The moderate increase in serum creatinine levels and the decrease in alkaline phosphatase activity indicate that the combined use of SnPP and photoirradiation may produce multiorgan damage in rats. The development of severe hypoglycemia was the most marked effect of SnPP and photoirradiation together. It seems unlikely that hypoglycemia is

causally related to the appearance of neurological symptoms because 1) the neurological signs appeared prior to the manifestation of hypoglycemia, and 2) the hypoglycemia occurred in 20-day-old rats (Table 1) that never show any neurological signs under these conditions (3). The hypoglycemia was found to increase with time and serum glucose levels fell rapidly to extremely low values during 2 to 3 h of photoirradiation. Most animals died after about 3.5 h photoirradiation. The severe hypoglycemia may be causally related to mortality. It remains unclear whether SnPP-sensitized photodynamic damage to B cells in the rat pancreas is responsible for the pathogenesis of progressive hypoglycemia. Sanfey *et al.* (6) reviewed oxygen-induced acute pancreatitis. It will be necessary to examine the pancreas of SnPP-injected, photoirradiated rats.

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