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Nephrotoxicity of Pyrroloquinoline Quinone in Rats

Akiharu WATANABE, Norio HOBARA, Toshiya OHSAWA,
Toshihiro HIGASHI and Takao TSUJI

The First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

ABSTRACT

When pyrroloquinoline quinone (PQQ) was intraperitoneally injected into rats daily for 4 days at a dose of 11.5 mg/kg body weight/injection, functional and morphologic changes of the kidneys were clearly observed. The most prominent finding was necrotic and degenerative changes of the proximal tubular epithelium as well as hematuria and an elevation of serum creatinine concentration.

Key words: *Nephrotoxicity, Pyrroloquinoline quinone, Renal tubules*

Pyrroloquinoline quinone (4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]-quinoline-2,7,9-tricarboxylic acid, PQQ), a newly discovered coenzyme, functions as the prosthetic group of several dehydrogenases and oxidases in microorganisms[1]. A stimulative effect of PQQ on microbial growth has been reported[2]. Moreover, a recent study revealed PQQ in mammalian amine oxidase[3]. Development of this agent as a new drug is probable, because of its interesting actions on microorganisms. Many toxicological studies should be performed for the general use.

During our study of the effects of PQQ on alcohol metabolism and on experimental liver injury in rats, we noticed that green-colored PQQ metabolites were excreted within 1 hr of an intraperitoneal injection of PQQ, indicating that PQQ and its metabolites are rapidly excreted through the kidneys. Therefore, in the present study, we examined the possibility of a toxic effect of PQQ on the kidneys prior to detailed studies of PQQ in mammals.

Male Wistar rats (Kyudo Co., Ltd., Kumamoto), weighing 360-430 g each, were used throughout the present study. The rats were kept at 24 ± 2°C and 55 ± 10% relative humidity under an alternating light-dark cycle of 12 hr each. PQQ was kindly provided by Ube Industries, Ltd., Ube, Japan. PQQ dissolved in 2% (w/v) $NaHCO_3$ solution (115 mg/dl) was administered intraperitoneally for 4 consecutive days at a daily dose of 11.5 mg/kg body weight (10 ml/kg) (PQQ group). The tentative dose of PQQ used in the present study was chosen out of consideration of the clinical dose of coenzyme Q_{10}, a clinically available but no nephrotoxic quinoline derivative. Control rats were similarly injected with 2% $NaHCO_3$ solution alone at a dose of 10 ml/kg body weight (Control group). Five rats were tested in each group (rat no. 1 to 5, Control group, and rat no. 6 to 10, PQQ group).

The rats were fed ad libitum during the experimental period. Blood was collected by venipuncture of the vena cava inferior, and urine were collected by applying pressure on the inguinal region, just before the rats were killed by a blow on head 24 hr following the last (4th) injection of PQQ. The wet weights of the kidneys, liver and spleen were determined, and the remaining tissues were further processed for light microscopic studies (hematoxylin and eosin). Urine was tested for pH,

Table 1. Blood biochemistry[a]

Group	BUN	Cr	GOT	GPT	ALP	LDH	Glc	TG
	(mg/dl)		(IU/l)				(mg/dl)	
Control[b]								
Mean	23.7	0.53	47	37	118	83	203	137
SD	2.3	0.07	10	11	24	40	27	38
PQQ[b]								
Mean	62.5[c]	1.48[c]	93[c]	71[d]	131	87	176	48[c]
SD	19.5	0.46	23	24	37	26	16	12

[a] Statistical differences between the mean values were determined by Student's t-test after analysis of variance. Cr, creatinine; Glc, glucose; and TG, triglyceride.
[b] There were 5 rats in each group.
[c] $p < 0.05$.
[d] $p < 0.001$.

Table 2. Organ weight

Group	Kidney		Liver		Spleen	
	(g)	(%)[a]	(g)	(%)[a]	(g)	(%)[a]
Control[b]						
Mean	2.72	0.65	16.4	3.91	0.93	0.22
SD	0.25	0.04	1.9	0.30	0.07	0.01
PQQ[b]						
Mean	3.43	0.91[c]	14.2	3.74	0.81	0.22
SD	0.23	0.07	2.6	0.23	0.04	0.01

[a] %: % of body weight.
[b] There were 5 rats in each group.
[c] $p < 0.001$.

sugar, protein, ketone bodies, bilirubin, urobilinogen and occult blood using Multi-Sticks III (Miles-Sankyo Co., Ltd., Tokyo). Serum glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), blood urea nitrogen (BUN), serum creatinine, triglyceride, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were routinely examined with a Gilford Auto-analyzer (Impact 400).

No significant difference of changes in the body weight of rats between the PQQ and Control groups was observed. Examination of the urine revealed an increased excretion of protein, glucose, ketone body and occult blood. BUN and serum creatinine levels were significantly higher in the PQQ group, and serum triglyceride contents were significantly lower in this group (Table 1). Serum GOT and GPT activities were also higher in the PQQ group than in the Control group, although ALP and LDH activities were not elevated. Swelling of the kidneys was observed macroscopically at autopsy, and the weight of the kidneys as well as % of body weight also increased, although only the latter was significant (Table 2).

In the PQQ group, vacuolar degeneration, atrophy and necrosis of the proximal tubular epithelium in the renal cortex were observed microscopically (Fig. 1). Dilatation and regeneration of the tubules were also observed in 2 rats of the PQQ group (Table 3). However, few changes of the glomerulus were observed in any of the rats treated with PQQ. A slight decrease in glycogen deposition and an increase in the mitotic process were observed also in the liver, but pathological findings of the spleen were not observed in the PQQ-treated rats.

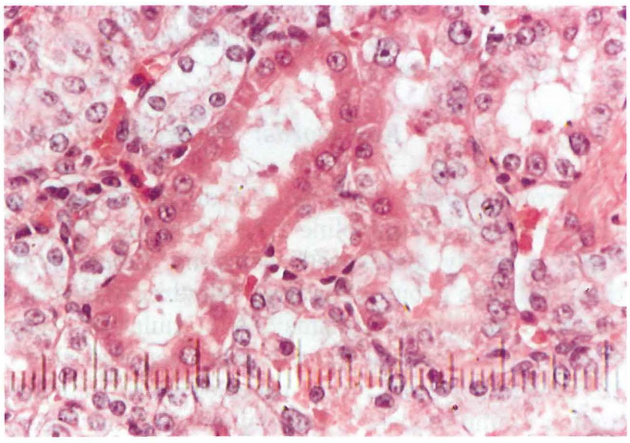

Fig. 1. Microscopic findings of PQQ-treated rats (No. 10).

Biological and toxic effects of PQQ on mammals have not been elucidated yet. PQQ contents in the animal tissues and the minimum toxic dose of PQQ are also unknown. The present preliminary study revealed that PQQ induced functional and histological changes in the kidneys, particularly proximal tubular changes, although the mechanism of PQQ nephrotoxicity is not clear. The high concentrations of PQQ metabolites in the kidneys may injury the proximal tubular component of the renal cortex. The further studies on the dose dependency of PQQ, the route of PQQ administration and the time

Table 3. Histological findings of the kidneys in PQQ-treated rats[a]

Rat No.	6	7	8	9	10
Glomerulus	—	—	—	—	—
Tubular (proximal) epithelium					
Dilatation	—	+++	—	—	+++
Necrosis	++	++	++	+++	+++
Vacuolar degeneration	+	++	+	—	+
Regeneration	—	++	++	—	—
Basophilic atrophy	+	+	+	++	++
Hyaline cast	+	+	+	++	++

[a] —: Negative, +: slight, ++: moderate and +++: severe changes.

course of renal injury in PQQ nephrotoxicity are now under study in our laboratory.

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