Note

Effect of Dietary Paraquat on the Enzyme Activities Involved in Tryptophan-Niacin Metabolism in Rats

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Brown *et al.*¹⁾ have reported that the intraperitoneal injection of paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) to rats caused death and that nicotinic acid reduced the paraquat toxicity. They have suggested, but did not prove, that damage by paraquat is a consequence of the poisoning of quinolinate phosphoribosyltransferase (EC 2.4.2.19),¹⁾ similar to *Escherichia coli* poisoned by hyperbaric oxygen,²⁾ because it is considered that superoxide is a common intermediate in the mechanisms of toxicity for paraquat and hyperbaric oxygen.³⁾ Pearl and Raffin⁴⁾ have also reported that nicotinic acid reduced oxygen toxicity in mouse alveolar macrophages by providing an alternate pathway for NAD biosynthesis; they speculated that quinolinate phosphoribosyltransferase, which is the rate-limiting enzyme in tryptophan-NAD metabolism,⁵⁾ was rapidly inactivated in mouse alveolar macrophages exposed to hyperbaric oxygen.

In order to prove the effect of dietary paraquat on the activities of quinolinate phosphoribosyltransferase and some other enzymes involved in tryptophan-niacin metabolism in rats, this experiment was carried out.

Male rats of the Sprague Dawley strain was purchased from Clea Japan Inc., the rats being individually kept in wire-bottomed cages. The room temperature was $22 \pm 2^\circ\text{C}$ and the humidity about 60%, with a 06:00-18:00 (light) and 18:00-06:00 (dark) schedule.

The liver was removed from each rat under light ether anesthesia at around 9:00 a.m., and homogenized in 5-volumes of cold 50 mM potassium phosphate buffer (pH 7.0) with a Yamato model LK-21 ultra-disperser. This homogenate was used for the determination of the protein and enzyme activities. Protein was measured by the method of Lowry *et al.*⁶⁾ with bovine serum albumin as the standard. The activities of kynureninase (EC 3.7.1.3), quinolinate phosphoribosyltransferase, NMN adenylyltransferase (EC 2.7.7.1), NAD⁺ synthetase (EC 6.3.5.1)

and nicotinamide methyltransferase (EC 2.1.1.1) were measured by the methods of Takeuchi *et al.*,⁷⁾ Iwai and Taguchi,⁸⁾ Kurokawa *et al.*,⁹⁾ Yu and Dietrich¹⁰⁾ and Cantoni,¹¹⁾ respectively.

Weanling male rats of the Sprague Dawley strain (3 weeks old) were fed *ad libitum* with a nicotinic acid-free, 20% casein diet (20% vitamin-free milk casein, 46% α -cornstarch, 23% sucrose, 5% corn oil, 0.1% choline-Cl, 5% Harper's salt mixture,¹²⁾ 1% nicotinic acid-free Harper's vitamin mixture,¹²⁾ 1 mg/100 g of diet retinol palmitate, 3.75 μg /100 g of diet ergocalciferol and 0.6 mg/100 g of diet *dl*- α -tocopherol) with or without 0.05% paraquat. At the 8th day, the rats were killed, and the livers were removed. The key enzyme activities for tryptophan-niacin metabolism were measured. As shown in Table I, the activity of quinolinate phosphoribosyltransferase was proved to decrease to about 60% by feeding the paraquat-containing diet, compared with that of the control, which is consistent with the speculation of Brown *et al.*¹⁾ Furthermore, the activities of kynureninase, NMN adenylyltransferase and NAD⁺ synthetase also decreased to about 25%, 62% and 21%, respectively, by the administration of the paraquat-containing diet. However, the activity of nicotinamide methyltransferase was not affected. From these findings, dietary paraquat was found to reduce the conversion ratio from tryptophan to NAD because the kynureninase activity was reduced to 25% and quinolinate phosphoribosyltransferase activity to 60%. The biosynthetic activity of NAD from nicotinic acid and nicotinamide was also found to be reduced by paraquat administration. Therefore, the niacin requirement would be increased when paraquat is taken. For the biosynthesis of NAD from nicotinic acid and tryptophan, NAD⁺ synthetase is involved as well as NMN adenylyltransferase. On the other hand, for the biosynthesis of NAD from nicotinamide, NMN adenylyltransferase is involved but not NAD⁺ synthetase. Accordingly, the reduction of the biosynthetic activity of NAD from nicotinic acid and tryptophan could be severer than that from nicotinamide because the NMN adenylyltransferase activity was reduced to 62% by paraquat and NAD⁺ synthetase activity to 21%. It has been reported that the NAD biosynthetic pathway from nicotinic acid and quinolinic acid mainly exists only in liver, and that from nicotinamide exists in all the tissues.¹³⁾ Brown *et al.*¹⁾ showed that the levels of liver NAD and niacin in paraquat-injected rats was significantly reduced compared to normal rats, and that when a large amount of nicotinic acid simultaneously with paraquat was injected, the levels of liver NAD and niacin remained in the normal range. Accordingly, it is possible that the administration of nicotinamide reduces the paraquat toxicity more than the administration of nicotinic acid, although the administration of nicotinic acid has been reported to reduce paraquat toxicity¹⁾ and hyperbaric oxygen toxicity.⁴⁾

TABLE I. EFFECT OF DIETARY PARAQUAT ON THE ENZYME ACTIVITIES INVOLVED IN TRYPTOPHAN-NIACIN METABOLISM

		Control	Paraquat*
Kynureninase	(nmol/hr/g of liver)	234.8 $\pm$ 23.8 ^a (100%)	60.3 $\pm$ 21.2 ^b (26%)
	(nmol/hr/mg of protein)	1.36 $\pm$ 0.15 ^a (100%)	0.38 $\pm$ 0.03 ^b (28%)
QPRase ¹	(nmol/hr/g of liver)	340.1 $\pm$ 54.2 ^a (100%)	198.4 $\pm$ 114.3 ^b (58%)
	(nmol/hr/mg of protein)	1.98 $\pm$ 0.36 ^a (100%)	1.18 $\pm$ 0.35 ^b (60%)
NMN ATase ²	(nmol/hr/g of liver)	6653.6 $\pm$ 644.4 ^a (100%)	4057.9 $\pm$ 2117.4 ^b (61%)
	(nmol/hr/mg of protein)	38.58 $\pm$ 2.78 ^a (100%)	24.44 $\pm$ 5.44 ^b (63%)
NAD ⁺ synthetase	(nmol/hr/g of liver)	457.9 $\pm$ 149.3 ^a (100%)	104.0 $\pm$ 100.7 ^b (23%)
	(nmol/hr/mg of protein)	2.65 $\pm$ 0.82 ^a (100%)	0.55 $\pm$ 0.50 ^b (21%)
NMTase ³	(nmol/hr/g of liver)	446.6 $\pm$ 84.1 (100%)	495.4 $\pm$ 184.0 (111%)
	(nmol/hr/mg of protein)	2.60 $\pm$ 0.51 (100%)	3.08 $\pm$ 0.38 (119%)

Values are means $\pm$ S.D. for 5 rats (control) and 3 rats (paraquat); means in the same line not sharing a common superscript differ significantly at $p < 0.05$.

The significance of differences between means having the same variance and the different variance analyses by an F -test were evaluated by Student's t test and Welch's test, respectively.

¹ QPRase = quinolinate phosphoribosyltransferase.

² NMN ATase = NMN adenylyltransferase.

³ NMTase = nicotinamide methyltransferase.

* In the paraquat group, two rats died on the 7th day.

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