Bull. Environ. Contam. Toxicol. (1985) 35:23–28

Bulletin of Environmental Contamination and Toxicology

Inhibition of Liver Glycolysis in Rats By Dietary Dichlone (2,3-Dichloro-1,4-Naphthoquinone)[1]

Chris A. Pritsos,[2] David E. Pisani, and Ronald S. Pardini[3]

Department of Biochemistry, Agricultural Experiment Station, University of Nevada, Reno, NV 89557

The fungicide Dichlone, 2,3-dichloro-1,4-naphthoquinone (CNQ), was shown to inhibit respiration, produce O_2^- and H_2O_2 and induce lipid peroxidation in isolated beef heart mitochondria (Pritsos et al. 1982). In addition, CNQ caused swelling of rat liver mitochondria, a process which was shown to be osmotic in nature, non-energy linked, cation non-specific, oxygen dependent (Pritsos et al. 1982) and inhibitable by exogenous α-tocopherol (Pritsos and Pardini 1982) and cysteine (Pritsos and Pardini 1981, Pritsos et al. 1982). CNQ also caused a depletion of DTNB reactable thiol groups (Pritsos and Pardini 1984) in isolated mitochondria. Consistent with these *in vitro* findings, CNQ feeding caused partial uncoupling of liver and heart mitochondria, elevation of hepatic and heart superoxide dismutase (SOD) and catalase activities and a depression of glutathione peroxidase and reductase activities (Pritsos et al. 1983). Subsequent *in vitro* studies showed that CNQ was a direct inhibitor of glutathione reductase in a fashion consistent with uncompetitive inhibition (Pritsos et al. 1983). These findings indicate that CNQ affects cellular energy metabolism by inducing oxidative stress at the organelle level and by interacting with key mitochondrial thiol groups. The glycolytic pathway is central to intracellular energy production, and its alteration by an exogenous oxidant such as Dichlone, could have widespread toxicological implications. In addition, *in vivo* exposure to CNQ could provide useful information on the hepatic response to quinone induced oxidative stress. Therefore, we elected to evaluate the effects of CNQ feeding on hepatic glycolysis in rats.

1 Contribution to Regional Research Project W-45; "Residues of Pesticides and Related Chemicals in the Agricultural Environment-Their Nature, Distribution, Persistence and Toxicological Implications", University of Nevada Agricultural Experiment Station, Reno, NV 89557.

2 Present address: Department of Pharmacology, Yale University, New Haven, CN 06510.

3 Author to whom all correspondence should be addressed.

MATERIALS AND METHODS

Female Wistar Rats (180-200 gm) were fed the various diets ad libitum. The vitamin E deficient base diet was "Vitamin E Test Diet - Rat" obtained in powdered form from the United States Biochemical Corp., and α-tocopherol stripped corn oil (10 ml/kg diet) was added as the lipid supplement. The diets were supplemented with D-α-tocopherol obtained from Sigma at a level of 271 units/kg diet. When employed, CNQ was added at a level of 2 gm/kg diet. The animals were fed the appropriate diets for 60 days and were then sacrificed by decapitation. Within 30 seconds, a lobe from the liver was frozen between aluminum plates which were precooled in liquid nitrogen, and the clamp and liver submerged in liquid nitrogen. The frozen tissue was powdered in a mortar and pestle in dry ice and then weighed to the nearest tenth of a mg. The protein was precipitated with $HClO_4$ as previously described (Williamson and Corkey 1969) and the various glycolytic intermediates were measured by established methods (Maitra and Estabrook 1964).

RESULTS AND DISCUSSION

The effects of 60 days of dietary exposure to high doses of CNQ on liver glycolysis were determined by measuring the concentrations of the various hepatic glycolytic intermediates. High doses of CNQ were included in this investigation to evaluate acute metabolic responses. The data shown in Table I demonstrate that CNQ feeding caused significant perturbation in the levels of the hepatic glycolytic intermediates. The concentration of glycolytic intermediates in the control group was consistent with published values (Faupe et al. 1972). In comparison, hepatic levels of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate were all significantly increased to 346, 258, 310, 187 and 302% of the control, respectively, in the CNQ fed group. Conversely, 2-phosphoglycerate, phosphoenolpyruvate and pyruvate were significantly depressed to 45, 9 and 7% of the control values respectively, in the CNQ fed group. The level of 3-phosphoglycerate was not affected by feeding CNQ. These findings demonstrate reversed crossover points at the glyceraldehyde-3-phosphate dehydrogenase (G-3PD) and enolase steps in the glycolytic pathway, indicating that ingestion of CNQ resulted in the inhibition of these two key glycolytic enzymes which catalyze the production of high energy intermediates. Glyceraldehyde-3-phosphate dehydrogenase is a well known sulfhydryl containing enzyme (Jocelyn 1972, Torchinsky 1981) and rabbit muscle enolase has been reported to contain 12 thiol groups, five of which are fast reacting and the seven remaining slow reacting thiols are essential for the maintenance of enzyme tertiary structure (Malmstrom 1962). In addition, both G-3PD (Kong and Davison 1980) and enolase (Jocelyn 1972) have been reported to be sensitive to radiation which in the presence of oxygen is known to result in the formation of toxic oxygen species including superoxide, hydrogen peroxide and hydroxyl radical. The observed apparent inhibition of thiol containing glycolytic

Table 1. Liver tissue glycolytic intermediates

Intermediate	CNQ in Diet*	Glycolytic Intermediates nmoles/gm liver wet weight ± S.D.†	Control %	Literature Values nmoles/gm liver wet weight ± S. D. (Faupel et al. 1972)
Glucose-1-Phosphate	-	0.9± 1.5		13± 3
	+	52.6± 14.5	5844.4**	
Glucose-6-Phosphate	-	300.9± 97.3		275±28
	+	1040.0±275.0	345.6**	
Fructose-6-Phosphate	-	70.3± 25.5		74± 7
	+	181.0± 46.	257.5**	
Fructose-1,6-Diphosphate	-	12.9± 12.9		27± 4
	+	40.0± 18.9	310.1**	
Dihydroxyacetone-Phosphate	-	29.9± 19.4		56± 9
	+	55.9± 27.2	187.0**	
Glyceraldehyde-3-Phosphate	-	11.7± 9.1		-
	+	35.3± 10.3	301.7**	
3-Phosphoglycerate	-	118.3± 42.2		273±29
	+	103.3± 17.3	87.3	
2-Phosphoglycerate	-	42.5± 10.8		-
	+	19.3± 8.5	45.4**	
Phosphoenolpyruvate	-	102.1± 35.8		145±12
	+	9.2± 2.2	9.0**	
Pyruvate	-	118.2± 45.0		129±18
	+	8.6± 3.0	7.3**	

* A (+) indicates that CNQ was in the diet at a concentration of 2 gm/kg diet. A (-) indicates control diet.

† Each value represents an average ± standard deviation of 9 animals.

** Indicates that the difference in values between enzyme levels in CNQ fed versus control is significant with $P < 95\%$ employing the students t test.

enzymes by dietary CNQ is consistent with its interaction with enzyme sulfhydryl groups.

CNQ is known to interact with protein thiol groups (Owens and Black 1960, Pritsos and Pardini 1981, Pritsos and Pardini 1984) and produce toxic oxygen radicals in isolated mitochondria (Pritsos et al. 1982, Pritsos and Pardini 1981); thus, the observed inhibition of glycolysis at G-3PD and enolase by dietary CNQ is consistent with known in vitro biochemical reactions of CNQ. The data in Table 1 do not address the mechanism of inhibition of G-3PD and enolase. However, extension of the data available on the in vitro effects of CNQ on thiol containing enzymes suggests that CNQ may act either by direct interaction with essential enzyme thiol groups (Owens and Black 1960, Ware 1975) or by the generation of toxic oxygen radicals (Pritsos et al. 1982, Pritsos and Pardini 1981). These radicals may in turn inactivate thiol containing enzymes, by oxidizing key sulfhydryl groups to the corresponding disulfide (Pritsos and Pardini 1984) or possibly by oxygenation of the disulfide to the corresponding sulfenic acid, sulfoxide or disulfoxide (Armstrong and Buchanan 1978, Kong and Davidson 1980, Lewis and Wills 1962, Packer 1974, Oae et al. 1981). As a consequence of either mechanism, formation of mixed disulfide links between proteins and glutathione could occur, (Brigelius et al. 1983). These modifications would be expected to cause enzyme inactivation.

Hepatic levels of glucose-1-phosphate were also significantly increased to 5844% of the controls when CNQ was fed (Table I). Since glucose-1-phosphate is the first metabolic intermediate on the glycogen side of the primary branch point between glycolysis and glycogen synthesis, this large accumulation of glucose-1-phosphate suggests that CNQ feeding alters glycogen metabolism in a way similar to the type VII Glycogen Storage disease (Tarui et al. 1965) which is caused by a deficiency of muscle phosphofructokinase (PFK) activity. Inhibition of PFK in glycogen storage disease or inhibition of G-3PD and enolase by dietary CNQ could effect intracellular glycogen stores in the same way. In addition, thiol reagents have been reported to cause dissociation of hepatic glycogen synthetase D into its subunits (Ernest and Kim 1974). Since CNQ interacts with thiol containing enzymes, we are evaluating the effects of prolonged CNQ feeding on hepatic glycogen levels.

REFERENCES

Armstong DA, Buchanan JD (1978) Reactions of O_2, H_2O_2 and other oxidants with sulfhydryl enzymes. Photochem Photobiol 28:743-755

Brigelius R, Akerboom TPM, Sies H (1983) Hepatic superoxide production by redox cycling. Paraquat and nitrofurantion effects on NADPH, glutathione and mixed disulfides. In: oxy radicals and their scavenger systems, Vol. II. Cellular and medical aspects (ed Greenwald RA and Cohen G) p 59-64

Ernest MF, Kim KH (1975) Regulation of rat liver glycogen synthetase D, role of glucose-6-phosphate and enzyme sulfhydryl groups in activity and glycogen binding, J Biol Chem 249:5011-5018

Faupel RP, Seitz HJ, Tarnowski W, Thiemann V, Weiss CH (1972) The problem of tissue sampling from experimental animals with respect to freezing technique, anoxia, stress and narcosis, a new method for sampling rat liver tissue and the physiological values of glycolytic intermediates and related compounds. Arch Biochem Biophys 148:509-522

Jocelyn PC (1972) Biochemistry of the SH group. Academic Press, New York, p 327-328

Kong S, Davison AJ (1980) The role of interactions between O_2, H_2O_2, .OH, e- and O_2- in free radical damage to biological systems. Arch Biochem Biophys 204:18-29

Lewis SE, Wills ED (1962) Destruction of -SH groups of proteins and amino acids by peroxides of unsaturated fatty acids. Biochem Pharmac 11:901-912

Maitra PK, Estabrook RW (1964) Fluorometric method for the enzymatic determination of glycolytic intermediates. Anal Biochem 7:472-484

Malmstrom BG (1962) Metal ion activation, metal binding and sulfhydryl groups in native and denatured enolase from rabbit muscle. Arch Biochem Biophys Suppl 1:247-259

Oae S, Takata T, Kim YH (1981) Reactions of organic sulfur compounds with superoxide anion III. Oxidation of organic sulfur compounds to sulfinic and sulfonic acids. Tetrahedron 37:37-44

Owens RG, Black G (1960) Chemistry of the reactions of dichlone and captan with thiols. Contrib Boyce Thomp Inst 20:475-497

Packer JE (1974) The chemistry of the thiol group part 2. Patai S (ed) Wiley, New York, p 481-517

Pritsos CA, Pardini RS (1981) Sufhydryl group redox state and superoxide: mediators of chloronaphthoquinone induced mitochondrial swelling. Fed Proc 40:1800

Pritsos CA, Pardini RS (1982) Physiological effects of a superoxide and hydrogen peroxide generating naphthoquinone. Fed Proc 41:1565

Pritsos CA, Pardini RS (1984) A redox cycling mechanism of action for 2,3-dichloro-1, 4-naphthoquinone with mitochondrial membranes and the role of sulfhydryl groups. Biochem Pharmac 33:3771-3777

Pritsos CA, Jensen DE, Pisani D, Pardini RS (1982) Involvement of superoxide in the interaction of 2,3-dichloro-1,4-naphthoquinone with mitochondrial membranes. Arch Biochem Biophys 217:98-109

Pritsos CA, Pisani D, Pardini RS (1983) Inhibition of glutathione reductase and peroxidase activity by 2,3-dichloro-1,4-naphthoquinone. Fed Proc 42:2109

Tarui S, Okuno G, Ikura Y, Tanaka T, Suda M, Nishikawa M (1965) Phosphofructokinase deficiency in skeletal muscle. A new type of glycogenosis. Biochem Biophys Res Comm 19:517-523

Torchinsky YM (1981) Sulfur in proteins. Pergamon Press, New York, p 154-179

Ware GW (1975) Pesticides, an auto-tutorial approach. WH Freeman and Co, San Francisco, p 124
Williamson JR, Corkey BE (1969) Assays of intermediates of the citric acid cycle and related compounds by fluorometric methods. Methods in Enzymol 13:434-513

Received September 4, 1984; accepted October 9, 1984.