Bull. Environ. Contam. Toxicol. (1992) 49:85–90

Environmental Contamination and Toxicology

Changes in Fatty Acid Composition of Rat Serum Induced by a Free Radical Generator

Valentina Ruiz-Gutierrez and Francisco J. G. Muriana

Instituto de la Grasa y sus Derivados (C.S.I.C.), Apdo. 1078, 41012 Sevilla, Spain

Compounds which can be reduced within cells, and whose reduced forms react rapidly with dioxygen, have the potential of diverting intracellular electron flow and thus of increasing the production of superoxide anion radical ($O_2^{\cdot-}$) (Cadenas et al. 1983; Rabinowitch and Fridovich 1983; Sandy et al. 1986). In mammalian organs such compounds have been shown to exert an oxygen-dependent toxicity which is due to enhanced $O_2^{\cdot-}$ production (Kappus 1987; Ruch and Klaunig 1988). Benzyl viologen is among compounds shown to exhibit this behaviour and the most toxic of the 1,1'-dialkyl-4,4'-bipyridylium salts tested (Ross and Krieger 1979).

In an earlier report, we have shown that intraperitoneal (i.p.) injection of benzyl viologen into experimental animals evoked important morphological alterations in liver, essentially characterized by lipid storage and lamellated inclusions (Muriana et al. 1987). On the other hand, changes in membrane fluidity, many of the enzymes of cholesterol and fatty acid metabolism (such as 3-hydroxy-3-methylglutaryl Coenzyme A reductase, acyl-CoA:cholesterol acyl transferase, Δ^9, Δ^6 and Δ^5 desaturases) and phospholipid fatty acid profile of hepatic microsomes were also observed after benzyl viologen treatment (Muriana et al. 1991). Thus, it is quite likely that these and other changes observed on the metabolism of lipids are reflected in the serum lipids of viologen-treated animals.

MATERIALS AND METHODS

Male Wistar rats (Iffa-Credo, Lyon, France) weighing initially from 265 to 275 g were housed in the same environmentally controlled animal room (23±1 °C; 50±10 % humidity; 12 h light cycles). Rats were randomly

Send reprint requests to V. Ruiz Gutierrez at the above address.

assigned to 2 groups each containing 12 animals. Each group was allowed free access to tap-water and was fed "ad libitum" on the standard diet (A-04 from Panlab, Barcelona, Spain) containing 19.0 % proteins, 3.5 % lipids, 66.0 % carbohydrates, 5.5 % minerals, 5.0 % cellulose and 1.0 % vitamin mix. First group was given i.p. injections (0.825 mg/kg of body weight) of benzyl viologen (dichloride salt; purchased from BDH Chemicals Ltd., England) in isotonic saline solution, in single daily dose for 21 days (Muriana et al. 1987). Control animals of the same average body weight were given i.p. injections of a comparable volume of isotonic saline solution.

After treatment, rats were starved overnight (with access to water only) and then killed by decapitation between 9 a.m. and 10 a.m. Blood was collected and the serum was separated (1,500 x g for 10 min). Lipids from serum were extracted by the method of Folch et al. (1957) and separated by thin layer chromatography on silica Gel H plates using a solvent system composed of hexane-ether-acetic acid (80:20:1). Spots were made visible in iodine vapour, and the rows of spots were delineated. Each row of spots (phospholipids, free cholesterol, esterified cholesterol, and triglycerides) were removed from the plate and eluted from the silica gel with 14 ml of either diethyl ether or chloroform/methanol (2:1, v/v) for free cholesterol and cholesteryl esters or phospholipids and triglycerides, respectively. Total lipids were estimated by a modification of the method of Amenta (1964), non-esterified cholesterol and cholesteryl esters by the method of Huang et al. (1961), and phospholipids and triglycerides by the Vioque and Holman (1962) method. High density lipoproteins in serum was assayed as described (Kingsley and Snyder 1988).

Fatty acid methyl esters of the major lipid fraction (triglycerides, esterified cholesterol, and phospholipids) were determined by gas-liquid chromatography. Silica gel spots containing the lipid fractions were scraped off and saponified by heating for 5 min with 5 ml of 0.2 M sodium methylate and obtained samples heated again at 80 °C for 5 min with 6 % (w/v) $H_2 SO_4$ in anhydrous methanol. The fatty acid methyl esters thus formed were eluted with hexane and analyzed with a gas chromatograph (Hewlett-Packard model 5710 A) equipped with a flame-ionization detector (FID). A 60 m x 0.25 mm I.D. fused silica capillary column (0.25 μm Supelcowax 10 film; purchased from Supelco Inc., USA) was used, and He (20 ml/min) served as the carrier gas. The temperature was programmed to rise from 190 °C to 220 °C at a rate of 2 °C per min.

All data are presented as the mean ± standard deviation. The effect of benzyl viologen was examined using analysis-of-variance (ANOVA) procedures. The unpaired student's t-test was used to test the significance of the difference between the means of control and treated groups.

RESULTS AND DISCUSSION

Although several studies on the effect of viologens, including benzyl viologen, on liver and lung pathology have been reported in recent years (Fukuda and Ferrans 1988; Muriana et al. 1987, 1991; Sandy et al. 1986), no information is available concerning the effect of "in vivo" administration of viologens on serum lipid composition. In this present study, we demonstrated several characteristic effects of benzyl viologen, the most toxic of the viologens tested (Ross and Krieger 1979), on serum lipid composition in rats. First, our results demonstrated that benzyl viologen treatment increased both the levels of serum phospholipids and cholesterol (free and esterified), but decreased the level of high density lipoproteins (HDL) (Table 1). Moreover, it was demonstrated that administration of the herbicide did not affect the amount of the triglycerides in serum, in spite of the changes in fatty acid composition of the triglycerides fraction (Table 2). As pointed out by Sandy et al. (1986), viologens are capable of stimulating oxygen free radical production, which may result in membrane damage (Kappus 1987). This correlates with known data from this laboratory that indicate the structure of rat liver membranes, as determined by electron microscopy, are severely affected by benzyl viologen treatment (Muriana et al. 1987). Therefore, it is possible that benzyl viologen treatment triggers an increase in hepatic phospholipids and cholesterol secretion and, by this, hyperphospholipidemia and hypercholesterolemia in serum.

Table 1. Changes in serum lipid composition by benzyl viologen treatment

Serum lipid (mg/dl)	Control	Benzyl viologen	P-value
Total lipids	248.3±9.9	459.3±9.8	<0.01
Triglycerides	71.6±5.9	74.3±4.8	NS
Phospholipids	105.3±8.1	258.2±9.3	<0.01
Free cholesterol	7.5±0.9	11.5±1.2	<0.05
Cholesteryl esters	55.6±3.1	95.1±4.8	<0.01
HDL	46.7±2.1	35.2±1.8	<0.05

Table 2. Effect of benzyl viologen treatment on fatty acid composition of serum triglycerides (%, W/W)

Fatty acid	Control	Benzyl viologen	P-value
12:0	1.8±0.3	2.1±0.4	NS
14:0	0.4±0.1	0.5±0.2	NS
16:0	24.9±2.1	22.2±0.9	NS
16:1(n-7)	2.9±0.1	2.6±0.3	NS
18:0	3.0±0.3	4.4±0.9	NS
18:1(n-9)	25.7±3.3	11.8±1.2	<0.001
18:1(n-7)	2.7±0.4	2.4±0.6	NS
18:2(n-6)	26.6±1.3	19.9±2.2	<0.01
20:4(n-6)	9.1±0.9	28.2±2.1	<0.001

Secondly, administration of benzyl viologen showed also different effects on the fatty acid composition of lipid fractions in rat serum. At the end of treatment, benzyl viologen decreased the proportion of some monounsaturated fatty acids [16:1(n-7), 18:1(n-9)], α-linolenic acid and long-chain fatty acids [22:5(n-3), 22:6(n-3)] in the phospholipid fraction (Table 3).

Table 3. Effect of benzyl viologen treatment on fatty acid composition of serum phospholipids (%, W/W)

Fatty acid	Control	Benzyl viologen	P-value
12:0	0.7±0.1	0.8±0.1	NS
14:0	0.4±0.1	0.4±0.1	NS
16:0	20.7±1.5	20.8±3.6	NS
16:1(n-7)	3.1±0.5	1.0±0.3	<0.01
18:0	19.6±1.1	26.5±0.9	<0.01
18:1(n-9)	8.9±1.1	5.2±0.3	<0.01
18:1(n-7)	1.3±0.2	2.5±0.1	<0.05
18:2(n-6)	16.2±1.1	17.2±6.1	NS
18:3(n-3)	2.2±0.1	1.3±0.2	<0.05
20:0	0.8±0.1	0.8±0.2	NS
20:1(n-9)	0.1±0.0	0.2±0.0	NS
20:1(n-7)	0.6±0.1	0.6±0.1	NS
20:3(n-9)	0.4±0.1	0.3±0.0	NS
20:3(n-6)	0.5±0.1	0.4±0.1	NS
20:4(n-6)	17.3±4.4	18.1±3.2	NS
22:5(n-6)	1.7±0.1	1.2±0.1	<0.05
22:5(n-3)	0.9±0.0	0.3±0.0	<0.01
22:6(n-3)	3.5±0.2	2.0±0.1	<0.01

The peculiar characteristic of the serum cholesteryl esters from viologen-treated rats was the very high content of eicosatrienoic acid [20:3(n-9)] (Table 4), an indicator of essential fatty acid (EFA) deficiency (Crofts et al. 1988). It is coincident with the notably

Table 4. Effect of benzyl viologen treatment on fatty acid composition of serum esterified cholesterol (%, W/W)

Fatty acid	Control	Benzyl viologen	P-value
12:0	0.5±0.1	0.6±0.1	NS
14:0	0.4±0.1	0.3±0.1	NS
16:0	11.7±0.3	14.8±0.5	<0.01
16:1(n-7)	8.1±0.2	8.2±0.3	NS
18:0	0.4±0.0	1.1±0.1	<0.01
18:1(n-9)	0.9±0.1	3.6±0.2	<0.001
18:1(n-7)	5.4±0.2	19.7±2.3	<0.001
18:2(n-6)	31.3±3.7	21.3±0.3	<0.01
20:3(n-9)	0.4±0.1	15.5±0.2	<0.001
20:4(n-6)	37.8±3.3	11.0±2.3	<0.001

low proportion of linoleic and arachidonic acids in cholesteryl ester fraction. Since the major part of the serum cholesteryl esters in the rat are contained in HDL, in which the proportion of arachidonic acid in fatty acids is greater than 50 % (Eisenberg 1984), decreased availability of arachidonic acid for cholesterol esterification could alter the amount of the HDL. Effectively, in accordance with previous studies on rat serum HDL after EFA-deficient diets (Lowe et al. 1988), our results indicate a decrease in serum HDL after benzyl viologen treatment. These findings are consistent with those obtained by direct hepatic microsomal studies (Muriana et al. 1991).

We have demonstrated recently that the major rate-controlling enzymes in polyunsaturated fatty acids and cholesterol metabolism decreased in liver microsomes from viologen-treated rats (Muriana et al. 1991). Therefore, it may be postulated that liver damage, followed by some metabolic changes in rat liver due to the herbicide, is responsible for the changes in serum lipid and fatty acid compositions. Further experimental work is needed to elucidate the mechanism of the changes in serum lipid composition in rats after administration of benzyl viologen.

REFERENCES

Amenta JS (1964) A rapid chemical method for quantification of lipids separated by thin-layer chromatography. J Lipid Res 5: 270-272

Cadenas E, Brigelius R, Akerboom T, Sies H (1983) Oxygen radicals and hydroperoxides in mammalian organs: aspects of redox cycling and hydrogen peroxide metabolism. Biol Oxidat 34: 288-310

Crofts JW, Ogburn PL, Johnson SB, Holman RT (1988) Polyunsaturated fatty acids of serum lipids in myocardial infarction. Lipids 23: 539-545
Eisenberg S (1984) High density lipoprotein metabolism. J Lipid Res 25: 1017-1058
Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 33: 497-509
Fukuda Y, Ferrans VJ (1988) Pulmonary elastic fiber degradation in paraquat toxicity. An electron microscopic immunohistochemical study. J Submicrosc Cytol Pathol 20: 15-23
Huang TC, Chem CP, Welfer W, Raftery A (1961) A stable reagent for the Lieberman-Buchard reaction. Anal Chem 33: 1405-1407
Kappus H (1987) Oxidative stress in chemical toxicity. Arch Toxicol 60: 144-149
Kingsley TR, Snyder DL (1988) Serum lipids in spontaneously hypertensive rats and sprague-dawley rats fed menhaden oil. Lipids 23: 564-567
Lowe KE, Pelkey S, Williams M, Nichols AV (1988) Effect of essential fatty acid deficiency on the size and distribution of rat plasma HDL. Lipids 23: 106-114
Muriana FJG, Ruiz-Gutierrez V, Vazquez CM (1991) Effect of benzyl viologen on the phospholipid fatty acid composition and some properties in hepatic microsomal membrane of rats. Mol Cell Biochem 108: 125-131
Muriana FJG, Santamaría C, Ruiz-Gutierrez V (1987) Ultrastructural alteration in the rat liver produced by benzyl viologen. Exp Pathol 32: 65-72
Rabinowitch HD, Fridovich I (1983) Superoxide radicals, superoxide dismutases and oxygen toxicity in plants. Photochem Photobiol 37: 679-690
Ross JH, Krieger RI (1979) Toxicity of 1,1'-alkyl-4,4'-bipyridylium salts in the rat. Drug Chem Toxicol 2: 207-222
Ruch RJ, Klaunig JE (1988) Inhibition of mouse hepatocyte intercellular communication by paraquat-generated oxygen free radicals. Toxicol Appl Pharmacol 94: 427-436
Sandy MS, Moldeus P, Ross D, Smith MT (1986) Role of redox cycling and lipid peroxidation in bipyridyl herbicide cytotoxicity. Studies with a compromised isolated hepatocyte model system. Biochem Pharmacol 35: 3095-3101
Vioque E, Holman RT (1962) Quantitative estimation of esters by thin layer chromatography. J Am Chem Soc 30: 63-66

Received January 15, 1991; accepted December 30, 1991.