

The Influence of Ascorbic Acid on the Hepatic Cytochrome P-450, and Glutathione in Guinea-Pigs Exposed to 2,4-dichlorophenol

A. NAGYOVÁ, E. GINTER

Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

Received April 18, 1995

Accepted May 31, 1995

Summary

Chlorophenols, mainly used as biocides, are compounds with a wide spectrum of toxic effects including teratogenic and carcinogenic actions. In this study, the effects of 2,4-dichlorophenol (2,4-DCP) on hepatic microsomal cytochrome P-450, NADPH-cytochrome c reductase activity, liver ascorbic acid (AA) and glutathione (GSH) content were studied in guinea-pigs with a low (2 mg/day/animal) or a high (50 mg/day/animal) ascorbic acid intake. The high AA intake significantly increased liver AA and GSH levels. There was a clear-cut correlation between liver AA and GSH levels. Administration of 2,4-DCP significantly decreased cytochrome P-450 and NADPH-cytochrome c reductase activity in hepatic microsomes isolated from guinea-pigs with the low AA intake. Such a reduction was not observed in intoxicated guinea-pigs with the high AA intake. The results suggest that AA can play a protective role in 2,4-DCP toxicity.

Key words

Cytochrome P-450 – Ascorbic acid – Glutathione – Guinea-pig liver – 2,4-dichlorophenol

Introduction

2,4-dichlorophenol (2,4-DCP) is used in the manufacture of herbicides, germicides, seed disinfectants, wood preservatives, etc. In general, the toxicity of chlorophenols increases with the chlorination of the phenol molecule. Although 2,4-DCP appears to be less toxic than the higher chlorinated phenols, its toxic effects on experimental animals as well as in professionally exposed workers have been studied by several authors (WHO 1989). Short-term exposures of experimental animals to 2,4-DCP have been associated with increased liver and spleen weights and, in some instances, with haematological or immunological effects (Exon and Koller 1985). Chronic exposure led to the accumulation of chlorophenols in the liver and kidney. The major metabolic transformation of lower chlorinated chlorophenols appears to be the conjugation with sulfate or glucuronate, prior to their clearance in the urine. Dechlorination and methylation reactions also serve to detoxify these compounds. In a 90-day study,

Borzelleca *et al.* (1985) exposed mice to 2,4-DCP in drinking water with daily doses ranging from 40 to 491 mg/kg body weight and found no significant effects on the activities of hepatic mixed-function oxidases (MFO) or serum enzymes. *In vitro* studies have shown that 2,4-DCP impairs liver microsomal detoxication functions by selective inhibition of cytochrome P-450 activity at the terminal oxygenation step of the MFO enzyme system by interfering with the coupling of flavin to this enzyme (Arrhenius *et al.* 1977).

Glutathione (GSH) and ascorbic acid are effective water-soluble cytoplasmic antioxidants participating in cellular protection against oxidative stress and toxic agents (Nagyová and Ginter 1994). GSH acts directly as a free radical scavenger or through the antioxidant enzyme system as the substrate for GSH-peroxidase and glutathione-S-transferase (Wefers and Sies 1983). Ascorbic acid, a natural antioxidant and free radical scavenger, is an important part of the antioxidant defence system (Bendich *et al.*

1986). A dysbalance between the generation of free radicals and the antioxidant defence system could result in an increased demand for vitamin C. The cooperation between ascorbic acid and GSH against oxidative stress was recently reviewed by Meister (1994 a,b). In experimental animals, the administration of ascorbate spares GSH (Martensson and Meister 1991) and conversely, the sparing effect of GSH in ascorbate deficiency has been demonstrated (Martensson *et al.* 1993). A low dietary intake of AA also decreased plasma GSH in man (Henning *et al.* 1991). Vitamin C supplementation (500 mg/day) to healthy adults elevated red blood cell GSH by 50 % (Johnston *et al.* 1993).

The aim of this study was to assess the possible protective effect of ascorbic acid against 2,4-DCP toxicity, with respect to hepatic microsomal monooxygenase activities and liver GSH availability in guinea-pigs which, like man, do not synthesize ascorbic acid.

Material and Methods

Male short-hair, three-coloured guinea-pigs (Velaz Prague) weighing 490 ± 93 g were used in the experiment. After two weeks of feeding on a standard laboratory diet with the addition of vegetables, the animals were randomly divided into four groups. Two control groups received either low or high AA concentration in their drinking water. The AA consumption in the drinking water was monitored for 4 months. The average intake of AA in groups with the low AA intake was 2 mg of AA/animal/day and 50 mg

of AA/animal/day in groups with the high AA intake. Two intoxicated groups were given 2,4-dichlorophenol p.o. in olive oil in six doses (one dose contained 50 mg/kg of body weight) during two weeks. The AA intake in these groups of guinea-pigs was the same as in the control groups. During the experiment, the animals received drinking water and a standard laboratory diet *ad libitum*. After overnight fasting and 24 hours after the last dose of 2,4-DCP, the guinea-pigs were killed by decapitation. The livers were quickly removed, weighed, chilled, and homogenized in ice-cold 0.15 M KCl containing 100 mM Tris-HCl and 10 mM EDTA (pH=7.4) using a Potter-Elvehjem glass homogenizer with a Teflon pestle. The 20 % (wt/vol) homogenate was centrifuged at 12 000xg for 15 min and liver microsomes were obtained by centrifugation of supernatant fraction at 100 000xg for 60 min. The microsomal pellet was resuspended in a buffer solution (100 mM Tris buffer, pH=7.4, containing 1 mM EDTA and 30 % glycerol) and stored at -80 °C together with liver samples for analysis of AA and the GSH content. In frozen liver microsomes, cytochrome P-450 (Omura and Sato 1964), NADPH-cytochrome c reductase activity (Vermilon and Coon 1978) and total GSH in the liver (Tietze 1969) were determined using a dual-wavelength spectrophotometer UV/VIS Pye Unicam. AA in the liver was determined by the dinitrophenylhydrazine method (Roe and Kuether 1943).

The results were evaluated statistically by the analysis of variance (ANOVA) and regression analysis (Statgraphic). The level of significance was set at $P < 0.05$.

Table 1

Effect of 2,4-DCP on hepatic monooxygenase activities and liver AA and GSH levels in guinea-pigs with low and high AA intake.

Group	Cytochrome P-450 [nmol/mg prot.]	NADPH-cytochrome c reductase [nmol/min/mg prot.]	Ascorbic acid [μ mol/g]	Glutathione [μ mol/g]
low AA	0.76 ± 0.03^a	133 ± 6^a	0.23 ± 0.02^a	4.07 ± 0.22^{ac}
low AA + 2,4-DCP	0.60 ± 0.05^b	113 ± 5^b	0.22 ± 0.01^a	3.30 ± 0.24^a
high AA	0.75 ± 0.06^{ab}	142 ± 6^a	1.26 ± 0.10^b	5.35 ± 0.48^b
high AA + 2,4-DCP	0.73 ± 0.06^{ab}	141 ± 7^a	1.24 ± 0.12^b	4.80 ± 0.44^{bc}

Data represent mean values \pm S.E.M. The number of animals in each group was ten. ^{a,b,c} Different superscripts indicate significantly different means ($P < 0.05$) in the same column.

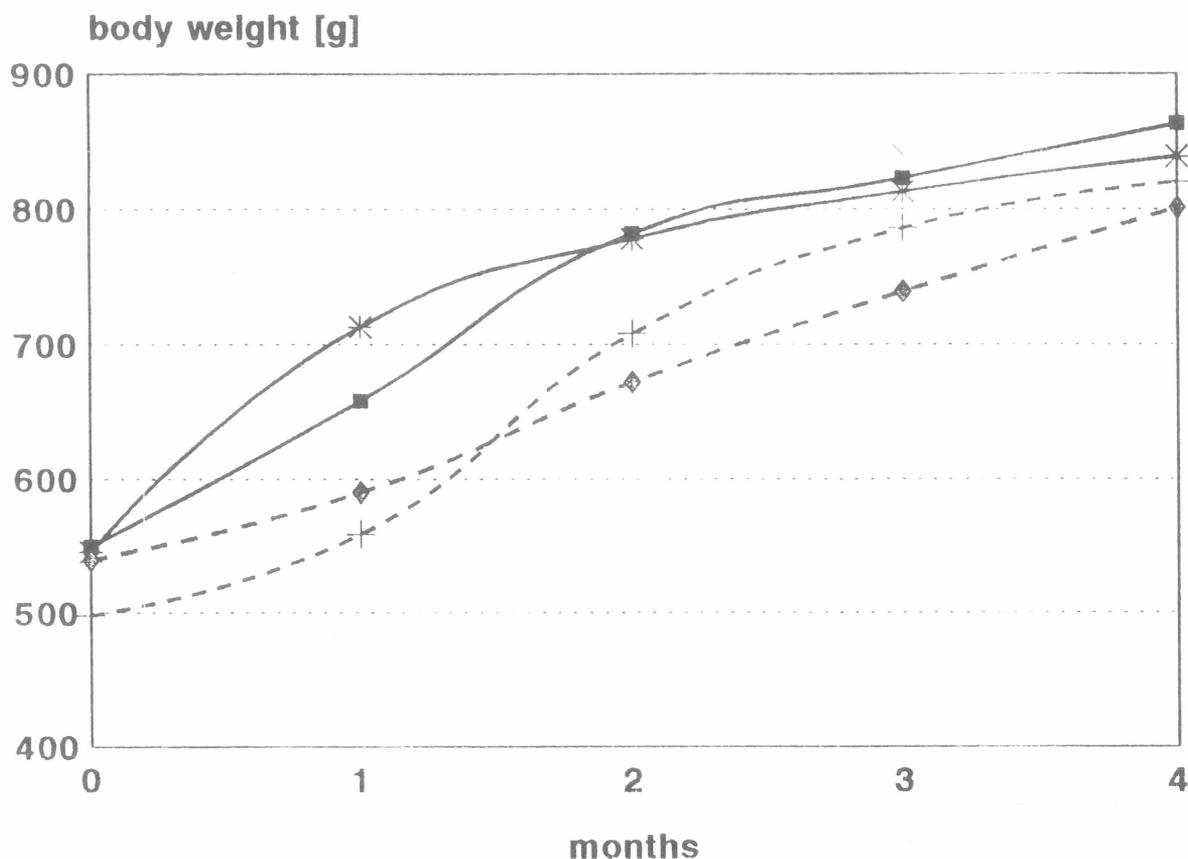


Fig. 1

Body weight of guinea-pigs with different ascorbic acid intake exposed to 2,4-DCP. Means for 10 animals. Groups: ◆ low AA intake, + low AA intake + 2,4-DCP, * high AA intake and ■ high AA intake + 2,4-DCP.

Results

The body weight of guinea-pigs increased in all groups during the experiment. There was no significant influence of different AA intake or 2,4-DCP on body weight (Fig. 1).

Table 1 shows that the high intake of AA significantly increased its concentration in the liver of both 2,4-DCP-treated and 2,4-DCP-untreated guinea-pigs. The high AA intake also significantly increased liver glutathione levels of intoxicated and control animals (by 28 % and 32 %, respectively). A highly significant correlation between AA concentration and GSH levels in the liver is shown in Fig. 2.

2,4-DCP administration significantly lowered cytochrome P-450 and NADPH-cytochrome c reductase activity in liver microsomes of guinea-pigs with the low AA intake (by 27 % and 18 %, respectively). Such decrease was not observed in guinea-pigs with the high AA intake. In the guinea-pigs exposed to 2,4-DCP, liver AA and GSH levels were not

apparently affected but exhibited a decreasing tendency compared with the control groups.

Discussion

Administration of 2,4-DCP to guinea-pigs with different AA intake decreased the hepatic cytochrome P-450 content and NADPH-cytochrome c reductase activity in the group of guinea-pigs with the low AA intake. The high AA intake prevented the depression of cytochrome P-450 and inhibition of reductase activity. In a 90-day study (Borzelleca *et al.* 1985), no significant effect of 2,4-DCP on mixed-function oxidase activities was observed. However, 2,4-DCP was administered in drinking water to male and female mice, which are capable to synthesize vitamin C. On the contrary, *in vitro* studies showed a specific inhibitory effect on the cytochrome P-450 enzyme or disturbance of the electron transfer from the flavin enzyme to P-450 (Arrhenius *et al.* 1977). Our results support the observation of *in vitro* studies.

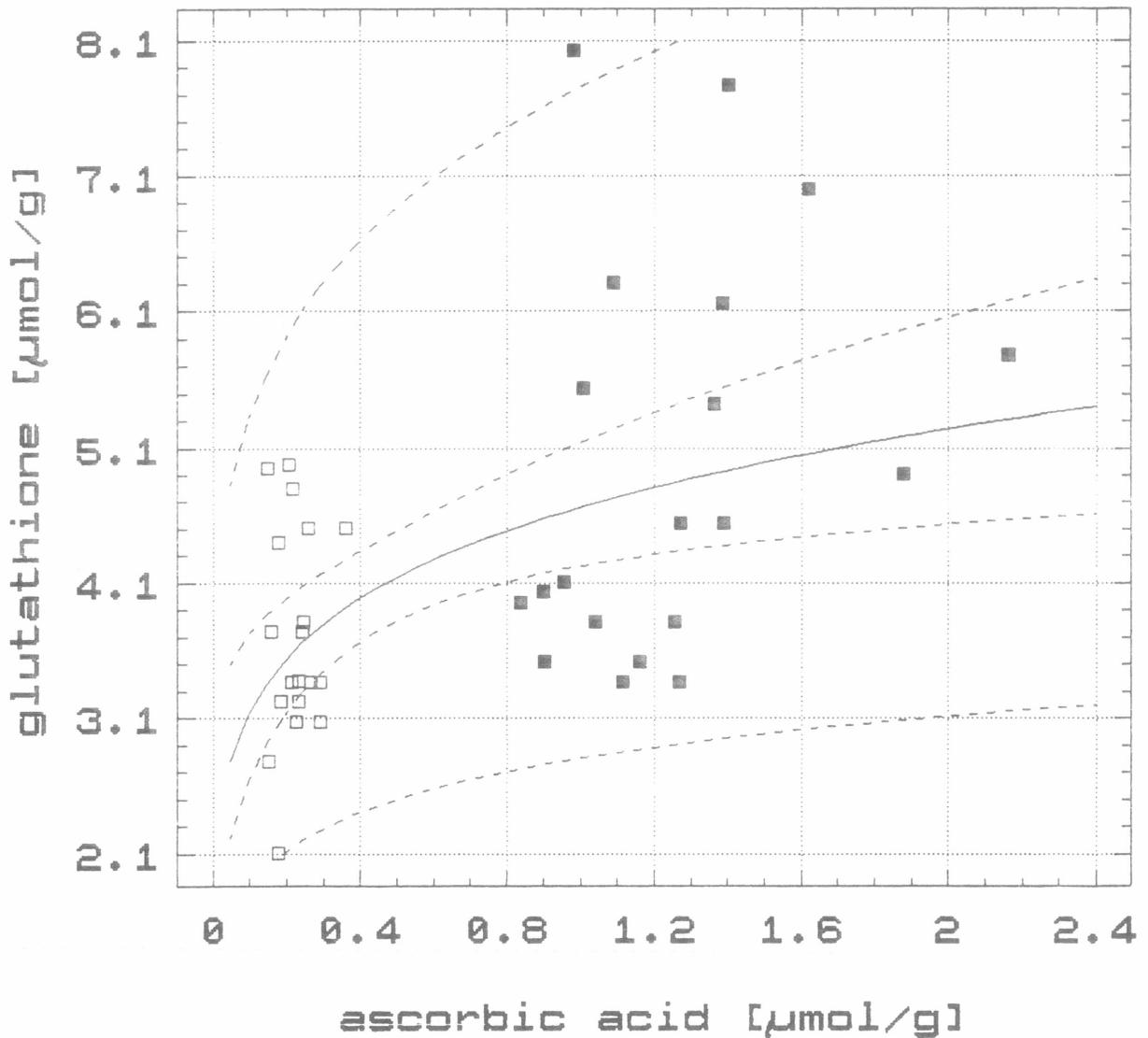


Fig. 2

Correlation between ascorbic acid and glutathione levels in the liver of guinea-pigs exposed to 2,4-DCP (multiplicative model: $r=0.531$; $P=0.00051$): groups with low AA intake (\square) and groups with high AA intake (\blacksquare).

In this study, a high intake of AA for four months significantly increased liver GSH levels in untreated as well as in 2,4-DCP-intoxicated animals. A common role of GSH and ascorbic acid, as two important water-soluble antioxidants, is the protection of cells against oxidative damage. Ascorbate administration to GSH-deficient newborn rats and adult mice prevented cellular damage and mortality and led to increased tissue and mitochondrial GSH levels (Martensson and Meister 1991, Jain *et al.* 1992). Thus, ascorbate spares glutathione *in vivo* and can serve as an essential antioxidant in the presence of severe GSH-deficiency. Conversely, GSH administration to guinea-pigs fed ascorbate-deficient diet significantly delayed the onset of scurvy and the decrease of tissue levels of ascorbate (Martensson *et al.*

1993). The observations of the above mentioned authors support the conclusion that one of GSH functions is to keep ascorbate in its reduced form. We have found a strong correlation between AA concentrations and GSH levels in the liver, which also supports the cooperation between ascorbic acid and glutathione.

Decreased liver GSH levels were observed in experimental animals exposed to many xenobiotics. The pronounced influence of 2,4-DCP administration on liver AA and GSH levels was not so evident in this study. We suppose that the dose of 2,4-DCP used, was insufficient to evoke more appreciable changes in antioxidant status. Ascorbic acid has been shown to protect against the toxicity of PCB (Kawai-Kobayashi and Yoshida 1986). If the same protective effects of

AA also take place in the toxicity of chlorophenols, is not known. Because of some similarities in the structure of PCB and 2,4-DCP protective effects of AA against chlorinated phenols could be expected. Lowered accumulation of 2,4-DCP observed in tissues of guinea-pigs with a high AA intake also suggests that

AA participates in 2,4-DCP metabolism and toxicity (unpublished results). The supplementation of AA in guinea-pigs in this study had a protective effect on cytochrome P-450 and NADPH-cytochrome c reductase and favourably affected the liver GSH levels in 2,4-DCP-intoxicated guinea-pigs.

References

- ARRHENIUS E., RENBERG L., JOHANSSON L., ZETTERQVIST M.A.: Disturbance of microsomal detoxication mechanisms in liver by chlorophenols pesticides. *Chem. Biol. Interact.* **18**: 35–46, 1977.
- BENDICH A., MACHLIN L.J., SCANDURRA O., BURTON G.W., WAYNER D.D.: The antioxidant role of vitamin C. *Adv. Free Radical Biol. Med.* **2**: 419–444, 1986.
- BORZELLECA J.F., HAYES J.R., COUDIE L.W., EGGLE J.L.Jr.: Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice. *Fundam. Appl. Toxicol.* **5**: 478–486, 1985.
- EXON J.H., KOLLER L.D.: Toxicity of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol. In: *Water Chlorination: Environmental Impact and Health Effects*. R.L. JOLLEY, R.J. BULL, W.P. DAVIS, S. KATZ, M.H.Jr. ROBERTS, V.H. JACOBS (eds), Lewis Publishers, Chelsea, Michigan, 1985, pp. 307–330.
- HENNING S.M., ZHANG Y.Z., MCKEE R.W., SWENDESEID M.E., JACOB R.A.: Glutathione blood levels and other oxidant defense indices in men fed diet low in vitamin C. *J. Nutr.* **121**: 1969–1975, 1991.
- JAIN A., MARTENSSON J., MEHTA T., KRAUSS A.N., AULD P.A., MEISTER A.: Ascorbic acid prevents oxidative stress in glutathione-deficient mice: effects on lung type-2 cell lamellar bodies, lung surfactant and skeletal muscle. *Proc. Natl. Acad. Sci. USA* **89**: 5093–5097, 1992.
- JOHNSTON C.S., MEYER C.G., SRILAKSHMI J.C.: Vitamin C elevates red blood cell glutathione in healthy adults. *Am. J. Clin. Nutr.* **58**: 103–105, 1993.
- KAWAI-KOBAYASHI K., YOSHIDA A.: Effect of dietary ascorbic acid and vitamin E on metabolic changes in rats and guinea-pigs exposed to PCB. *J. Nutr.* **116**: 98–106, 1986.
- MARTENSSON J., MEISTER A.: Glutathione-deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. *Proc. Natl. Acad. Sci. USA* **88**: 4656–4660, 1991.
- MARTENSSON J., HAN J.H., GRIFFITH O.W., MEISTER A.: Glutathione ester delays the onset of scurvy in ascorbate-deficient guinea-pigs. *Proc. Natl. Acad. Sci. USA* **90**: 317–321, 1993.
- MEISTER A.: Glutathione-ascorbic acid antioxidant system in animals. *J. Biol. Chem.* **269**: 9397–9400, 1994a.
- MEISTER A.: Glutathione, ascorbate, and cellular protection. *Cancer Res.* **54** (Suppl): 1969–1975, 1994b.
- NAGYOVÁ A., GINTER E.: Interaction between hepatic ascorbic acid, cytochrome P-450 and lipids in female guinea-pigs with different ascorbic acid intake. *Physiol. Res.* **43**: 307–312, 1994.
- OMURA T., SATO R.: The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239**: 2370–2378, 1964.
- ROE J.H., KUETHER C.A.: The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivate of dehydroascorbic acid. *J. Biol. Chem.* **147**: 399–407, 1943.
- TIETZE F.: Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal. Biochem.* **27**: 502–522, 1969.
- VERMILON J., COON M.: Purified liver microsomal NADPH-cytochrome P-450 reductase. *J. Biol. Chem.* **253**: 2694–2704, 1978.
- WEFERS H., SIES H.: Oxidation of glutathione by the superoxide radical to the disulfide and the sulfonate yielding singlet oxygen. *Eur. J. Biochem.* **137**: 29–36, 1983.
- WHO, ENVIRONMENTAL HEALTH CRITERIA 93: Chlorophenols other than pentachlorophenol, WHO, Geneva, 20–23, 1989.

Reprint Requests

A. Nagyová, Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic.