Z Ernährungswiss 34: 220–223 (1995)


ORIGINAL CONTRIBUTION

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# The effect of graded ascorbic acid intake on the activity of GSH-Px in the liver of female guinea pigs

**Einfluß der Fütterung steigender Ascorbinsäuremengen auf die Aktivität der GSH-Peroxidase in der Leber von Meerschweinchen**

**Summary** Differing antioxidant potentials created by graded ascorbic acid supplementation (1, 10, 100 mg per animal daily) evoked changes in the level of glutathione peroxidase activity and lipid peroxides in the liver of female guinea pigs. The group with the lowest ascorbic acid intake (1 mg) had the lowest activity of glutathione peroxidase and the highest level of lipid peroxides. The two other groups (10 and 100 mg) showed enhancement of glutathione peroxidase activity and decline in lipid peroxides. There was no difference between the groups with 10 and 100 mg ascorbic acid intake.

Received: 11 March 1994
Accepted: 27 April 1995

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**Zusammenfassung** Verschiedene Werte an antioxidativem Potential, erzeugt mit Hilfe verschiedener Konzentrationsstufen an Ascorbinsäure (1, 10, 100 mg/Tier/Tag) führten zu Veränderungen in der GSH-Px Aktivität und der Menge den Lipidperoxiden in der Leber von Meerschweinchen. Die Gruppe mit der kleinsten Dosierung (1 mg) von Ascorbinsäure hatte die niedrigste GSH-Px Aktivität und den höchsten Anteil an Lipidperoxiden. Die zwei anderen Gruppen zeigten eine Erhöhung der GSH-Px Aktivität und Senkung von Lipidperoxiden auf. Es bestand kein Unterschied zwischen den Gruppen mit der Dosis von 10 und 100 mg Ascorbinsäure.

**Key words** Ascorbic acid – glutathioneperoxidase – lipid peroxides – liver

**Schlüsselwörter** Ascorbinsäure – Glutathion-Peroxidase – Lipidperoxiden – Leber

**Abbreviations** *AA* = ascorbic acid · *GSH*-Px = glutathione peroxidase · LPO = lipid peroxides · *MDA* = malone dialdehyde

## Introduction

Optimal cell and tissue functioning requires control of oxidative reactions. The control is mediated through an oxidant defense system present in all cells and comprised of oxidant scavengering molecules and enzymes. The activity of enzymes involved in detoxification of products arising from free-radical metabolism may be the key factor with regards to the manifestation of toxic effects. Glutathione peroxidase (GSH-Px) is considered to be very important in protecting cells from oxidative injury since it can catalyze the reduction of both $H_2O_2$ and lipid hydroperoxides (3).

The relationship between GSH-Px and the level of selenium in organism is well known, but the role of other antioxidants in controlling GSH-Px activity is still not clear. Ascorbic acid (AA) is an important contributor to the oxidant defense system in body tissues. The present study examined the effect of AA level in the diet of guinea pigs – which, as humans, are not able to synthetize ascorbate – on GSH-Px activity and lipid peroxides (LPO) in the liver, the major site of detoxification.

## Materials and methods

Tricoloured female guinea pigs (Velaz, Prague) with an initial body weight of about 400 g were housed under standard laboratory conditions in plastic cages with wood chip bedding. During the adaptation period animals were fed ad libitum a standard laboratory chow for guinea pigs (MOK, Velaz). Animals had free access to drinking water with 20 mg/l AA. AA was purchased from Farmakon (Olomouc), glutathione, glutathione reductase, and NADPH from Sigma, and thiobarbituric acid from Merck.

After a 4-week adaptation period the animals, with body weight of about 500 g, were randomly divided into three groups and fed ad libitum a vitamin C free diet (6). Group 1 was given drinking water that provided a mean dose of 1 mg AA per animal per day, group 2 10 mg, and group 3 100 mg. After 9, 10, and 11 weeks 4 animals from each group were decapitated after overnight fast. Thus a total of 12 animals per group were analyzed.

AA was measured in liver spectrophotometrically by 2,4-dinitrophenylhydrazine (14). Measurement in 105 000 *g* supernatant from liver homogenate was based on the procedure of Paglia and Valentine (12). The reaction was started with the addition of cumene hydroperoxide; one unit is the quantity of enzyme which converts 1 μmol NADPH in 1 min. Protein content was determined using the method of Lowry et al. (9). LPO were determined using malone dialdehyde after the reaction with thiobarbituric acid and HPLC (Hewlett-Packard 1090) separation of the reaction product (11, 15). Selenium was determined by atomic absorption spectrometry in the mineralized sample of 0.5 g liver. PU 9400 flame atomic absorption spectrophotometer with deuterium background correction equipped with a PU 9360 continuous flow vapor system (Unicam Analytical System) was used (10).

The results were evaluated statistically by analysis of variance and regression analysis (Statgraphic).

## Results and discussion

The animals in each of the three groups gained weight similarly. There was no significant difference in body weight, liver weight, or the liver to body weight ratio at the end of the experiment. The concentration of AA reached a steady state after 9 weeks. All studied parameters were the same in each killed after 9, 10, or 11 weeks, and the results from the three different intervals were therefore pooled.

The content of AA in the liver increased proportionally with the amount of AA in the drinking water (Table 1). The activity of GSH-Px in the liver was significantly decreased in the group with low AA intake (Table 1). There was no difference between the groups with medium and high AA intake, but they both differed significantly from the group with low intake. The positive correlation between AA levels and GSH-Px activity in the liver is shown in Fig. 1.

The relationship between the amount of LPO in the liver and AA intake was precisely the reverse: the group with low AA intake had the highest content of LPO, and the LPO content in the two other groups was the same. The inverse correlation of LPO and GSH-Px activity is demonstrated in Fig. 2. Selenium content in the liver of guinea pigs with different AA intake was the same in all three groups.

In this experiment we evoked in guinea pigs different antioxidant potentials by graded supplementation of AA. The group with low AA intake was near to the state of marginal vitamin C deficiency (4). The daily dose of 1 mg AA per animal was still enough for normal growth. The proportionality between AA intake and its level in the liver was clearly indicated. The aim of the study was to evaluate the influence of AA – a nonenzymatic antioxidant present normally in the diet – on the enzymatic antioxidant GSH-Px and on the level of lipid peroxidation in the liver.

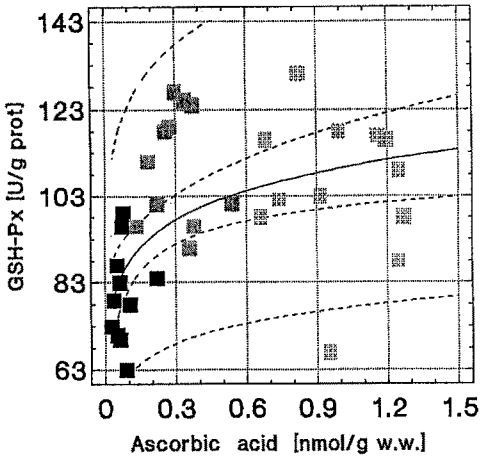
**Table 1** Influence of graded ascorbic acid intake on ascorbic acid, lipid peroxides, glutathione peroxidase and selenium in guinea pig liver ($n = 12$)

| Ascorbic acid intake (mg/day) | Relative weight of liver (%) | Ascorbic acid (nmol/g w.w.) | Lipid peroxides (nmol MDA/g w.w.) | Glutathione peroxidase (U/g protein) | Selenium (ng/g w.w.) |
|---|---|---|---|---|---|
| 1 | 3.56 ± 0.38[a] | 74 ± 17[a] | 28.64 ± 9.64[a] | 79.73 ± 1.06[a] | 195.76 ± 39.11[a] |
| 10 | 3.21 ± 0.49[a] | 295 ± 34[b] | 19.57 ± 4.32[b] | 109.91 ± 13.28[b] | 188.78 ± 46.36[a] |
| 100 | 3.13 ± 0.33[a] | 939 ± 68[c] | 20.26 ± 7.74[b] | 108.77 ± 12.19[b] | 190.51 ± 40.40[a] |

w.w. wet weight

[a,b,c] Different superscripts indicate significantly different means ($P < 0.01$) in the same column.

**Fig. 1** Direct correlation between the amount of AA and GSH-Px activity in the liver of guinea pigs (multiplicative model, $r = 0.581$, $P = 0.00025$). Confidence and prediction limits appear on the regression plot as the pair of dashed lines closest to and farthest from the regression curves respectives. Daily AA intake per animal: ■, 1mg; ▩, 10 mg; ▦, 100 mg




GSH-Px occurs mainly in the liver cytosol. The activity measured in this study with cumene hydroperoxide corresponds to the sum of Se-dependent and Se-independent activities (8) and is important in vivo in the protection of tissue from oxidative damage. Non-Se/GSH-Px activity is associated with glutathione S-transferase, a group of multifunctional enzymes involved in the biotransformation and detoxification of a broad spectrum of xenobiotic and endogenous compounds (13). In liver of various species the Se-dependent GSH-Px (measured with $H_2O_2$) represents only a small part of the total activity measured with cumene peroxide (1); for this reason we studied total GSH-Px activity. The amount of selenium differed neither in the diet nor in the livers of guinea pigs with differing AA intake. We presume that the enhancement of activity was due to the Se-independent GSH-Px.

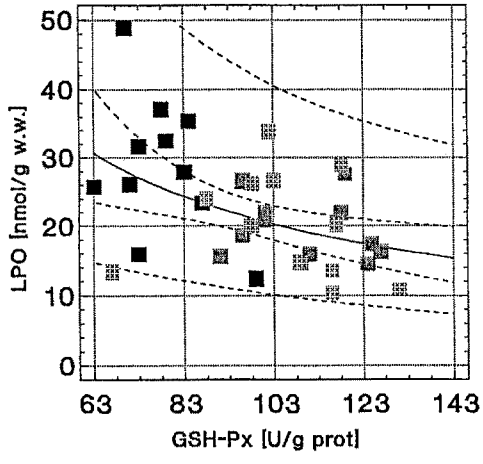



**Fig. 2** Inverse correlation between GSH-Px activity and amount of LPO in the liver of guinea pigs (multiplicative model, $r = -0.458$, $P = 0.0056$). Confidence and prediction limits appear on the regression plot as the pair of dashed lines closest to and farthest from the regression curve respectives. Daily AA intake per animal: ■, 1 mg; ▩, 10 mg; ▦, 100 mg

Koul et al. (7) studied also the effect of AA on the antioxidant defense system and lipid peroxidation in guinea pigs. Their results are in concordance with ours concerning the GSH-Px activity. Surprisingly, they found no difference in the levels of LPO. The discrepancy in results may have been caused by the different methods used to measure LPO. In our experiments tissue levels of LPO were measured after the reaction with thiobarbituric acid and subsequent HPLC separation of the reaction product, while Koul et al. followed NADPH-dependent lipid peroxidation in vitro. Cadenas et al. (2) used the same method as ours and had found the same alterations in lipid peroxidation. Henning et al. (5) in experiments on human volunteers found no influence of low AA status on Se-dependent GSH-Px in the erythrocytes. We cannot explain the different reaction of Se-dependent and Se-independent enzymes at different levels of AA, but we regard the vitamin C deficiency in our guinea pigs as more pronounced than that in human volunteers (5).

The liver, a major site of detoxification and the target tissue of ingested xenobiotics and oxidants, is important for studying the role of GSH-Px in detoxification of products arising from free-radical metabolism. Our results show that GSH-Px activity and LPO level are affected by the level of AA in the tissue. The decrease in GSH-Px activity and LPO level in the group of animals with low AA intake was achieved in seemingly healthy animals. In the case of intoxication the high intake of AA, which affects GSH-Px activity and the level of LPO, increases the global antioxidant capacity. On the other hand, the low intake of AA does not protect against the oxidant injury. The toxic effect of cadmium – reflected in elevated lipid peroxidation – was supressed in animals with high AA supplementation, while the low dose of AA shows no protective effect (6). The results demonstrate that low antioxidant defense in guinea pigs with marginal vitamin C deficiency is conditioned not only by low tissue levels of AA but also by low GSH-Px activity in the liver and support the protective value of dietary antioxidant supplementation.

**Acknowledgement** The authors wish to thank Ing. A. Hudecová for LPO estimation.

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