Protective role of brain ascorbic acid content against lipid peroxidation

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Summary. The high ascorbic acid concentration in the brain inhibits lipid peroxidation induced by various agents in rat brain microsomes. The physiological importance of the fact is discussed.

The brain subcellular fractions were shown to be sensitive to lipid peroxidation[2,3]. The diluted cytoplasm of the brain was known to contain a factor stimulating the phospholipid peroxidation of brain subcellular particles[4], and the prooxidant factor was identified as ascorbic acid[5]. At the same time, the brain lacks mechanisms which in other tissues protect against peroxidation[6]. Lipid peroxidation induced by ascorbic acid is known to depend on ascorbic acid concentration, and high concentrations of ascorbic acid inhibit the lipid peroxide formation induced by various agents in liver[7,8] and brain[3] preparations. We have stated earlier that physiological level of ascorbic acid in the brain corresponds to a concentration at which ascorbic acid acts as a protective compound only[9]. Thus, it may prevent a peroxidative damage of brain tissues. The experiments now reported support this hypothesis and give further information on the mode of action of large amounts of ascorbic acid.

Materials and methods. CFY rats of both sexes weighing 160–180 g were used. The microsomal fraction of the brain was isolated in 0.25 M sucrose as described previously[10]. The 100,000 × g supernatant was used as soluble fraction. The microsomes were suspended in 0.25 M sucrose to 1.2–1.9 mg/ml final protein content and kept at −16 °C. Incubation was carried out in a reaction mixture of 2 ml total volume containing 50 mM tris-HCl (pH 7.4), 0.2 ml microsomal suspension, and alternatively various amounts of investigated substances and inducers as indicated in the text, at 37 °C for 10 min. The controls contained the above substances expect for the inducers. The reaction was stopped by the addition of 1 ml of 20% trichloroacetic acid. Lipid peroxide was measured by the thiobarbituric acid test[11]. The amount of malonyldialdehyde formed was calculated by the molar extinction coefficient $\epsilon_{530} = 1.56 \times 10^5$ cm/mmole[12]. Protein was determined by the method of Lowry et al.[13], ascorbic acid according to Maickel[14].

The materials used were: L-ascorbic acid (Merck, Darmstadt), dehydroascorbic acid, reduced glutathion, (Fluka, Buchs). Other chemicals were brought from Reanal, Budapest.

Results. The ascorbic acid concentration in the brain soluble fraction was found to be $1.44 \pm 0.07 \times 10^{-4}$ M (n = 5), in accord with earlier results[10,15]. Considering this concentration and the fact that during preparation the cytoplasm of the brain cells was diluted 10fold to obtain the soluble fraction, the ascorbic acid concentration of the brain cytoplasm was calculated to amount to about 1.5×10^{-3} M. This concentration was tested for its protective effect on peroxidation. Lipid peroxidation was stimulated by small amounts of ascorbic acid, NADH[3,8], brain soluble fraction[4,16] containing endogenous ascorbic acid[5,10] and by the mixture of reduced and oxidized glutathione[17]. Ascorbic acid at a physiological concentration inhibited the peroxidation of brain microsomes induced by the agents applied (table). The same quantity of dehydroascorbic acid prevented lipid peroxide formation induced by the soluble fraction, or by the corresponding small amount of exogenous ascorbic acid. It was remarkable that dehydroascorbic acid considerably potentiated the effect of the mixture of reduced and oxidized glutathione (GSSG + GSH).

Discussion. Lipid peroxide formation is inhibited in various tissues by ascorbic acid at high concentrations[7,8], but the physiological significance of the finding has not been adequately stressed. The fact is of especial importance in connection with the brain, a tissue most sensitive for lipid peroxidation and known to contain a prooxidant factor but no such protective mechanisms as exist in other tissues. Thus e.g. its glutathione peroxidase activity is insufficient[6], it has no or very low catalase and peroxidase activity[18,19], and is apparently devoid of cytochrome P-450 and P-420 enzymes[20]. It is hardly acceptable that the brain tissue should not have some protective mechanisms capable of preventing the oxidative deterioration of phospholipids.

Bishayee and Balasubramanian[3] examined the inhibitory effect of ascorbic acid on lipid peroxidation of rat brain microsomes up to 1 mM. At higher ascorbic acid concentrations, we could find hardly any peroxidation. At 1.5×10^{-3} M concentration, one corresponding to the endogenous ascorbic acid level of the rat brain cytoplasm, no lipid peroxidation was induced by the soluble fraction, mixture

Effect of ascorbic acid at physiological concentration and of the same amount of dehydroascorbic acid on microsomal lipid peroxidation induced by various stimulators

Lipid peroxidation induced by	Malonyldialdehyde formed (nmoles/mg protein) None	In the presence of 1.5×10^{-3} M ascorbic acid	1.5×10^{-3} M dehydroascorbic acid	Time of incubation (min)
0.2 ml soluble fraction*	2.30 ± 0.11	0.51 ± 0.17	0.38 ± 0.12	10
1.5×10^{-5} M ascorbic acid	2.75 ± 0.30	0.65 ± 0.06	0.56 ± 0.09	10
5×10^{-3} M GSSG 5×10^{-4} M GSH	3.53 ± 0.15	0	12.11 ± 1.96	10
10^{-4} M NADH	1.49 ± 0.15	0.20 ± 0.10	1.54 ± 0.07	30

The reaction mixture contained 315 µg microsomal protein and inducers at concentrations as indicated in the table, in a total volume of 2 ml. For details, see Materials and methods. The results are the means of 3 experiments in duplicate ± SEM. *Final ascorbic acid concentration in 2 ml total volume was $1.44 \pm 0.07 \times 10^{-5}$ M.

of reduced and oxidized glutathione or by NADH in brain microsomes (table). Considering these facts, we suggest that the physiological amount of ascorbic acid present in the brain cytoplasm may be one of the factors protecting the membrane structures against peroxidative disintegration in the CNS.

Wills[8] explains the inhibitory effect of high ascorbic acid concentrations on lipid peroxide formation by assuming that, owing to their reductivity, they upset the balance between ferric and ferrous iron, essential for auto-oxidative processes. In the present experiments lipid peroxidation induced by ascorbic acid could be inhibited also by dehydroascorbic acid, showing that its structural properties rather than its reductivity are responsible for the inhibitory effect of large amounts of ascorbic acid. This, however, does not rule out the possibility that high concentrations of ascorbic acid, as an antioxidant agent, would inhibit the other types of peroxidation.

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Toxic substances produced by *Fusarium*. VII. Control of fusarial wilt of safflower by root exudates and extractives of *Ruellia tuberosa*

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Summary. Exudates and extractives of roots of *Ruellia tuberosa*, containing 2,6-dimethoxyquinone, acacetin and a C_{16}-quinone, have been shown to produce significant protective and curative actions against *Fusarium oxysporum*-incited wilt of safflower. The potentiality of the root extractives as a foliar fungicide is appraised.

Fusarium oxysporum f.sp. *carthami*-incited wilt of safflower in India was sometimes reported[2] to be as high as 25%. Another alarming aspect of the disease, reported recently[3-5], was the secretion of a number of mycotoxins by the fungus on safflower seeds in fields and during storage. Nonhost crops, e.g., maize, jawar, etc. were tried[6], from time to time, for reducing the fungal population in wilt-sick soil without achieving any great success. Recently, we reported[7] on the potentiality of 2,2',4-trihydroxybenzophenone as a foliar fungicide against this pathogen.

A remarkable observation was made by the present investigators while surveying the wilting of safflower in fields. The incidence of the disease was found to be very low in safflower plants fortuitously grown in the colony of *Ruellia tuberosa* L. (Acanthaceae). This observation prompted us to examine the effect of exudates and solvent extractives of roots of *R. tuberosa* on the most virulent strain of the pathogen, viz., *F. oxysporum* f.sp. *carthami* (IMI-186539)[2]. The details of these findings are reported in this communication.

Material and methods. Root portions of mature intact plants (65 g) of *R. tuberosa* were kept immersed (12 h) in sterile distilled water (100 ml). A portion of the aqueous solution (50 ml), containing the root exudates, was added to Richard's medium (100 ml). In the control, only distilled water (50 ml) was added. The 2 solutions were inoculated with the fungus and the mixtures were incubated at 21 °C for 7 days. The mycelial dry weight from the 2 cultures was determined. The mean of 3 experiments was recorded.

100 young plants of *R. tuberosa* were transplanted into 10 sterilized potting soil (ca. 2 kg, each). After acclimatization (4 weeks), the fungal mat (5 g), grown in Richard's medium, was added to each lot of potting soil. Subsequently, after 1 week, 10 surface-sterilized healthy safflower seeds were sown in each of the 10 lots of potting soil. The number of seedlings emerged with the fungal infection was recorded. The cotyledonary leaves of mildly affected safflower seedlings were sprayed with the aqueous solution of root exudates of *R. tuberosa*. After 2 weeks, the safflower seedlings were uprooted from the potting soil and a second batch of seeds was sown. For control experiments, susceptible safflower plants were used in place of *R. tuberosa* plants. Finally, the safflower seedlings and the *R. tuberosa* plants were uprooted and the fungal concentration in potting soil was determined by the Soil Smear technique[8] and by the Standard Soil Plate method[9].

In another set of experiments, aqueous sodium carbonate (1%) solution of petroleum ether (b.p. 60–80°) extracts of *R. tuberosa* roots (hereafter referred to as '*Ruellia* extracts'), mainly consisting of 3 chemical constituents in nearly equimolar quantities (average concentration ca. 1×10^{-3} M), was used for determining the antifungal activity. A portion (250 ml) of the aqueous sodium carbonate solution