The effects of vitamin A nutritional status on microsomal lipid peroxidation and α-tocopherol level in rat liver

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Received 26 October 1988; accepted 13 December 1988

Summary. In vitamin A-deficient rats, liver glutathione peroxidase activity was decreased, α-tocopherol content was strongly enhanced, but microsomal liquid peroxidation remained unchanged.
Key words. Vitamin A; vitamin E; liquid peroxidation; liver.

The formation of oxygen radicals and lipid peroxidation (LP) have been recognized as deleterious processes leading to cell damage in various tissues, and they are probably involved in aging and cancer[1]. Mammalian cells contain powerful defence systems including enzyme systems and nonenzymatic antioxidants[2], among which α-tocopherol plays the major role. Glutathione peroxidases detoxify hydrogen peroxide and lipid peroxides; the flavoprotein glutathione reductase regenerates reduced glutathione, maintaining the redox state of the cell. β-carotene is known as a scavenger of singlet oxygen[4] and as a radical trapping antioxidant[5]. However, the in vivo preventive role of retinoids against the damage elicited by free radicals and LP remains largely unknown. In a recent study, Tom et al.[6] found that LP in rat liver was inversely related to vitamin A intake, but these authors did not observe any increase in the generation of active oxygen species ($O_2^{\cdot -}$, O_2^{2-}) in the case of vitamin A deficiency.

The purpose of this work is to investigate the effect of a vitamin A-free diet on liver microsomal LP, in relation to other cell defences namely α-tocopherol, glutathione peroxidases and glutathione reductase.

Material and methods. Weanling male Sprague-Dawley rats (50–60 g) were maintained for eight weeks either on a vitamin A-free semi-synthetic diet (deficient) or the same diet supplemented with 20 IU/g of retinyl acetate (control); the α-tocopherol content was 0.17 mg/g diet in both experimental groups. These diets were prepared by UAR, Villemoisson 91360, France. Rats were killed by decapitation after overnight fasting. Livers were perfused in situ with ice-cold physiological saline, rapidly excised and weighed. Liver samples from the left lobe (1 g) were extracted essentially as described by Olson[7]. Retinol, retinyl palmitate and α-tocopherol were quantified by HPLC using a C18 column (Waters μBondapack), a ternary gradient (CH_3OH, H_2O, CH_3CN) and a spectrophotometric detector set at 290 nm. Elution was performed at 40 °C and at a flow rate of 2.0 ml/min. Retinyl acetate was used as an internal standard. The detection limits for retinol and retinyl palmitate were 5 and 10 ng, respectively. The day-to-day precision was 5%. The remaining liver was homogenized in 3 vols of 10 mM Tris-HCl buffer containing 150 mM KCl and 0.1 mM EDTA, pH 7.4. The homogenates were centrifuged for 15 min at 12,500 × g, the postmitochondrial supernatants at 105,000 × g for 60 min. Microsomal pellets were washed and finally resuspended in the same buffer without EDTA. All procedures were carried out at 2 °C.

Microsomal and cytosolic protein were determined according to Lowry at al.[8] with bovine serum albumin as a standard. Total and selenium-dependent glutathione peroxidase activities were estimated by the method of Burk et al.[9], using tertio-butyl hydroperoxide and hydrogen peroxide as substrates respectively. Glutathione reductase was assayed by the method of Carlberg and Mannervik[10]. 'Spontaneous' or iron-stimulated (ADP-Fe II, 0.5 mM-5 μM) microsomal LP were assessed by the production of malonic dialdehyde[11]. Enzymatic LP was measured in the presence of 0.25 mM NADPH and non-enzymatic LP in the presence of 0.50 mM L-ascorbic acid, tetra 1,1,3,3 ethoxypropane was used as an external standard. Statistical comparisons were made by analysis of variance (ANOVA).

Results and discussion. Final body weight in the vitamin A-deficient group was slightly decreased (table 1).

Table 1. Effects of vitamin A deficiency on body weight, liver cytosolic and microsomal protein, vitamin A and vitamin E status

	Control	Deficient
Body weight[a]	356±8	337±4.6**
Cytosolic protein[b]	77.6±2.5	72.9±1.8
Microsomal protein[b]	38.1±2.7	39.9±1.9
Retinol[c]	13.8±0.9	ND**
Retinyl palmitate[c]	148±11	ND**
α-tocopherol[c]	24.7±1.3	41.7±3.6**

Results are quoted as mean + SEM from 16 rats. [a] g; [b] mg per g liver, [c] μg per g liver. ** $p<0.01$; ND, not detected.

Table 2. Effects of vitamin A deficiency on liver microsomal lipid peroxidation

	Control	Deficient
Enzymatic		
'spontaneous'[a]	18.4±4.2	16.8±2.06
with ADP-Fe II[b]	5.39±0.23	5.67±0.43
Non-enzymatic		
'spontaneous'[a]	108±26	89±22
with ADP-Fe II[b]	7.15±0.40	7.42±0.45

Results are quoted as mean + SEM from 16 rats. [a] pmoles malonic dialdehyde formed per mg microsomal protein per min; [b] nmoles malonic dialdehyde formed per mg microsomal protein per min.

Table 3. Effects of vitamin A deficiency on liver cytosolic glutathione peroxidases and reductase

	Control	Deficient
Total glutathione peroxidase[a]	64.0 ± 4.0	50.3 ± 3.9*
Selenium-dependent glutathione peroxidase[a]	45.1 ± 3.5	32.7 ± 3.6*
Glutathione reductase[a]	26.5 ± 1.7	24.3 ± 1.2

Results are quoted as mean + SEM. [a] nmoles NADPH oxidized per mg cytosolic protein per min. * $p < 0.05$.

Using the same experimental design, Siddik et al.[12] and de Waziers and Albrecht[13] showed that liver vitamin A level began to decline soon after the animals were maintained on a vitamin A-free diet. In the present study retinol and retinyl palmitate were undetectable in the livers of deficient rats. Interestingly, vitamin A deficiency led to a marked increase in α-tocopherol (+69%). Previously Bieri et al.[14,15] reported that retinoids lower intestinal absorption of α-tocopherol. Recently Weiser et al.[16] showed in broiler chickens that increasing vitamin A doses reduce α-tocopherol levels in plasma and liver. Tom et al.[6,17] found an increased LP in liver and lung microsomes. Under our conditions, such an effect was not observed (table 2). This apparent discrepancy could originate from differences in the experimental procedures; the mode of administration of the vitamin, the duration of the deprivation, the extent of contamination of cell fractions by free iron. In the present study, despite the lower activities of both glutathione peroxidases (table 3), (−21% and −28%, for total and seleno enzyme respectively) the large increase in α-tocopherol hepatic stores can prevent peroxidation breakdown of microsomal lipids. One other possible explanation is that vitamin A does not contribute to protection against microsomal lipid peroxidation.

Thus the dietary intake of a single vitamin not only affects its own accumulation in the liver but also the bioavailability and storage of other vitamins. The efficiency of cell defence systems against oxidative stress probably depends upon the interaction between the anti-electrophilic compounds of the diet.

Acknowledgment. This work was supported by PIREN-CNRS grants nr. 955495 and 508005.

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0014-4754/89/040342-02$1.50 + 0.20/0

Latent iron deficiency alters gamma-aminobutyric acid and glutamate metabolism in rat brain

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Received 5 October 1988; accepted 12 January 1989

Summary. A diet containing 18–20 mg iron/kg to young weaned rats for 8 weeks altered the metabolism of gamma-aminobutyric acid and glutamate in the central nervous system without affecting blood hemoglobin. Subsequent rehabilitation with 390 mg iron/kg diet for 2 weeks normalized these changes.
Key words. Latent iron deficiency; brain; GABA; glutamate; enzymes; rehabilitation.

Dietary iron deficiency progresses in a sequence of three overlapping stages. In the first two stages iron stores and transport iron concentrations are depleted and the last stage occurs with diminished production of iron proteins that serve known physiological functions[1]. The early stage of iron deficiency is known as latent iron deficiency and the manifest or last stage is called anemia. Iron deficiency is well known to produce a variety of biological dysfunctions and of particular interest to us is its effects on the central nervous system which may be asso-