Pyridoxine Deficiency-Associated Peroxidative Changes in Rats and the Salubrious Effects of Structurally Related Triterpenes

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Summary Pyridoxine-deficient rats showed increased lipid peroxidation in the kidney when compared with control rats, and the lipid peroxidation was enhanced in the presence of inducers such as ascorbate and hydrogen peroxide. Pyridoxine-deficient kidney showed decreased levels of enzymic antioxidants such as glutathione peroxidase, superoxide dismutase, catalase, glutathione reductase, and glucose-6-phosphate dehydrogenase and non-enzymic antioxidants including ascorbate, glutathione and vitamin E. Treatment with the triterpenes—betulin and lupeol—restored the levels to normal, the latter being more effective in bringing about the changes.

Key Words: lipid peroxidation, triterpenes, antioxidants, betulin, lupeol

Vitamin B₆ plays a central role in amino acid metabolism. Apart from that, oxalate metabolism is the major pathway in which pyridoxine plays a pivotal role. It acts as a cofactor in the transamination of glyoxylate, the precursor of oxalate, to glycine. A deficiency in vitamin B₆ manifests itself as hyperoxaluria due to increased oxalate synthesis in the liver by glycolic acid oxidase [1]. Crystal deposition in the kidney has been reported to induce lipid peroxidation [2]. Triterpenes such as oleanolic and ursolic acids have been shown to protect against peroxidative damage [3]. Hence, evaluation of the related triterpenes—betulin and lupeol—on the peroxidative status in hyperoxaluric rats was undertaken in the present study.

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MATERIALS AND METHODS

Betulin was obtained from Sigma Chemicals, St. Louis, MO, USA; and lupeol was isolated in our laboratory from the stem bark of *Crataeva nurvala* [4].

Male albino rats of the Wistar strain (150–180 g) were used. The animals were housed under standard conditions of temperature ($23 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 10\%$), 12-h light/12-h dark cycle. They were divided into six groups of six rats each. Group I served as controls. Hyperoxaluria was induced in Group II rats by feeding a diet deficient in pyridoxine and containing 3% glycolic acid [5]. Groups III and IV served as drug controls and were each given 35 mg/kg b.wt./d/rat, p.o., of betulin and lupeol, respectively. Groups V and VI were maintained on the pyridoxine-deficient diet and simultaneously treated with betulin and lupeol (35 mg/kg b.wt./d/rat, p.o.), respectively. The control rats were maintained on pyridoxine-supplemented feed (4 mg/kg feed), described by Chow *et al.* [5]. The animal experiments were conducted according to the guidelines for the care and use of laboratory animals in our university.

At the end of 21 days, the rats were decapitated, and their kidneys removed and homogenized in Tris-HCl buffer (0.1 M, pH 7.4). A 10% homogenate was used to estimate lipid peroxidation levels and the antioxidant status [6]. The enzymic antioxidants including glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR), and glutathione-S-transferase (GST) and non-enzymic antioxidant such as

Table 1. Effect of PDD and triterpenes on oxalate levels and lipid peroxidation.

Constituent	Group I C	Group II PDD	Group III C+B	Group IV C+L	Group V PDD+B	Group VI PDD+L
Oxalate (urine)	2.83 $\pm$ 0.12	8.47 $\pm$ 0.65 a*	2.64 $\pm$ 0.11 b*	2.74 $\pm$ 0.10 b*	3.64 $\pm$ 0.28 a*b*c*d*	3.52 $\pm$ 0.21 a # b*c*d #
Oxalate (kidney)	4.89 $\pm$ 0.30	10.13 $\pm$ 0.94 a*	5.11 $\pm$ 0.26 b*	5.31 $\pm$ 0.31 b*	6.78 $\pm$ 0.38 a*b*c*d*	6.4 $\pm$ 0.51 a*b*c # d #
Basal	4.10 $\pm$ 0.26	5.63 $\pm$ 0.38 a*	3.98 $\pm$ 0.28 b*	4.20 $\pm$ 0.27 b*	3.93 $\pm$ 0.21 b*	4.39 $\pm$ 0.30 b*
1 mM ascorbate	6.43 $\pm$ 0.41	7.86 $\pm$ 0.52 a*	6.16 $\pm$ 0.38 b*	6.34 $\pm$ 0.44 b*	5.26 $\pm$ 0.38 a # b*c # d #	6.33 $\pm$ 0.35 b*e #
10 mM FeSO ₄	7.79 $\pm$ 0.55	8.18 $\pm$ 0.48	7.50 $\pm$ 0.56	7.80 $\pm$ 0.49	6.30 $\pm$ 0.36 a*b*c*d*	7.80 $\pm$ 0.51 e*
10 mM H ₂ O ₂	4.55 $\pm$ 0.31	6.10 $\pm$ 0.52 a*	4.21 $\pm$ 0.36 b*	4.44 $\pm$ 0.28 b*	5.07 $\pm$ 0.35 a @ b*c # d @	6.02 $\pm$ 0.45 a*c*d*e*

Values represent mean $\pm$ SD for six rats. Treatment of groups: Group I, control (C); Group II, pyridoxine-deficient (PDD) rats; Group III, control+betulin (B); Group IV, control+lupeol (L); Group V, pyridoxine-deficient rats+betulin; Group VI, pyridoxine-deficient rats+lupeol. Units: Lipid peroxidation in kidney, nmol of MDA formed/min/mg protein; oxalate, mg/24 h urine and mg/g wet tissue. Comparisons are made: a, with Group I; b, with Group II; c, with Group III; d, with Group IV; e, with Group V. Symbols represent statistical significance: @ $p < 0.05$; # $p < 0.01$; * $p < 0.001$.

glutathione (GSH) were estimated by standard protocols followed in our laboratory [6]. Ascorbic acid and vitamin E were quantified by the methods adopted by Omaye *et al.* [7] and Quaife and Dju [8], respectively.

RESULTS

Oxalate levels in the urine and kidney of the pyridoxine-deficient rats indicated hyperoxaluria and crystal deposition (Table 1). There was a marked reduction in the oxalate levels on treatment with the triterpenes. The level of malondialdehyde (MDA) released, which indicates the extent of lipid peroxidation in the kidney of the experimental animals, is also shown in Table 1. Basal, ascorbate-, and hydrogen peroxide-induced lipid peroxidation was found to be increased in the pyridoxine-deficient animals when compared with their control levels. Restoration of normal levels was seen only in the lupeol-treated animals. The antioxidant status of the kidney was decreased as evidenced from the decreased activities of the enzymic antioxidants GPx, catalase, SOD, GR, and G6PD and of the non-enzymic antioxidants GSH, ascorbate, and vitamin E. GST levels were found to be unaffected (Table 2). Administration of lupeol and betulin brought about a

Table 2. Effect of betulin and lupeol on antioxidant status in kidney of PDD fed rats.

Constituent (units/min/mg protein)	Group I C	Group II PDD	Group III C+B	Group IV C+L	Group V PDD+B	Group VI PDD+L
GPx	17.17±1.23	12.45±1.10 a*	16.97±1.41 b*	16.48±1.52 b*	16.70±1.08 b*	15.88±1.14 b*
Catalase	242±20	107±8 a*	236±19 b*	240±20 b*	222±20 b*	231±19 b*
SOD	4.20±0.31	3.24±0.25 a*	4.08±0.39 b*	4.44±0.37 b*	4.02±0.29 b*	4.14±0.32 b*
GR	2.34±0.14	1.93±0.16 a [#]	2.11±0.18 a [@]	2.38±0.15 b*c [@]	2.52±0.18 b*c [#]	2.48±0.17 b*c [#]
GST	28.50±1.84	25.25±2.46	26.75±2.12	27.21±2.31	25.80±2.11	27.25±1.68
G6PD	2.36±0.22	1.96±0.07 a [#]	2.21±0.15	2.16±0.17	2.14±0.18	2.13±0.15
<hr/> (μg/mg protein) <hr/>						
GSH	3.99±0.21	2.14±0.16 a*	3.65±0.25 b*	3.85±0.31 b*	3.46±0.26 a [#] b*d [@] a*b*c [#] d*e [@]	3.06±0.25
Vitamin C	1.50±0.08	1.23±0.08 a*	1.41±0.08 b [#]	1.56±0.07 b*c [@]	1.30±0.08 a [#] c [@] d*	1.41±0.07 b [#] d [@]
Vitamin E	0.46±0.03	0.29±0.01 a*	0.48±0.03 b*	0.43±0.04 a*b*c [#]	0.32±0.02 a*c*d*	0.38±0.02 a*b*c*d [#] e*

Values represent mean±SD for six rats. Treatment of groups: as in Table 1. Unit of enzyme activity: GPx, μmol of GSH; catalase, μmol of H₂O₂; SOD, amount of enzyme required to bring about 50% inhibition of autoxidation of pyrogallol; GR and G6PD, nmol of NADPH; GST, nmol of CDNB-GSH conjugate. Comparisons are made: as in Table 1. Symbols represent statistical significance: [@] *p*<0.05; [#] *p*<0.01; **p*<0.001.

significant change towards normal in the treated animals.

DISCUSSION

Feeding the rats a diet deficient in pyridoxine for 21 days resulted in hyperoxaluria and crystal deposition, which deficiency is known to induce lipid peroxidation. The increased susceptibility of the kidney to peroxidative damage might be due to the high vascularity of the kidney.

Increased lipid peroxidation is associated with the depletion of the antioxidant status in the tissue. Replenishing the GSH levels is necessary for maintenance of the overall thiol status in the cell. The observed decrease in the GSH levels in the kidney of the hyperoxaluric rats might have been due to its increased conversion to GSSG. The consequence of this is the reduction in the regeneration of ascorbate from dehydroascorbate. Consequently, there is a hindrance to the regeneration of vitamin E. This simultaneous decrease in the two vitamins leads to elevated lipid peroxidation [9]. The regeneration of GSH requires NADPH supplied by G6PD. A decrease in the G6PD activity seen in the pyridoxine-deficient rats explains the decreased GSH level. This also accounts for the decreased GR activity in these animals. Moreover, oxidative assault inhibits both GR and G6PD activities, as reported by Brin and Yonimoto [10].

Accumulation of hydrogen peroxide is injurious to cells. The increase in hydrogen peroxide levels in the cell can be supposed by the observed decrease in the GPx and catalase activities, the two enzymes responsible for the decomposition of hydrogen peroxide [11]. The reduction in the availability of the substrate of GPx might be the reason for the decrease in its activity. The results showed that the catalase activity was reduced by more than half in the pyridoxine-deficient animals. This might be a cumulative effect of the nephrotoxic effect of oxalate [12] and the decreased availability of NADPH, which is necessary for its regeneration from inactive forms [13]. On the other hand, Selvam and Ravichandran [14] also observed decreased SOD levels in pyridoxine-deficient rats. The combined effect of the three led to enhanced lipid peroxidation in the group II animals.

Lipid peroxidation levels were lowered remarkably, and also the antioxidant status of the kidney was restored, on treatment. Though the resultant change towards normal was similar with both lupeol and betulin treatment, overall lupeol was more efficacious; for a more significant change was observed in some parameters such as vitamins E and C and peroxidation in the presence of inducers such as ascorbate and ferrous sulphate. Earlier reports from our laboratory also showed that lupeol was a good cytoprotective agent against free radical-induced toxicity, in both urolithiasis and arthritis [4, 15]. The present results further emphasize its efficacy against peroxidative damage to tissues.

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