Prevention of Orotic Acid-Induced Fatty Liver with Allopurinol (35203)

H. G. WINDMUELLER AND LEO H. VON EULER
(Introduced by J. G. Bieri)

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

Standerfer and Handler (1) observed that rats develop grossly fatty livers when fed a nutritionally adequate semisynthetic diet supplemented with 1% orotic acid, an intermediate on the biosynthetic pathway to all pyrimidine nucleotides. This observation has since been confirmed and extended in several laboratories [see Ref. (2) for a review]. It has been established that the hepatic steatosis results from a total block in release of triglycerides and low-density or β-lipoproteins from the liver into the blood (3, 4) and is therefore accompanied by greatly reduced concentrations of circulating lipids, particularly triglycerides (5, 6). In addition to producing a massive accumulation of hepatic triglycerides, orotic acid also depresses the steady-state concentration of acid-soluble adenosine nucleotides in the liver and increases greatly the concentrations of the uridine nucleotides (7, 8). The block in hepatic lipid transport and also the altered hepatic nucleotide concentrations are prevented, as well as reversed, when the orotic acid-containing diet is supplemented with 0.25% adenine sulfate (7, 9). An adenine precursor, 4-amino-5-imidazolecarboxamide, also protects against orotic acid fatty liver (10), but two other purines, hypoxanthine and xanthine, are not protective (9). We have now found another purine, allopurinol (4-hydroxypyrazolo-[3,4-*d*]-pyrimidine), which protects rats completely from the known effects of orotic acid. Allopurinol, a structural analogue of hypoxanthine, is an inhibitor of the enzyme xanthine oxidase (11) and is widely used clinically in the treatment of hyperuricemia and gout (12).

Materials and Methods. The rats used were 80 to 200 g males of the National Institutes of Health Osborne-Mendel or Sprague-Dawley strains. They were caged individually in wire-bottomed cages and supplied with food and water *ad libitum*. The basal diet (W-7) contained 68% glucose monohydrate, 20% casein, 5% corn oil, 0.3% DL-methionine, 0.2% choline chloride plus adequate amounts of vitamins and minerals (13). Orotic acid (General Biochemicals Corp.) and allopurinol (Calbiochem) were added at the expense of the whole diet.

The procedures used for determining the total fatty acid content of liver and plasma were described previously (3). For the determination of hepatic acid-soluble nucleotides, portions of liver removed from ether-anesthetized rats were immediately homogenized in 0.5 *M* perchloric acid. The extracts were adjusted to pH 8 with KOH and, after centrifugation, a portion of the supernatant fraction was applied to a 1 $\times$ 25-cm column of Dowex-1(X 10), 200 to 400 mesh, in the formate form. The adsorbed nucleotides were eluted with a nonlinear gradient (approx. 0.1 to 1.0 *M*) of ammonium formate, pH 5.2. Fractions of 5 to 10 ml were collected and the absorbancy determined at 260 nm. Nucleotides in the fractions were identified from their absorption spectra and from elution patterns obtained with mixtures of known reference compounds.

Results. As little as 0.05% allopurinol in the diet completely prevented the accumulation of liver fat and the weight gain depression produced by 1% orotic acid (Table I). Even 0.01% allopurinol appeared to have a slight protective action. In another experiment (Table II), 0.075% allopurinol when added to the diet alone did not influence the circulating total fatty acid concentration, but when added to the diet containing 1% orotic acid it prevented the reduction in circulating

TABLE I. Liver Total Fatty Acids After Feeding Orotic Acid and Allopurinol.

Additions to basal diet	Body wt gain, 14 days[a] (g)	Total fatty acid content of livers[a] (μmoles/g)
None	101 ± 4.6	90.4 ± 3.6
Orotic acid, 1%	73 ± 1.3	768 ± 51
+ allopurinol, 0.1%	100 ± 3.7	92.6 ± 3.8
0.05%	107 ± 3.6	94.9 ± 5.1
0.01%	93 ± 2.7	530 ± 36

[a] Mean values ± standard error for 4 animals/group fed the respective diet for 14 days.

lipid and the fatty liver produced by orotic acid alone.

The data in Table III illustrate the typical depression in the hepatic steady-state concentrations of adenosine nucleotides and the elevation in the concentration of uridine nucleotides produced by 1% orotic acid. Allopurinol alone (0.05%) appeared to depress the concentration of UMP + UDP + UTP. When added to a diet containing 1% orotic acid, allopurinol (0.075% and 0.05%) effectively prevented the large concentration changes in adenosine as well as uridine nucleotides produced by orotic acid alone. The lowest level of allopurinol tested (0.01%), which gave only slight protection against the fatty liver (Table I), inhibited only slightly the orotic acid-induced nucleotide changes, particularly the elevated uridine nucleotide concentrations.

Discussion. These studies show that a small amount of allopurinol, 5 mg or less/day for a 100 g rat, is completely effective in preventing the total block in liver-to-plasma triglyceride transport and the altered hepatic nucleotide concentrations produced by orotic acid. Similar preventive effects are obtained with adenine (7, 9). 4-Amino-5-imidazolecarboxamide also prevents the fatty liver (10), and although its effect on nucleotide pools has not been reported, it would be expected to behave like adenine since it is converted efficiently into this purine by the rat (14). The present results, therefore, provide further support for a biochemical link between the nucleotide imbalance and the block in lipid transport in livers of orotic acid-fed rats.

In this connection, several mechanisms may contribute to the observed action of allopurinol. Orotic acid causes an increase in the activity of hepatic adenosine deaminase and xanthine oxidase (15). Furthermore, from studies *in vivo* with formate-^{14}C and glycine-2-^{14}C, it has been shown that orotic acid increases the rate of purine biosynthesis *de novo* and the turnover rate of the acid-soluble purine nucleotide pool (16). Together, these results suggest that contraction of the purine nucleotide pool in orotic acid-fed rats results from accelerated catabolism of purines in the liver. By inhibiting xanthine oxidase (11) allopurinol reduces catabolism by slowing the conversion of hypoxanthine to xanthine and of xanthine to uric acid, thereby promoting the reutilization of both hypoxanthine (17) and xanthine (18) for nucleotide synthesis. At the same time, allopurinol reduces somewhat the biosynthesis of purines *de novo*, an effect which may in part be independent of xanthine oxidase inhibition (19). Unless xanthine oxidase is inhibited, exogenous hypoxanthine and xanthine are rapidly catabolized in the rat and contribute little to the hepatic purine nucleotide pool (17, 18), which may explain why these purines failed to protect against the fatty liver

TABLE II. Plasma Total Fatty Acids After Feeding Orotic Acid and Allopurinol.

Additions to basal diet	Body wt gain, 7 days (g)	Plasma total fatty acids[a] (μmoles/ml)	Gross appearance of livers
None	53	5.8	All normal
Allopurinol, 0.075%	48	5.8	All normal
Orotic acid, 1%	41	2.7	All fatty
+ allopurinol, 0.075%	54	5.0	All normal

[a] Mean values for 3 animals/group fed the respective diet for 7 days.

TABLE III. Liver Acid-Soluble Nucleotides After Feeding Orotic Acid and Allopurinol.

Additions to basal diet[a]	N[c]	Nucleotide (μmoles/g of liver)[b] AMP + ADP + ATP	UMP + UDP + UTP	UDP-N-acetyl-glucosamine	UDP-glucose
None	2	2.65	1.39	0.42	0.72
Orotic acid, 1%	5	0.88	3.98	1.65	1.82
Allopurinol, 0.05%	1	2.13	0.71	0.43	0.66
Orotic acid, 1%					
+ allopurinol, 0.075%	1	2.89	0.81	0.51	0.61
0.05%	1	1.88	1.82	0.61	0.81
0.01%	1	1.56	3.45	1.01	2.08

[a] Diets fed for 7 to 11 days before livers were analyzed.

[b] AMP, ADP, and ATP are adenosine-5'-mono-, di-, and triphosphate, respectively. UMP, UDP, and UTP are uridine-5'-mono-, di-, and triphosphate, respectively.

[c] Number of animals/group.

when they were added (0.5%) to a diet containing 1% orotic acid (9).

In addition to its effect on xanthine oxidase, allopurinol may prevent the fatty liver through a direct effect on the metabolism of orotic acid. In man (20, 21) as well as in the rat (20) administration of allopurinol produces a marked orotic aciduria and orotidinuria. From studies *in vitro* allopurinol appears to act as a competitive inhibitor with respect to orotic acid in the phosphoribosyltransferase reaction which converts orotic acid to orotidylic acid (20), while the ribonucleotide of allopurinol inhibits the decarboxylation of orotidylic acid to UMP (20, 21).

Allopurinol may also have indirect effects on orotic acid metabolism. The allopurinol-stimulated reutilization of hypoxanthine and xanthine by the action of a phosphoribosyltransferase would require equimolar quantities of ribosylpyrophosphate 5-phosphate (PP-ribose-P). If the salvage rate were sufficiently great, competition for limiting amounts of PP-ribose-P might restrict the production of UMP from orotic acid (7), a conversion also requiring PP-ribose-P. The conversion of allopurinol itself to a nucleotide, which would also drain PP-ribose-P, may possibly contribute to this effect, although compared with hypoxanthine, allopurinol was shown to be a very poor substrate *in vitro* for the phosphoribosyltransferase from human erythrocytes (22), and after exposure to allopurinol, human fibroblasts in culture accumulate little if any allopurinol ribonucleotide (19).

Thus, known effects of allopurinol on purine metabolism or on pyrimidine nucleotide biosynthesis, or both, could explain the protective action against orotic acid fatty liver. Allopurinol may prove useful in future studies to elucidate the mechanism of orotic acid action.

Summary. Allopurinol in small amounts (0.05% of the diet) is completely effective in preventing the fatty liver and the changes in hepatic adenosine and uridine nucleotide concentrations produced in rats by feeding orotic acid (1% of the diet). The action of allopurinol may be explained by known effects on both purine and pyrimidine metabolism. The results provide further support for a biochemical link between the altered nucleotide levels and the block in hepatic lipid transport produced by orotic acid.

1. Standerfer, S. B., and Handler, P., Proc. Soc. Exp. Biol. Med. **90**, 270 (1955).

2. Windmueller, H. G., Levy, R. I., and Spaeth, A. E., Perspect. Biol. Med. 4, 365 (1969).

3. Windmueller, H. G., J. Biol. Chem. **239**, 530 (1964).

4. Windmueller, H. G., and Levy, R. I., J. Biol. Chem. **242**, 2246 (1967).

5. Creasey, W. A., Hankin, L., and Handschumacher, R. E., J. Biol. Chem. **236**, 2064 (1961).

6. Windmueller, H. G., Biochem. Biophys. Res. Commun. **11**, 496 (1963).

7. von Euler, L. H., Rubin, R. J., and Handschumacher, R. E., J. Biol. Chem. **238**, 2464 (1963).

8. Marchetti, M., Puddu, P., and Caldarera, C. M., Biochim. Biophys. Acta **61**, 826 (1962).

9. Handschumacher, R. E., Creasey, W. A., Jaffe, J. J., Pasternak, C. A., and Hankin, L., Proc. Nat. Acad. Sci. U.S.A. **46**, 178 (1960).

10. Hayano, K., Tsubone, T., Azuma, I., and Yamamura, Y., J. Biochem. (Tokyo) **52**, 379 (1962).

11. Lorz, D. C., and Hitchings, G. H., Fed. Soc., Fed. Amer. Soc. Exp. Biol. **9**, 197 (1950).

12. Rundles, R. W., Wyngaarden, J. B., Hitchings, G. H., and Elion, G. B., Annu. Rev. Pharmacol. **9**, 345 (1969).

13. von Euler, L. H., and Windmueller, H. G., Proc. Soc. Biol. Med. **125**, 1251 (1967).

14. Miller, C. S., Gurin, S., and Wilson, D. W., Science **112**, 654 (1950).

15. Marchetti, M., Puddu, P., and Caldarera, C. M., Biochem. J. **92**, 46 (1964).

16. Windmueller, H. G., and Spaeth, A. E., J. Biol. Chem. **240**, 4398 (1965).

17. Pomales, R., Bieber, S., Friedman, R., and Hitchings, G. H., Biochim. Biophys. Acta **72**, 119 (1963).

18. Pomales, R., Elion, G. B., and Hitchings, G. H., Biochim. Biophys. Acta **95**, 505 (1965).

19. Kelley, W. N., and Wyngaarden, J. B., J. Clin. Invest. **49**, 602 (1970).

20. Fox, R. M., Royse-Smith, D., and O'Sullivan, W. J., Science **168**, 861 (1970).

21. Kelley, W. N., and Beardmore, T. D., Science **169**, 388 (1970).

22. Krenitsky, T. A., Papaioannou, R., and Elion, G. B., J. Biol. Chem. **244**, 1263 (1969).

Received Sept. 1, 1970. P.S.E.BM., 1971, Vol. 136.