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INHIBITION OF PURINE CATABOLISM BY BENZBROMARONE IN ISOLATED RAT LIVER CELLS

COMPARISON WITH ALLOPURINOL AND PROBENECID

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Abstract—Benzbromarone, a potent uricosuric agent, inhibited allantoin production in isolated hepatocytes at concentrations half to ten times greater than therapeutic plasma levels of the drug. In addition, the drug at these concentrations also markedly inhibited xanthine oxidase (EC 1.2.1.37), an enzyme involved in the regulation of this pathway. We found that allopurinol is several times superior to benzbromarone in the lowering of allantoin production (if they are compared in terms of their relative therapeutic levels), and that probenecid had no effect on it.

Benzbromarone is a benzofuran derivative (Fig. 1) which lowers serum urate by its potent uricosuric properties. Although some authors have demonstrated a significant effect on enzymes involved in purine metabolism, others have reported the opposite and thus no definite conclusions can be drawn from studies available at present. Some urate-lowering activity in a small number of anephric patients supports the possible existence of an extrarenal mechanism of action (i.e. other than increased uricosuria) but further systematic investigation is needed to confirm this [1].

The purpose of this study was to determine the effect of benzbromarone on purine catabolism in isolated hepatocytes as an alternative mechanism of action of the drug, comparing it with the effect of probenecid, a uricosuric drug, and allopurinol, a potent xanthine oxidase inhibitor.

MATERIALS AND METHODS

Animals. Male Wistar rats, maintained on standard laboratory diet and weighing 250–300 g were used. Rats were fasted for 24 hr before experiments.

Chemicals. Probenecid, xanthine, guanine and NAD were purchased from Sigma Chemical Company (St. Louis, MO). Collagenase was obtained from Boehringer GmbH (Mannheim, F.R.G.). Benzbromarone and allopurinol were generously donated by the laboratories Labaz and Fides (both from Barcelona, Spain) respectively. All other reagents were of analytical grade.

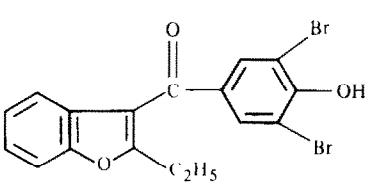

Fig. 1. Chemical structure of benzbromarone: 2-ethyl-3-(4-hydroxy-3,5-dibromobenzoyl)-benzofuran.

Isolation and incubation of hepatocytes. Isolated hepatocytes were prepared from 24-hr starved rats as described in [2], with the modifications suggested in [3, 4]. The cells (70–100 mg wet wt) in a final volume of 4 ml Krebs–Henseleit bicarbonate buffer pH 7.4 [5] containing the appropriate substrates were incubated at 37° in 25 ml Erlenmeyer flasks in a shaking bath for 30 min [6]; the flask atmosphere contained 95% O_2:5% CO_2. More than 85% of the cells excluded Trypan blue at the end of the incubation. The reactions were stopped with perchloric acid (2% w/v final concentration) and the precipitate was removed by centrifugation. The supernatant was used for the assays.

Biochemical determinations. Xanthine oxidase (EC 1.2.1.37) was assayed in whole rat liver homogenates in the presence and absence of the drugs according to [7]. Allantoin was measured colorimetrically on perchloric acid extracts [8]. Protein was determined as in [9] using bovine serum albumin as standard.

Statistical analysis of data was carried out using Student's *t*-test.

RESULTS

Figures 2 and 3 show the effect of benzbromarone on allantoin production by isolated hepatocytes in a given time (the 30-min period between the 15 and 45 min of the incubation).

As previously established [10] the rate of allantoin production from any precursor depends on the importance of its salvage pathway, so that the major allantoin production was reached by using xanthine and guanine as precursors. These substances are

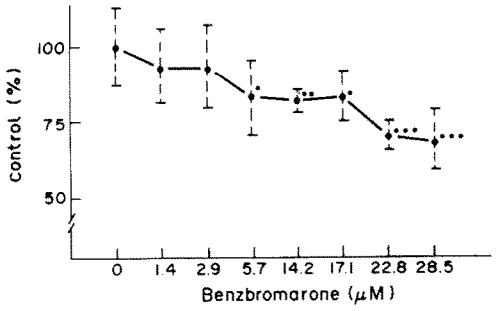

Fig. 2. Effect (% of control) of benzbromarone on purine catabolism from xanthine in isolated hepatocytes. The absolute control value (100%) was 0.041 ± 0.005 mg allantoin/g wet wt/min. Xanthine concentration was 1 mM. The results are means ± SD for 6 or 7 experiences. Results that are significantly different from those of control are shown: *P < 0.05, **P < 0.01, ***P < 0.001.

rapidly catabolized to allantoin, whereas nucleotide synthesis is low [10]; therefore they were chosen as precursors in our experiences. In both cases, allantoin production was linear with time during the incubation period studied (data not shown).

Benzbromarone clearly inhibited purine catabolism in isolated hepatocytes. This marked inhibition

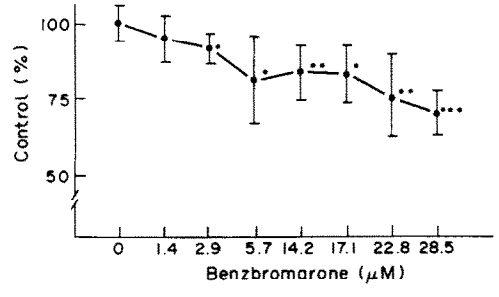

Fig. 3. Effect (% of control) of benzbromarone on purine catabolism from guanine in isolated hepatocytes. The absolute control value (100%) was 0.042 ± 0.003 mg allantoin/g wet wt/min. Guanine concentration was 1 mM. The results are means ± SD for 6 or 7 experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

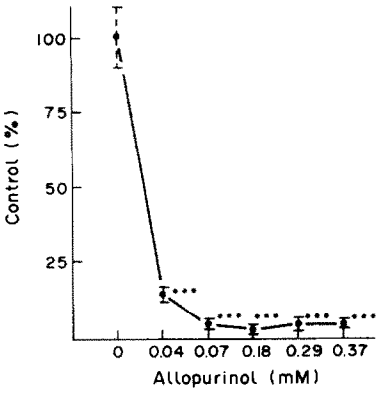

Fig. 5. Effect (% of control) of allopurinol on purine catabolism from xanthine in isolated hepatocytes. The absolute control value was 0.041 ± 0.004 mg allantoin/g wet wt/min. Xanthine concentration was 1 mM. Results are means ± SD for 6 experiments. ***P < 0.001.

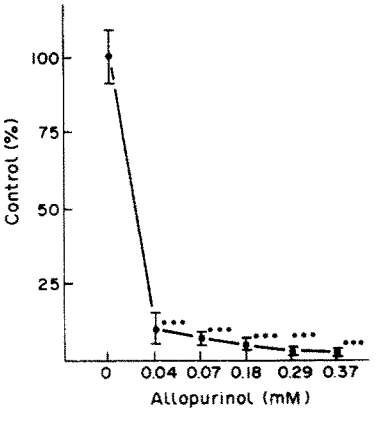

Fig. 6. Effect (% of control) of allopurinol on purine catabolism from guanine in isolated hepatocytes. The absolute control value was 0.042 ± 0.004 mg allantoin/g wet wt/min. Guanine concentration was 1 mM. Results are means ± SD for 6 experiments. ***P < 0.001.

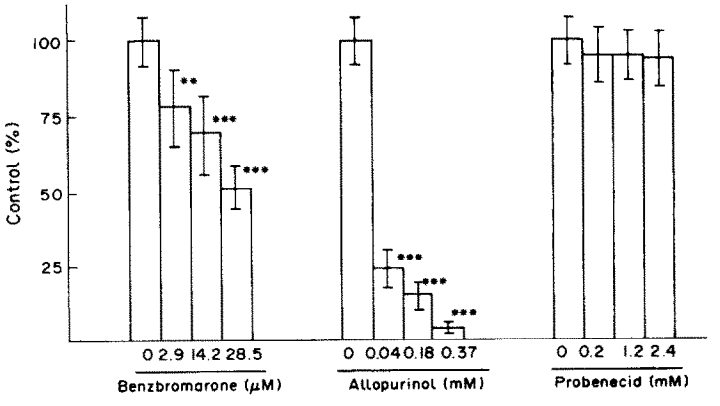

Fig. 4. Effect (% of control) of benzbromarone, allopurinol and probenecid on xanthine oxidase activity in rat liver homogenate. The absolute control value was 1.70 ± 0.13 nmol uric acid/min/mg liver protein. The homogenates were preincubated with the drugs for 30 min. The activity was assayed as described in Methods. Results are expressed as means ± SD for 5 or 6 experiments. **P < 0.01, ***P < 0.001.

Table 1. Effect of probenecid on purine catabolism from xanthine and guanine in isolated hepatocytes

Concentration of probenecid (mM)	Precursors Xanthine	Guanine
0	0.039 ± 0.009	0.041 ± 0.005
0.1	0.039 ± 0.009	0.038 ± 0.008
0.2	0.037 ± 0.004	0.038 ± 0.004
0.5	0.035 ± 0.005	0.035 ± 0.006
1.2	0.033 ± 0.006	0.037 ± 0.005
2.4	0.032 ± 0.007	0.036 ± 0.007

Xanthine and guanine concentration were 1 mM. Allantoin production is expressed as mg/g wet wt/min. Results are expressed as means ± SD for 6 experiments.

(*ca.* 34% at 28.5 μM of benzbromarone) suggests an inhibitory effect of the drug on the steps catalyzed by xanthine oxidase, since both precursors, xanthine and guanine, enter into the pathway before the mentioned steps. On this point, Fig. 4 shows that xanthine oxidase is inhibited markedly by the addition of benzbromarone. Under the same conditions, the enzyme was inhibited by allopurinol, as previously described [11], but it was not affected by probenecid.

Figures 5 and 6 show the effect of allopurinol at concentrations one to ten times greater than therapeutic plasma levels of the drug [12], in isolated hepatocytes. As expected from xanthine oxidase inhibition, allopurinol markedly decreased allantoin production from both precursors. When data from allopurinol and benzbromarone are compared, it appears that inhibition of the pathway by benzbromarone is due to a direct inhibition of xanthine oxidase.

In contrast, probenecid used at the same relative levels as allopurinol [13] had no effect on purine catabolism in isolated hepatocytes (Table 1), as also expected since it produced no inhibition on xanthine oxidase.

DISCUSSION

Hyperuricemia is a common clinical disorder, which occurs in as many as 13 per cent of a hospitalized population [14]. The lowering of serum uric acid (end product of purine catabolism in man and primates because of their lack in uricase) to normal is necessary in patients with clinical symptoms associated with hyperuricemia and occasionally in certain asymptomatic subjects. The serum uric acid may be diminished by drugs which act either by decreasing the production of uric acid (allopurinol) or by increasing its renal excretion (uricosurics: probenecid, benzbromarone). Our results demonstrate that benzbromarone not only acts by a uricosuric mechanism but by decreasing uric acid production as well.

The potential clinical implications of our study deserve consideration. In this study, benzbromarone has been used at concentrations half to ten times greater than the therapeutic plasma levels of the drug [15]. Therefore a significant inhibition of xanthine oxidase activity and of uric acid synthesis might occur during the course of treatment with benzbromarone. This could explain the lowering effect of benzbromarone on serum uric acid in anephric patients.

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