*J. Pharm. Pharmac.*, 1971, **23**, 931–935 *Received May* 13, 1971

# The action of azapropazone, oxyphenbutazone and phenylbutazone on lysosomes

*D. A. LEWIS, R. B. CAPSTICK AND R. J. ANCILL

*Pharmacology Group, School of Pharmacy, University of Bath, Claverton Down, Bath BA2 7AY, U.K.*

Azapropazone, oxyphenbutazone and phenylbutazone have a stabilizing action on isolated lysosomes over a wide concentration range but a lytic action at high concentrations. The lytic action of phenylbutazone was greater than the other drugs. Phenylbutazone at a high concentration was found to accelerate the breakdown of lysosomes in isolated stomach preparations *in vitro*. Phenylbutazone was found to have a greater ulcerogenic action than azapropazone in rats and rabbits. Tissues removed from rats and rabbits dosed with phenylbutazone showed evidence of lysosomal damage when examined histochemically for acid phosphatase. In contrast, tissues from control and azapropazone-treated rats showed no evidence of lysosomal damage. Sections of rat gut incubated with azapropazone and phenylbutazone *in vitro* showed similar results. The possibility is discussed that drugs in high concentration may damage lysosomes in the gastrointestinal tract. It is suggested that lysosomal damage may contribute to the ulcerogenic action of the drugs *in vivo*.

The actions of anti-inflammatory drugs on lysosomes are of interest since lysosomal enzymes are suspected mediators of inflammation (Weissman, 1967). The actions of some anti-inflammatory aromatic acids and anti-inflammatory steroids are concentration-dependent (Lewis, 1970; Lewis, Symons & Ancill, 1970). This investigation was concerned with the effect of concentration of azapropazone, oxyphenbutazone and phenylbutazone on lysosomes. The actions of the drugs on lysosomes was compared with that of prednisolone since the anti-inflammatory action of steroids has been related to their stabilizing actions on lysosomes (Weissmann & Dingle, 1961). The ulcerogenic action of phenylbutazone in rats was also investigated to examine the possibility that lysosomal damage at high drug concentrations may be involved in the development of ulcers *in vivo*.

## METHODS

### *Isolated lysosome experiments*

Lysosomes were isolated from rat and rabbit liver by methods previously described for rabbit liver (Weissmann, 1965). An identical procedure was used to prepare lysosomes from rat ileum. The lysosomes were finally suspended in 0·05M-tris-acetate buffer (pH 7·4), sucrose (0·25M) and the protein concentration determined (Lowry, Rosebrough & others, 1951). The drugs were dissolved in dimethyl sulphoxide and 0·1 ml portions added to 5 ml of the lysosome suspension. Dimethyl

* Present address: Department of Pharmacy, The University of Aston in Birmingham, Gosta Green, Birmingham, U.K.

sulphoxide was added to the controls. The suspensions were incubated with shaking at 37° for 90 min. After incubation the lysosomes were removed and the acid phosphatase (EC 3.1.3.2) and β-acetylglucosaminase (EC 3.2.1.30) activity of the supernatants were determined (Symons & others, 1969; Lewis & others, 1970).

*Isolated tissue experiments*

Six female rats (200 g) were killed by a blow to the head and the stomachs removed and placed on cracked ice. Each stomach was slit open and placed in 22 ml of oxygenated Ringer-Tyrode buffered at pH 6 with 0·1M phosphate containing *p*-nitrophenyl phosphate (0·015M) in stoppered flasks. The flasks were gently shaken and samples of the medium (1 ml) taken at intervals. The samples were centrifuged and 0·5 ml portions of the supernatants transferred to test tubes and 5 ml of 0·1N sodium hydroxide added. The absorbances of the solutions were then determined at 420 nm. After the stomachs had been incubating for 75 min, phenylbutazone dissolved in dimethyl sulphoxide was added to three flasks to give a final concentration of $4{\cdot}4 \times 10^{-3}$M. The same volume (0·5 ml) of dimethyl sulphoxide was added to the three control flasks. The flasks were incubated for 6 h, samples of the medium being taken at intervals and assayed for acid phosphatase. In a second experiment female rats (250–300 g) were killed by a blow to the head and 5 cm ileum segments removed. After slitting longitudinally these were placed in oxygenated Ringer-Tyrode solution buffered at pH 6 with 0·1M phosphate, and containing either phenylbutazone or azapropazone at a concentration of $4 \times 10^{-3}$M. The flasks were incubated at 37° for 90 min, after which time the segments were removed and plunged into acetone-solid $CO_2$ at −70°. The segments were then examined histochemically for acid phosphatase by the Gomori (1941) procedure except that the incubation time with sodium β-glycerophosphate was reduced to 2 h instead of 4 h.

*Whole animal experiments*

*Rabbits.* Azapropazone and phenylbutazone were separately granulated and mixed with animal meal and subsequently pelleted for feeding to rabbits. Preliminary experiments had established that a rabbit would eat 200–250 g of pellets each day. During the experimental period of 5 days they were given 100 g of the pellets containing drugs and it was found that this was completely eaten. This was equivalent to a drug dose of 500 mg/kg per day. The rabbits selected were of similar weight (1 kg). Two were treated with phenylbutazone and two with azapropazone. After five days they were killed and the stomach and gut removed for examination. Segments were frozen in acetone-solid $CO_2$ and subsequently examined histochemically as described by the Gomori (1941) procedure for acid phosphatase.

*Rats.* Azapropazone or phenylbutazone was suspended (20%, w/v) in syrup B.P. and separately administered to Wistar rats (250 g). In one experiment drug, 500 mg/kg was administered daily for five days. In a second experiment drug, 250 mg/kg, was administered at 48 h intervals. In this experiment the rats were dosed on four occasions. Six h after the rats had their final dose they were killed and the digestive systems were examined for signs of ulceration.

In the second experiment segments of the ileum were plunged into acetone-solid $CO_2$ at −70° and then examined for acid phosphatase (Gomori, 1941).

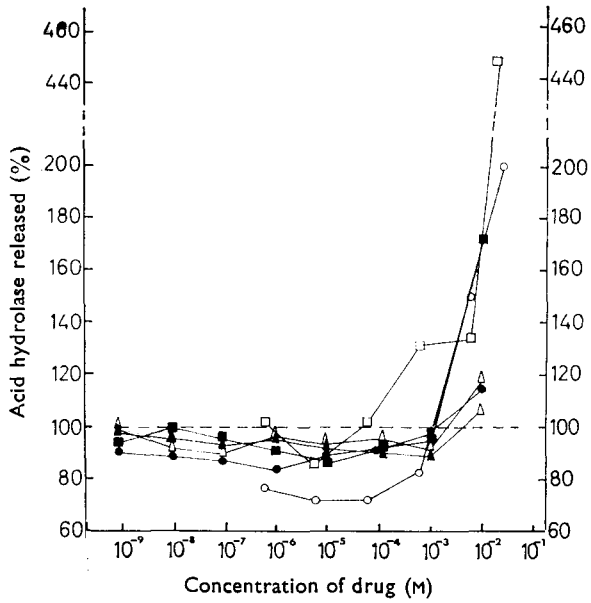


FIG. 1. Action of azapropazone, oxyphenbutazone and phenylbutazone on the release of acid phosphatase from lysosomes. The control valves have been adjusted to 100. Values below 100 represent a stabilizing action and values above 100 represent a lytic action by the drugs. The results represent the mean values of four determinations for the liver lysosomes and two determinations for the ileum lysosomes. Legends. ●—Azapropazone; ▲—oxyphenbutazone; ■—phenylbutazone; △—prednisolone (rabbit liver lysosomes); ○—azapropazone; □—phenylbutazone (rat ileum lysosomes). Although not plotted the standard error of the mean did not exceed ±2·4 for any value. Most values were less than ±0·5.

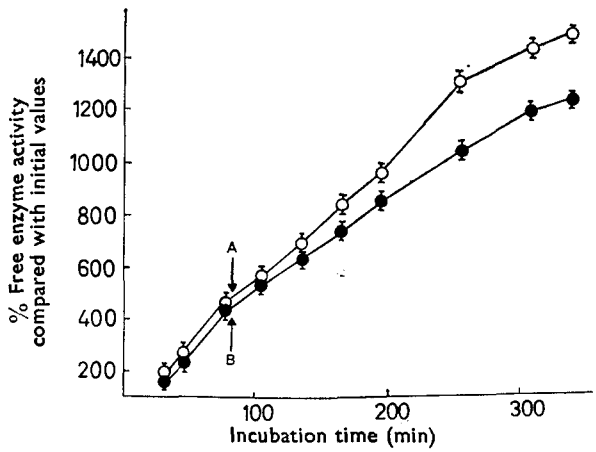


FIG. 2. The effect of phenylbutazone on the release of acid phosphatase from rat stomachs *in vitro*. Each value is the mean ± standard error of the mean of three experiments. ○—experimental; ●—control. Initial values have been adjusted to 100%. A, addition of DMSO to controls. B, addition of phenylbutazone to tests.

## RESULTS

### *Effects of the drugs on lysosomes*

The action of the three drugs on rabbit liver lysosomes and rat ileum lysosomes is shown in Fig. 1. Clearly the drugs have stabilized the lysosomes at the lower

concentrations; the % stabilizing action on liver lysosomes is similar to that of prednisolone. At higher concentrations the drugs have a lytic action on lysosomes, phenylbutazone being the most lytic. A similar type of stabilization-lysis curve was obtained when rat liver was used in place of rabbit liver and where β-acetyl glucosaminase activity was plotted in place of acid phosphatase.

The protein content of the ileum and liver lysosome suspensions was 3·5–6 mg/ml.

*Effects of the drugs on isolated tissues*

The results of the experiments where stomachs were incubated with *p*-nitrophenyl phosphate in the presence of phenylbutazone is shown in Fig. 2. It is clear that phenylbutazone has accelerated the rate of hydrolysis of *p*-nitrophenyl phosphate.

Evidence that the drugs damage lysosomes in tissues was provided by the experiment where gut segments were incubated *in vitro* with phenylbutazone and azapropazone. Segments that had been incubated with phenylbutazone stained heavily and diffusely in patches, for acid phosphatase, whilst the controls and azapropazone-treated segments were only lightly stained in discrete 'point-like' granules.

*Whole animal experiments*

*Rats.* Phenylbutazone was found to have a greater ulcerogenic action in rats than azapropazone. Four out of six rats survived the phenylbutazone treatment, and five out of six rats survived the azapropazone treatment. This was at a dose of 500 mg/kg. Numerous ulcers were present in the ileum and caecum of the phenylbutazone-treated rats and stomach ulcers were found in two rats. A few ulcers were found in the caecae of two rats treated with azapropazone but the ilea and stomachs were not ulcerated. At the lower dose the ilea and caecae of the phenylbutazone-treated rats were still ulcerated although the ulcers were much smaller and fewer than those found at the higher dose. The stomachs were free from ulcers. No ulcers were found where the rats were treated with azapropazone at the lower dose. Ilea segments were examined by the Gomori procedure for acid phosphatase. Tissue taken from a non-ulcerated region of a segment of ileum removed from a rat that has been dosed with phenylbutazone shows the staining to be heavy and diffuse. In contrast the ileum from azapropazone-treated rats and the controls were only slightly stained and no diffused staining was observed. When segments were incubated with sodium β-glycerophosphate for 4 h periods some diffuse staining was found in the control as well as the experimental segments. Presumably this was due to the breakdown of the lysosomes over the longer period. Although a large number of controls were incubated for 2 h with sodium β-glycerophosphate no lysosomal damage was observed in these specimens. It is therefore unlikely that the procedure described was responsible for the lysosomal damage observed in the phenylbutazone-treated specimens.

*Rabbits.* A similar result was found with rabbits. Both drugs induced stomach ulceration in the pyloric region and segments of this area, when examined by the modified Gomori (1941) procedure for acid phosphatase, stained heavily and diffusely compared with a light staining pattern for controls.

The duodenum of phenylbutazone dosed rabbits was also ulcerated but not that of the azapropazone-treated animals or the controls. The ulcerogenic action of phenylbutazone in rabbits was greater than that of azapropazone.

## DISCUSSION

Azapropazone, oxyphenbutazone and phenylbutazone clearly stabilize lysosomes at concentrations of physiological interest, and it is possible that this property may be the basis of their anti-inflammatory action. At high concentrations the drugs induced lysis of the lysosomes; phenylbutazone has a much stronger lytic action than the other drugs. Phenylbutazone also appears to have a much stronger ulcerogenic action than azapropazone in rats and rabbits. In addition, phenylbutazone, at high concentrations *in vitro* appears to damage lysosomes in the stomach. Some additional histochemical evidence that phenylbutazone may damage lysosomes was found where the phenylbutazone-treated ileal sections showed evidence of lysosomal damage whereas the azapropazone and control sections did not. Although histochemical evidence is often difficult to evaluate since the staining process probably damages lysosomes it was consistently found that when the incubation time with the substrate was reduced to 2 h the phenylbutazone-treated sections stained heavily but the control and azapropazone sections did not. This suggests that the lysosomes in the phenylbutazone-treated tissues were more fragile than in the other sections. Lysosomal damage was observed in the ileal sections taken from rats dosed with phenylbutazone. No lysosomal damage was observed in the sections taken from the azapropazone and control rats. However, since inflammation is associated with lysosomal damage it may be that the lysosomal damage observed histochemically was part of the inflammatory response associated with ulceration. It is also possible that it was caused directly by the phenylbutazone. The diffuse staining was general and not specifically located in the ulcers and this may have represented a pre-ulcerogenic condition. In a previous paper (Lewis, 1970) it was suggested that lysosomal damage induced by high concentrations of drugs may result in the intracellular release of acid hydrolases such as cathepsins with consequential damage to surrounding tissue. Such local high concentrations may occur after oral administration. Therefore lysosomal damage may be involved, at least in part, with gastrointestinal ulceration.

The additional evidence in this paper is consistent with this suggestion.

*Acknowledgements*

The authors with to thank Messrs. Geigy Ltd. for a gift of phenylbutazone and oxyphenbutazone and A. H. Robins Company Ltd., for a gift of azapropazone and for financial assistance with the project. We also thank Mr. F. Ring, of the Royal National Hospital for Rheumatic Diseases, Bath, for helpful discussions concerning the histology, and Miss J. M. Heal for technical assistance.

## REFERENCES

GOMORI, G. (1941). *Arch. Path.*, **32**, 180–210.

LEWIS, D. A. (1970). *J. Pharm. Pharmac.*, **22**, 909–912.

LEWIS, D. A., SYMONS, A. M. & ANCILL, R. J. (1970). *Ibid.*, **22**, 902–908.

LOWRY, O. M., ROSEBROUGH, N. J., FARR, A. H. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.

SYMONS, A. M., LEWIS, D. A. & ANCILL, R. J. (1969). *Biochem. Pharmac.*, **18**, 2581–2582.

WEISSMANN, G. (1965). *Biochem. Pharmac.*, **14**, 525–535.

WEISSMANN, G. (1967). *A. Rev. Med.*, **18**, 97–101.

WEISSMANN, G. & DINGLE, J. T. (1961). *Expl. Cell. Res.*, **25**, 207–210.