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# FURTHER STUDIES ON THE *IN VIVO* EFFECT OF CEPHALORIDINE ON THE STABILITY OF RAT KIDNEY LYSOSOMES

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**Abstract**—The stabilizing effect of cephaloridine, an antibiotic, on rat kidney lysosomal membranes was tested by a single subcutaneous injection. The release of two lysososomal enzymes, acid phosphatase and muramidase, was used as an index of lysosome membrane integrity. The levels of these enzymes in the kidney extracts as well as in the isolated kidney lysosome fractions were found to be raised considerably, compared to the controls. In rats treated with cephaloridine, the supernatant fraction obtained from the kidney homogenates, after centrifugation at 15,000 $g_{av}$, contained lower enzyme activities than were found in the control animals. It is suggested that cephaloridine may inhibit the release of acid phosphatase and muramidase from rat kidney lysosomes and, therefore, may exert a stabilizing effect on the lysosomal membrane system. The possible mechanism of interaction of this antibiotic with rat kidney lysosomal membranes is proposed.

Lysosomes have been shown to play important roles in cellular and tissue injury, and lysosomal acid hydrolase enzymes are mediators of numerous pathological processes [1–6]. Many compounds of physiological and clinical importance have been shown to affect the stability of lysosomal membranes [7]. Detergent-like molecules are known to render the lysosomal membrane more permeable and may, eventually, lead to complete lysis, whereas a number of anti-inflammatory agents protect lysosomes against these disrupting forces [8–10].

Cephaloridine, a semi-synthetic antibiotic, widely used for the treatment of a wide range of bacterial infections, has been reported recently to suppress urinary acid phosphatase excretion in the rat [11, 12]. In these recent observations, urinary acid phosphatase activity was found to be significantly lower than the control levels after a subcutaneous administration of cephaloridine to male rats. Following these findings, it was thought that cephaloridine might play a role in the stabilization of the rat kidney lysosomal membrane system and the suppression of urinary acid phosphatase release from the renal system. The present investigation was an effort to further extend and confirm these earlier findings, since extrapolation to the *in vivo* situation is usually a difficult task. The suggestion that cephaloridine could stabilize and protect lysosomal membrane systems in an intact cell is very crucial in view of the roles played by the lysosomes in a number of disease conditions [4, 6].

## MATERIALS AND METHODS

*Micrococcus lysodeikticus* (freeze-dried) was purchased from the Sigma Chemical Co., London, and 4-nitrophenyl orthophosphate was obtained from British Drug Houses, Poole, England. Cephaloridine (Ceporin, Glaxo) was obtained from Glaxo Laboratories Ltd., Greenford, England. All other reagents used were of analytical grade and were prepared in double-distilled water.

Male albino rats were obtained from the breeding stock at the Faculty of Health Sciences, University of Ife.

*Administration of cephaloridine.* Cephaloridine was dissolved in sterile physiological saline (200 mg/ml) and administered to rats by subcutaneous injection. Seventy-two male albino rats weighing between 190 and 230 g were divided into six groups of twelve rats each. Group 1 received 1.0 ml of sterile physiological saline and, serving as controls, were killed on day 1 and day 5 (six rats in each). The remaining five groups were injected with a single dose of the antibiotic using the appropriate volume for each rat to give the desired dose of 1.0 g/kg body weight (2.4 mmoles/kg). The rats were killed on days 1, 2, 3, 4 and 5, respectively, after injection.

*Preparation of tissue extracts and homogenates.* Rat kidney tissue extracts and lysosomes were prepared, and the lysosome pellets were checked for purity by enzyme assay and light microscopy as previously described [13] and modified as follows. Animals were killed by cervical dislocation, and the kidneys were removed, weighed and placed in ice-cold 0.25 M sucrose. The tissue was then cut finely with clean scissors, and portions were homogenized in 0.25 M sucrose using a TRI-R STIR-S model K43 homogenizer. All operations were carried out at 0–4°. This initial suspension was diluted to a final concentration of 10% (w/v) with 0.25 M sucrose, and an aliquot was placed in a clean pre-cooled tube for enzyme assay and protein measurement. The remaining portion was then centrifuged at 3000 $g_{av}$ for 15 min in the cold room to sediment nuclei and cell debris. The resulting supernatant fraction was then centrifuged at 15,000 $g_{av}$ for 20 min to sediment a large granule fraction containing lysosomes. The remaining supernatant solution was used for assay

of the enzymes and proteins in the soluble fraction. The lysosomal pellets were then resuspended by directing a stream of sucrose solution from a pipette on to them. This method yielded a suspension of undamaged lysosomes. The pellets were then finally suspended in 0.25 M sucrose containing 0.05 M Tris–HCl buffer (pH 7.4). Two milliliters of sucrose–buffer solution was added to every gram of kidney used. This final suspension was used for subsequent determinations.

The purity of the lysosomal preparations was checked visually by fluorescence microscopy with acridine orange. By this method the lysosomes appear bright orange and the mitochondria green. Microscopy examination showed the preparation to be satisfactory, and this was confirmed by the enzyme assays.

*Enzymes and protein measurements.* Aliquots of the crude kidney homogenate with or without Triton X-100 (1% final concentration) were used for enzyme and protein determinations. Acid phosphatase and muramidase activities, and proteins in the lysosome pellets, were measured in the presence of Triton X-100 (1%). Acid phosphatase assay was carried out as previously described [14], muramidase activity was determined following the method of Litwack [15], and protein was estimated by the biuret method [16]. A Pye Unicam SP 1800 double beam spectrophotometer was used for all measurements.

## RESULTS

The effects of cephaloridine on acid phosphatase and muramidase levels in the crude kidney homogenate are shown in Fig. 1. The levels of both of these enzymes had risen 1 day after cephaloridine injection, reaching their peaks 2 days after. Acid phosphatase activity remained much higher than normal 5 days after cephaloridine, but muramidase activity dropped to nearly normal 5 days after. The specific activities for the two enzymes were increased (Fig. 2). Muramidase activity reached a peak on day 2, but fell on day 3; acid phosphatase activity remained elevated up to 5 days.

The enzyme levels in the supernatant soluble fractions of the kidney after lysosome pelleting are shown in Fig. 3. The activities of both enzymes dropped below the control levels 1 day after the chemical agent. The acid phosphatase level was greatly diminished, compared to the controls, up to 5 days following cephaloridine, but muramidase activity rose slightly above the control level 5 days after.

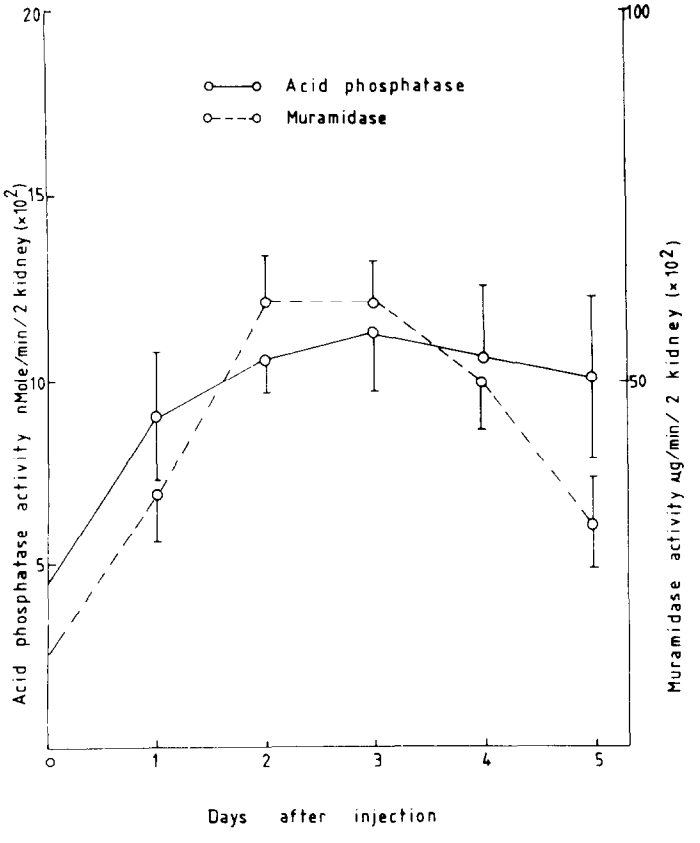


Fig. 1. Effect of cephaloridine on rat kidney lysosomal acid hydrolases. Results are expressed as total enzyme activity/2 g kidney ± S.D. Key: (○——○) acid phosphatase, and (○---○) muramidase. Two groups of six rats each were used as controls; one group was killed on day 1 (4.5 ± 2.1 acid phosphatase; 2.6 ± 1.7 muramidase), while the second group was killed on day 5 (6.8 ± 3.7 acid phosphatase; 2.9 ± 1.3 muramidase).

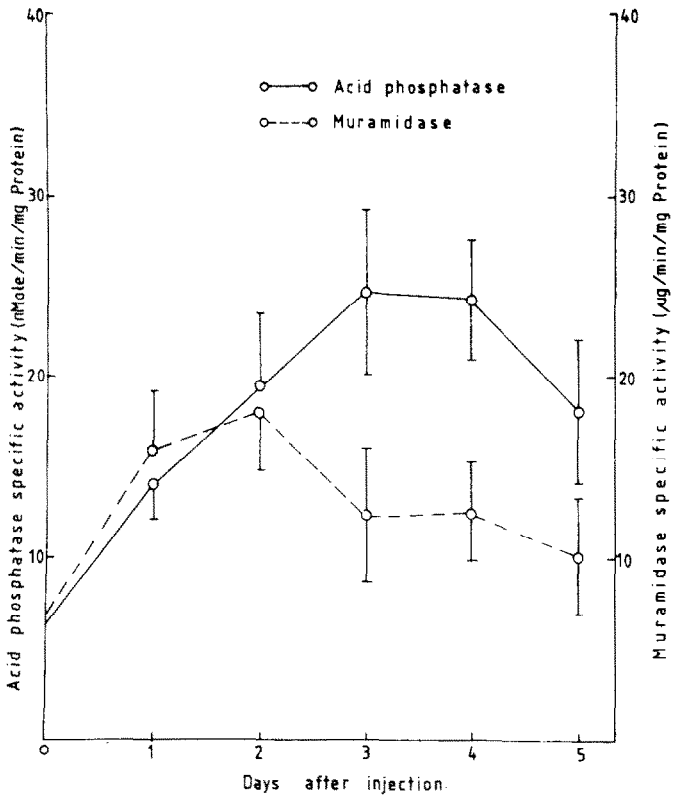


Fig. 2. Effect of cephaloridine on rat kidney lysosomal acid hydrolases. Results are expressed as specific activities in nmoles/per mg protein/per whole kidney ± S.D. Key: (○——○) acid phosphatase, and (○---○) muramidase. Two groups of six rats each were used as controls; one group was killed on day 1 (6.2 ± 2.9 acid phosphatase; 6.4 ± 3.3 muramidase), while the second group was killed on day 5 (5.8 ± 2.7 acid phosphatase; 7.3 ± 3.8 muramidase).

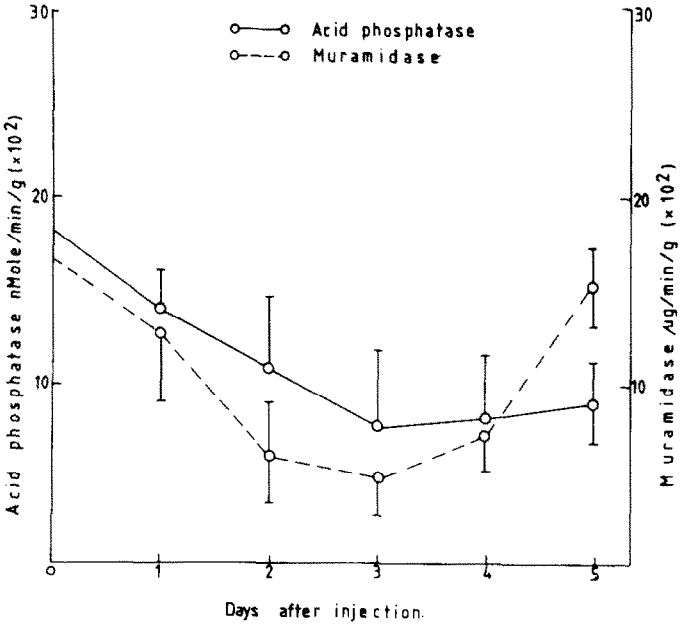


Fig. 3. Effect of cephaloridine on rat kidney 15,000 $g_{av}$ supernatant fraction. Results are expressed in nmoles/per min/per whole kidney ± S.D. Key: (○——○) acid phosphatase, and (○---○) muramidase. Two groups of six rats each were used as controls: one group was killed on day 1 (18.1 ± 5.2 acid phosphatase; 16.3 ± 5.9 muramidase), while the second group was killed on day 5 (21.4 ± 4.0 acid phosphatase; 19.8 ± 5.4 muramidase).

The specific activities of acid phosphatase observed in the rat kidney lysosomal fraction are shown in Table 1. A sharp rise in the activity was observed 1 day after cephaloridine injection, and the level reached a peak on day 2. This level remained well above the controls for the rest of the investigation.

## DISCUSSION

The present findings and those of earlier reports [11, 12, 17, 18] clearly indicate that cephaloridine exerts some stabilizing action on rat kidney lysosomal membrane *in vivo* and *in vitro*. A previous investigation has also demonstrated that cephaloridine (0.5 mmole/l) protected isolated rat kidney lysosomes against thermal disruption [12].

The release of two lysosomal enzymes, acid phosphatase and muramidase [19] was used as an index of lysosomal membrane integrity in these studies. The activities of these two enzymes in the crude extracts of rat kidney were raised above the control levels following the administration of cephaloridine (Fig. 1). The specific activities of these enzymes in the crude kidney homogenate were elevated far above the normal levels (Fig. 2). These observations indicate that the cephaloridine and/or its metabolites may have interacted with the lipoprotein membrane structure of the lysosomes in some as yet unknown way. This interaction might have led to some modification of the membrane system *in vivo*, in such a manner as to inhibit the release of acid hydrolase enzymes into the extracellular environment.

Previous reports [12, 17, 18] have shown that cephaloridine decreases the release of acid phosphatase *in vitro* from isolated lysosomes during prolonged incubation and that the injection of this antibiotic in rats (2 g/kg) causes a significant depression in the urinary excretion of acid phosphatase. The presence of inhibitors or activators was not detected in the urines of rats treated with cephaloridine. Electron microscopic studies of the treated rat kidney preparations did not show any evidence of damage to the cellular structures.

The reduced acid phosphatase and muramidase activities observed in the 15,000 $g_{av}$ supernatant fraction of the treated rats (Fig. 3), in spite of possible contributions by such cellular organelles as the golgi components as well as the variables in the homogenization process, could have reflected an inhibition of the release of these enzymes from the lysosomes and could also give further support to the concept of stabilization by this antibiotic of the lysosomal membrane system. The elevated specific activity of acid phosphatase in the lysosomal fraction (Table 1) may have been an indication of a diminished release of this enzyme from the lysosomes.

Table 1. Specific activities of acid phosphatase following cephaloridine (1.0 g/kg)*

| Days after injection | Specific activity |
|---|---|
| Control | 11.8 ± 4.34 (7)† |
| 1 | 45.30 ± 7.39 (6) |
| 2 | 40.16 ± 8.84 (7) |
| 3 | 31.77 ± 5.26 (6) |
| 4 | 26.16 ± 5.04 (10) |
| 5 | 18.94 ± 3.96 (6) |

* Results are expressed in nmoles per min per mg protein ± S.D.

† Figures in parentheses indicate the number of rats used.

The specific activities of acid phosphatase enzyme alone in the control and treated rats were determined in order to confirm the stabilizing action of cephaloridine on the lysosomal membranes. This was necessary since acid phosphatase shows a notably higher proportion in the insoluble membrane fraction, compared to any other lysosomal enzyme after lysosomal disruption [20].

Bangham *et al.* [21] have proposed a mechanism of action of certain chemical agents, such as the steroids, on membranes and have stated that any active stabilizing molecule must have a co-planar ring system, must be capable of hydrophilic, hydrophobic and hydrogen bonding, and must possess charge-transfer properties. The chemical structure of the cephaloridine molecule has some of the above attributes. It would not be surprising, then, that this molecule should exert some stabilizing effect on the lysosomal membrane system by a simple hydrogen-bonding process. For example, the peptide bond present in the cephaloridine molecule is capable of hydrogen bonding with the lipoprotein membrane of lysosomes and could bring about a clustering of protein molecules which are normally dispersed in the lipid bilayer. The nitrogen atom of the pyridine ring of the cephaloridine molecule, the ketone and the carboxyl groups, and the sulfur atoms, all could possibly participate in some manner of hydrogen bonding with the lipoprotein membrane of the lysosomes. These bonding forces could then play a very significant role in the stability of the rat kidney lysosomal membrane.

It appears, therefore, that lysosome membrane stabilization could be a mechanism of action specific for certain chemical compounds with specific molecular configurations and, in the interaction of cephaloridine with rat kidney lysosomes, it looks as though hydrogen bonding could be an important factor.

Further studies in this laboratory are intended to show whether more frequent injection of cephaloridine, other than the single dose so far administered to rats, will sustain the stability of the lysosomal membrane system for a longer period than was the case in the present studies.

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