Degradation of oxalate in rats implanted with immobilized oxalate oxidase

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Accumulation of oxalate leads to hyperoxaluria and calcium oxalate nephrolithiasis in man. Since oxalate is a metabolic end product in mammals, the feasibility of its enzymic degradation has been tested in vivo in rats by administering exogenous oxalate oxidase. Oxalate oxidase, isolated from banana fruit peels, in its native form was found to be non-active at the physiological pH of the recipient animal. However, its functional viability in the recipient animal was ensured by its prior binding with ethylenemaleic anhydride, thus shifting its pH activity curve towards the alkaline range. Rats implanted with dialysis membrane capsules containing such immobilized oxalate oxidase in their peritoneal cavities effectively metabolized intraperitoneally injected [^{14}C]oxalate as well as its precursor [^{14}C]glyoxalate. The implantation of capsules containing coentrapped multienzyme preparations of oxalate oxidase, catalase and peroxidase led to a further degradation of administered [^{14}C]oxalate in rats.

Oxalate oxidase *Nephrolithiasis* *Enzyme therapy* *Immobilized enzyme*

1. INTRODUCTION

In man, oxalate is a metabolic end product with no enzyme present to act on it [1]. Consequently, in conditions of its enhanced endogenous synthesis or increased absorption from the diet, oxalate can accumulate and lead to hyperoxaluria [1–5]. Regardless of causes, the major clinical manifestation of hyperoxaluria is calcium oxalate nephrolithiasis [1]. A variety of therapeutic measures have been recommended for hyperoxaluric syndromes. Depending upon the specific pathophysiology, treatments have resorted to the restriction of dietary calcium and oxalate intake, use of oxalate-trapping agents, intake of larger volume of fluid and chemical inhibitors of oxalate precipitation and haemodialysis [1,2].

The manifestation of hyperoxaluria, in a wider sense, can be considered as a metabolic disorder resulting from the absence of an oxalate-decomposing enzyme. One of the newer approaches of curing such metabolic diseases is the application of enzyme supplementation therapy [6–8]. Though mammalian systems do not possess oxalate-degrading enzymes, oxalate oxidases capable of breaking down oxalate into CO_2 and H_2O_2 are known to occur in the plant kingdom [1]. We have therefore examined the attractive possibilities of employing enzyme therapy for degrading endogenous oxalate in hyperoxaluric conditions. As a prerequisite, we modified oxalate oxidase, isolated from banana fruit peels [9], by covalently binding it to a polyanionic electrolyte, ethylenemaleic anhydride (EMA), shifted its pH activity curve towards the alkaline range, and thus rendered it functional at the physiological pH of 7.4 of the recipient animal. Here we report the feasibility of reducing the endogenous concentration of oxalate in rats by implanting dialysis membrane capsules containing EMA-oxalate oxidase in their peritoneal cavities.

2. MATERIALS AND METHODS

[^{14}C]Oxalic acid (spec. act. 8.8 mCi/mmol) and [1-^{14}C]glyoxalic acid (spec. act. 7.33 mCi/mmol)

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were obtained from Amersham. Oxalate decarboxylase was purchased from Sigma. Oxalate oxidase was isolated from banana fruit peels (*Musa paradiasica* cv. French plaintain) as described [9].

2.1. *Covalent binding of oxalate oxidase to polyanionic electrolyte*

The EMA derivative of banana oxalate oxidase was prepared according to Goldstein [10]. About 500 mg (wet wt) of banana oxalate oxidase preparation [9] (1.8 U/mg protein) was used for derivatisation. 1 U activity is defined as the amount of enzyme required to convert 1 μmol substrate to product in 1 min at pH 5.2. The recovery of enzyme activity in the EMA derivative ranged between 72 and 80%. In the case of multienzyme derivatisation, a solution containing 300 mg oxalate oxidase [9], 10 mg catalase (Sigma, 2000 U/mg protein) and 100 mg peroxidase (Sigma type I) was used.

2.2. *Encapsulation of EMA-enzyme in dialysis membranes and their surgical implantation in rats*

Dialysis tubing of 50 cm (flat width 10 mm, molecular mass cut off value 14 kDa) was sterilised prior to use and filled with EMA-oxalate oxidase suspended in 01. M potassium phosphate buffer, pH 7.5. Several capsules (10 mm, 1 × 6 mm diameter) were prepared from this and each contained approx. 12 units enzyme.

Male Wistar rats (180–200 g) were fasted for 24 h prior to treatment, but had free access to water. Laparotomy was performed, under mild ether anesthesia, by making a 1.5 cm dorsal incision. Enzyme capsules were inserted into the peritoneal cavity, one on either side, and incisions were sutured. Control animals received the same treatment except for the presence of bovine serum albumin instead of oxalate oxidase in the capsules. To individual rats, 2 h post-surgery, 2 μmol [U-^{14}C]oxalate, [^{14}C]glyoxalate or labelled glycolate in saline was administered intraperitoneally. The treated animals were housed in metabolic cages for urine collection.

2.3. *Analytical methods*

The amount of labelled oxalate present in the urine sample was determined using oxalate decarboxylase. The $^{14}CO_2$ liberated from the labelled oxalate was determined after trapping it as described [4,5]. The activities of banana oxalate oxidase preparations were determined spectrophotometrically [9].

3. RESULTS

The pH profiles of oxalate oxidase and EMA-oxalate oxidase are shown in fig.1. The native oxalate oxidase exhibited optimal activity at pH 5.2, the enzyme being non-active beyond pH 6.5. However, oxalate oxidase on derivatisation with EMA showed a marked shift in its pH activity curve, more towards the alkaline range. The extent of broadening of the pH activity and the amount of catalytic activity displayed at a specific pH by the enzyme depended upon the ionic molarity of the buffer employed. Thus, at an alkaline pH of 7.5 with 0.01 M phosphate buffer, EMA-oxalate oxidase displayed 30% of its optimal activity. When the molarity of the phosphate buffer was raised to 0.1, 9–12% of its optimal activity could be observed at the same pH.

Fig.2 shows the rate at which the membrane-encapsulated EMA-oxalate oxidase could decom-

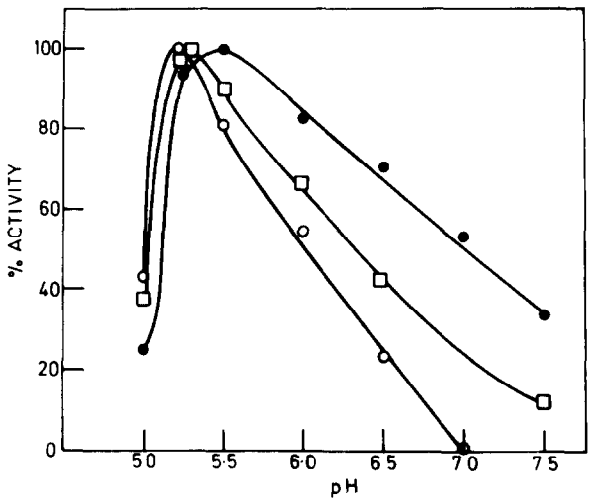

Fig.1. The pH profiles of native oxalate oxidase and EMA-oxalate oxidase at different buffer molarity. Buffers used: pH 5–6 (K_2HPO_4-H_3PO_4); pH 6.5–7.5 (K_2HPO_4-KH_2PO_4). (○—○) Native oxalate oxidase; (●—●) EMA-oxalate oxidase activity with 0.01 M buffer; (□—□) EMA-oxalate oxidase activity with 0.1 M buffer.

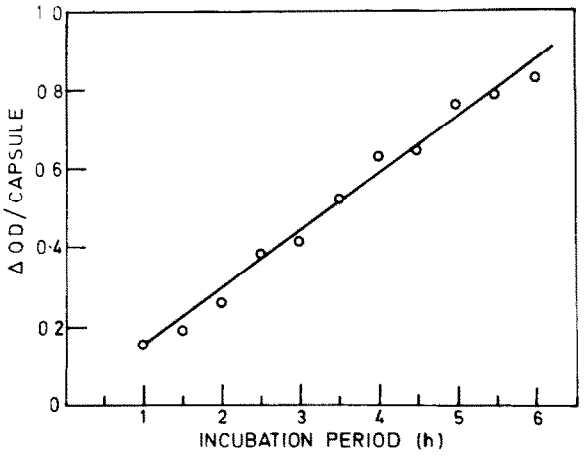

Fig.2. The rate of degradation of oxalate in vitro by capsule containing EMA-oxalate oxidase. An enzyme capsule was placed in a 25 ml conical flask containing 5 μmol oxalic acid in 10 ml of 0.1 M phosphate buffer (pH 7.5) and incubated in a shaker water bath at 37°C. H_2O_2 formed in the dialysate was measured as described [9].

pose oxalate, presented in an external medium having a pH of 7.5. For this purpose, the rate of formation of H_2O_2 in the dialysate was determined at different time intervals. After an initial lag of 45–50 min, the oxalate oxidase activity was found to be essentially linear for 6 h, under the conditions employed.

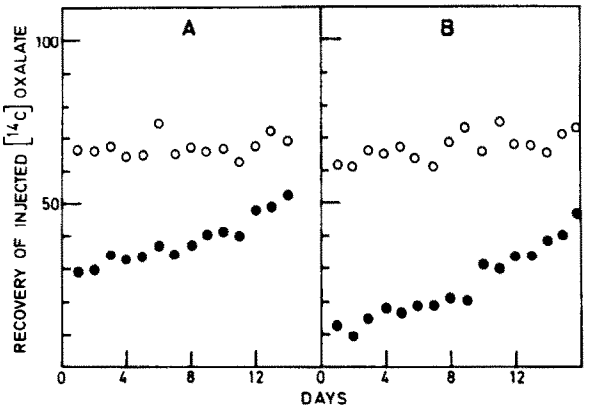

Fig.3. The percent recovery of labelled oxalate in 24 h urine in control and enzyme-treated animals following daily injection of [1-^{14}C]oxalate. Panel A: (○) control rat; (●) EMA-oxalate oxidase-treated animals; panel B: (○) control rat; (●) multienzyme-treated rats. Each value is an average of 4 experiments.

Rat, like man, cannot metabolize oxalate and therefore excretes major amounts of ingested, synthesized or administered oxalate in its urine. However, rats when implanted with EMA-oxalate oxidase were able to decompose large amounts of injected [^{14}C]oxalate (fig.3A). Thus, compared with controls, oxalate oxidase-treated animals consistently excreted lesser amounts of unmetabolized oxalate in 24 h urine following daily administration of [^{14}C]oxalate, during a 15 day period of investigation. While the control animals excreted as high as 64–76% of the injected oxalate, the enzyme-treated rats excreted merely 28–37% during the first 8 days post-implantation and 40–52% on subsequent days.

The implantation of multienzyme capsules containing EMA derivatives of oxalate oxidase, catalase and peroxidase resulted in a higher rate of metabolic degradation of administered [^{14}C]oxalate. Such multienzyme-treated rats excreted a meagre amount of 9–20% of the injected dose during the first 8 days post-implantation and on subsequent days in the range 19–44% (fig.3B). Though a reduction in the functional performance of the implanted multienzyme capsule was observed in due course, the loss of enzyme activity was less marked when compared with the monoenzyme preparation.

In mammals glyoxalate is the immediate major precursor of oxalate [2] whereas glycolate is shown to be both a direct as well as an intermediate precursor of oxalate via glyoxalate in in vitro ex-

Table 1

[^{14}C]Oxalate recoveries in 24 h urine of multienzyme-implanted rats following intraperitoneal injection of precursors

Compound injected	Percent recovery of injected ^{14}C-compound as [^{14}C]oxalate in 24 h urine	
	Control	Multienzyme-treated
[1-^{14}C]Glyoxalate	13.4 ± 1.1	5.2 ± 0.4[a]
[1-^{14}C]Glycolate	9.0 ± 0.8	3.1 ± 0.6[a]

[a] $P < 0.001$, as compared with control

Each value represents mean ± SE of 4 animals

periments with human and rat livers [2,11]. The multienzyme-treated rats were able to degrade newly formed [^{14}C]oxalate from the intraperitoneally injected [^{14}C]glyoxalate or [1-^{14}C]-glycolate (table 1).

4. DISCUSSION

Most pathological conditions in man involving oxalic acid are associated with an increased urinary excretion of oxalate [1]. Oxalate once formed or absorbed cannot be metabolized further owing to the absence of an oxalate-metabolizing enzyme thus leading to hyperoxaluria. The idea of curing such metabolic disorders by administering the required enzymes in immobilized form is gaining wider recognition. However, enzymes in their native forms are unsuitable for therapeutic studies in animals since they become inactivated, provoke immune reactions and fail to act at pH other than their own activity ranges [7,8,12]. Other factors that still restrict its practical applicability include (i) the cost of the enzyme, (ii) the isolation of the protein in large quantities and (iii) the non-availability of suitable animal models to evaluate the efficacy of enzyme therapy [7,13,14].

In this study, some of these have been overcome to a large extent. We have already developed an inexpensive and rapid procedure for the isolation of oxalate oxidase from banana peels [9]. Rat, like man, is incapable of metabolizing oxalate and therefore serves as a good model for studies on disorders of oxalate metabolism. Though oxalate oxidase in the native form is non-active beyond pH 6.5, we have rendered it suitable for therapeutic studies by binding it to the polyionic carrier EMA and extending its pH activity curve close to the physiological pH of the animal. Banana enzyme, in soluble as well as in EMA derivative form, elicits a specific immune response in rats, but failed to do so when implanted in animals after encapsulation in dialysis membranes, at least for a period of 30 days (not shown).

Our results demonstrate that the concentration of the endogenous oxalate could be effectively reduced in rats by implanting EMA-oxalate oxidase capsules in the peritoneal cavity. The synthesis or ingestion of oxalate in hyperoxaluric conditions is a chronic process. Therefore, for the enzyme therapy to be effective, it is essential that the supplemented oxalate oxidase sustains its catalytic activity for longer duration. Thus, here the animals that were implanted with EMA-oxalate oxidase were able to degrade continuously [^{14}C]oxalate, administered daily for a period of 2 weeks though the functional performance of the implanted enzyme tended to decline in due course.

The inclusion of EMA derivatives of catalase and peroxidase along with oxalate oxidase in the capsules has a potentiating effect on the activity of oxalate oxidase in the rat. As a result, the multiple enzyme-treated rats excreted lesser amounts of oxalate for a longer period in contrast to animals which received oxalate oxidase alone. These enzyme preparations have also been found to be effective in eliminating newly formed oxalate from administered precursors like glyoxalate and glycolate.

Current techniques in enzyme therapy exploit a variety of novel supports such as semipermeable membranes, artificial red blood cells, collodion and liposomes for this purpose [6–8]. The binding of oxalate oxidase to EMA and subsequent encapsulation in dialysis membranes offer several advantages. Such immobilized enzyme preparations are easy to handle, implant and also immunologically biocompatible. The encapsulated enzyme is also inaccessible to proteolytic enzymes and microorganisms due to permeability barriers. In addition, several enzymes could be easily encapsulated to carry out a series of metabolic reactions.

The observed decline in the functional performance of oxalate oxidase in due course appears to be caused by the product H_2O_2. Such local poisoning of immobilized glucose oxidase by H_2O_2 has been reported [15]. Though H_2O_2 is the substrate for both catalase and peroxidase, these enzymes themselves are highly susceptible to local poisoning by H_2O_2. In this study, this effect has been minimized by simultaneously co-entrapping catalase and peroxidase along with oxalate oxidase in the polyionic carrier.

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