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# EFFECTS OF NEPHROTOXIC COMPOUNDS ON ACTIVE UPTAKE OF DRUGS IN ISOLATED RENAL TUBULES IN RABBITS

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**Abstract**—The uptake of sulfonamides and phenolsulfonphthalein (PSP) was examined *in vitro* using isolated renal proximal tubule suspension, and the effects of nephrotoxic compounds on the uptake of sulfamethizole (SMZ) were studied. The uptake of SMZ and PSP was energy dependent and was inhibited competitively by iodopyracet (IP), which is transported actively by the *p*-aminohippurate mechanism. The uptake of sulfamethoxazole was also reduced by IP but that of sulfanilamide was negligible. The present results correspond well with those of *in vitro* experiments reported previously.

Nephrotoxic compounds, mercuric chloride, neomycin, viomycin and kanamycin, decreased the uptake of SMZ non-competitively. The inhibitory action of the three antibiotics corresponds with *in vivo* potency, suggesting that this renal tubule preparation may provide a simple method for predicting the nephrotoxicity of drugs.

The effects of disease states on renal handling of drugs have been investigated extensively [1–5]. However, the quantitative aspects of these problems have not been completely elucidated. In previous papers [6–8], we have reported a new and simple analytical method for quantitative investigation of the renal handling of drugs *in vivo* in normal rabbits, dogs, and humans. We have also investigated the quantitative changes in the disease state in dogs and humans [9, 10]. To confirm those results and to predict the *in vivo* changes in renal insufficiency, we examined the uptake of drugs by the kidney *in vitro*.

Isolated renal tubules are known to reflect the *in vivo* state physiologically [11–13], and to be a good preparation for kinetic study [12, 14]. In this paper, we determined the active transport of drugs in isolated tubules and evaluated its correspondency to *in vivo* data. Moreover, the effects of nephrotoxic compounds on uptake were investigated and the predictability of *in vivo* results in disease state using isolated tubule preparation were examined.

## MATERIALS AND METHODS

*Uptake experiments in isolated rabbit renal tubules.* New Zealand male albino rabbits weighing 2.2–3.2 kg were killed by carotid exsanguination under pentobarbital anesthesia (27 mg/kg, i.v.). The technique of preparing isolated renal tubules was almost the same as that of Burg and Orloff [11]. Microscopic examination of the final tubule suspension revealed excellent preservation of the tubules. A 5% (v/v) suspension of tubules was prepared with modified Ringer solution containing 5% (v/v) rabbit serum to which an appropriate concn of drugs was added and then incubated at 25 ± 1° for 30 min with continuous bubbling with a gas mixture of $O_2$–$CO_2$ (95:5). The 30 min incubation time was selected on the basis of the following evidence: the uptake reached to steady-state within 15 min and the viability of tubules was maintained within 60 min. After incubation, the samples were centrifuged at 2000 rpm for 2 min. The supernatant was removed and assayed for unbound (protein-free) concn of the drug. Ultrafiltrate of the supernatant was obtained by centrifugation using a Centriflo membrane cone (CF-50A, Amicon Co., Denvers, MA, U.S.A.). The wet weight of the pellet of tubules was determined and then the pellet was dissolved in 0.5 N NaOH. The sample solution was assayed for drug concn after deproteinization with trichloroacetic acid. Inulin was used to estimate the volume of trapped water, termed the "inulin space" [11]. Tubule water was calculated by the difference between wet and dry weight after correction for the inulin space. The concn of the drug in the tubule water was calculated by determination of the amount in the tubule pellet minus that in the inulin space, and dividing this by the volume of tubule water.

*Analytical methods.* Sulfamethizole (SMZ), sulfamethoxazole (SMX), sulfanilamide (SA) and *p*-aminohippuric acid (PAH) were assayed by the procedure of Bratton and Marshall [15] using 2-diethylaminoethyl-1-naphtylamine as the coupling reagent. Phenolsulfonphthalein (PSP) was determined colorimetrically after the addition of 1 N NaOH. Inulin was analyzed by the method of Roe *et al.* [16].

*Materials.* SMZ (Eizai Co., Tokyo, Japan) and SMX (Shionogi & Co., Osaka, Japan) were of JP IX grade. Iodopyracet (Daiichi Seiyaku Co., Tokyo, Japan) (IP), neomycin sulfate (Takeda Chemical Industries, Osaka, Japan) (NM), viomycin sulfate (Sankyo Co., Tokyo, Japan) (VM) and kanamycin sulfate (Meiji Seika Co., Tokyo, Japan) (KM) were kindly supplied by the respective companies. Collagenase (Type I) (EC 3.4.24.3) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals used were of analytical grade.

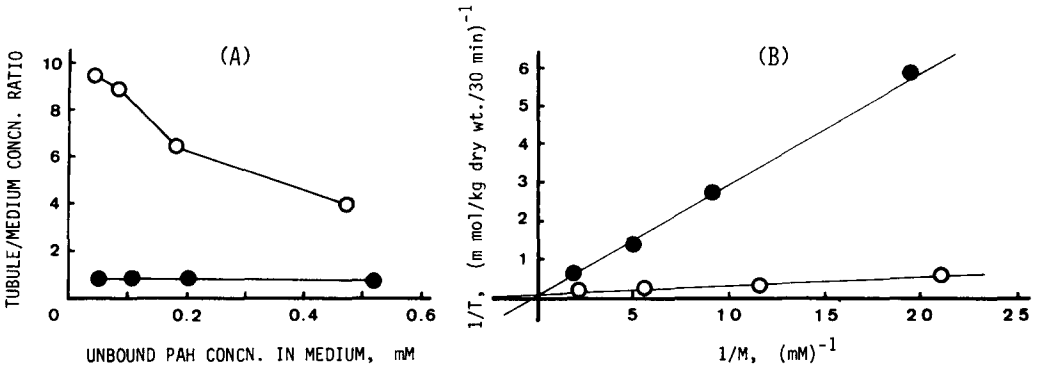


Fig. 1. Uptake (A) and Lineweaver–Burk plot (B) of *p*-aminohippuric acid (PAH) in isolated renal tubules. T and M represent PAH uptake in tubules and unbound PAH concn in medium. Experiments were performed with (●) or without (○) iodopyracet (2 mM).

## RESULTS AND DISCUSSION

In a previous paper, we reported active renal tubular secretion and secretory inhibition by IP of sulfonamides and PSP using an *in vivo* clearance technique in rabbits [7]. In this study, the details of active transport of these drugs were investigated *in vitro*.

The experiments were performed using isolated renal tubule suspension, as this preparation is useful for kinetic study [12, 14]. Active PAH uptake in isolated tubules in rabbits and its competitive inhibition by probenecid are well documented [14, 17]. However, as the effects of IP on PAH uptake in isolated tubule suspension in rabbits are unknown, we studied the inhibitory effects of IP on PAH accumulation. As shown in Fig. 1, PAH was accumulated in the tubules against the concn gradient, and tubule/medium concn ratio (T/M ratio) of PAH decreased as the PAH concn in medium increased. Further, T/M ratio decreased to nearly 1.0 with IP addition to the incubation medium, and active PAH uptake was considered to be inhibited almost completely. Lineweaver–Burk plot confirmed that this inhibition was competitive (Fig. 1B). Thus, the competitive inhibition of active renal tubular transport of PAH by IP could be demonstrated using isolated renal tubule suspension. Therefore, using the four drugs, SMZ, SMX, SA and PSP, studied *in vivo* in the previous paper [7], their respective uptakes into the isolated renal tubules and the effects of IP on them were examined.

The uptake of PSP and SMZ proceeded against the concn gradient of the drugs, and a dependency on drug concn in the medium and uptake inhibition by IP, as in the case of PAH, were observed (Fig. 2A and B). Double reciprocal plot also clearly demonstrated that the uptake of both drugs by the

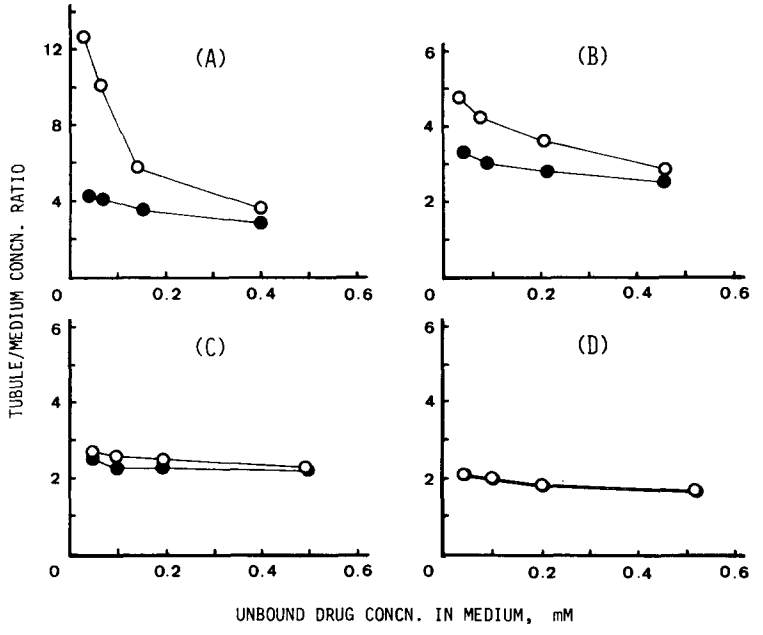


Fig. 2. Uptake of drugs in isolated renal tubules with (●) or without (○) iodopyracet (2 mM). (A) Phenolsulfonphthalein, (B) sulfamethizole, (C) sulfamethoxazole, (D) sulfanilamide.

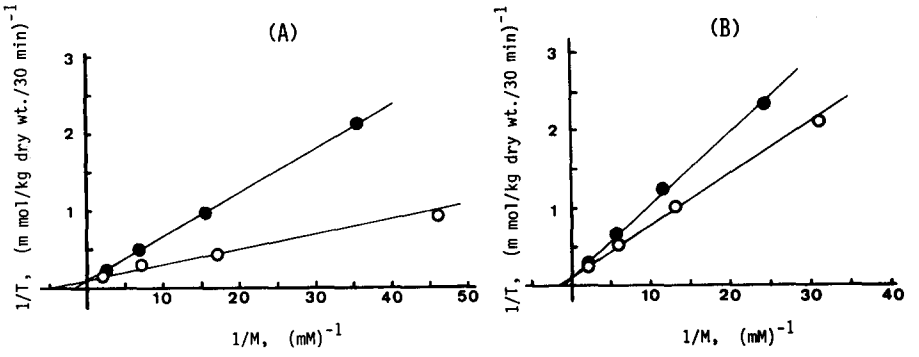


Fig. 3. Lineweaver–Burk plot of phenolsulfonphthalein (A) and sulfamethizole (B) uptake in isolated renal tubules with (●) or without (○) iodopyracet (2 mM). T and M represent drug uptake in tubules and unbound drug concn in medium.

Table 1. Michaelis–Menten kinetic parameters for active renal transport in isolated tubules *in vitro* and in renal clearance method *in vivo* in rabbits

| | *in vitro* | | *in vivo** | |
|---|---|---|---|---|
| | $V_{max}$ (mmole/kg dry wt/30 min) | $K_m$ (mM) | $V_{max}$ (μmole/min) | $K_m$ (mM) |
| Sulfamethizole | 10.4 | 0.63 | 33 | 1.7 |
| Phenolsulfonphthalein | 7.4 | 0.095 | 9.0 | 0.15 |
| Sulfamethoxazole | small† | † | 2.1 | 0.04 |
| Sulfanilamide | 0 | — | 0 | — |

* *Chem. Pharm. Bull.* **26**, 740 (1978).
† $V_{max}$ and $K_m$ could not be calculated.

tubules was competitively inhibited by IP (Fig. 3). In the case of SMX, the tubular uptake was slightly inhibited by IP (Fig. 2C), suggesting active transport, but its contribution to SMX uptake might be small. As inhibition of SA uptake was not detected by IP, it was concluded that active transport did not contribute to SA uptake (Fig. 2D). Comparison of the *in vitro* data with the previously reported *in vivo* data [7] is shown in Table 1. The order of $V_{max}$ of active tubular transport was SMZ > PSP > SMX > SA = 0 in both experiments. As the isolated renal tubules mainly consisted of proximal tubules, the $V_{max}$'s of secretion of these drugs may reflect the value originating in the proximal tubules. The agreement in results of both experiments also supported the validity of our analytical method of renal handling of drugs *in vivo* [7], and suggests that the extent of secretion is predictable from the findings of the isolated renal tubules.

On the basis of these results, the changes in renal tubular uptake of drugs were followed using this preparation as a method for elucidating the reaction mechanism of nephrotoxic compounds which pose many problems in clinical drug therapy. We used

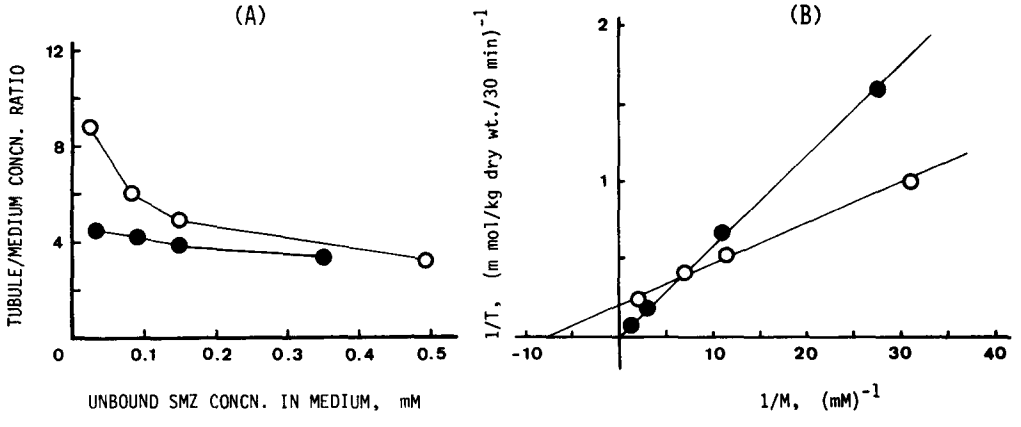


Fig. 4. Effect of mercuric chloride ($HgCl_2$) (2 mM) on sulfamethizole (SMZ) uptake (A) and its Lineweaver–Burk plot (B) in isolated renal tubules. T and M represent SMZ uptake in tubules and unbound SMZ concn in medium. Experiments were performed with (●) or without (○) $HgCl_2$.

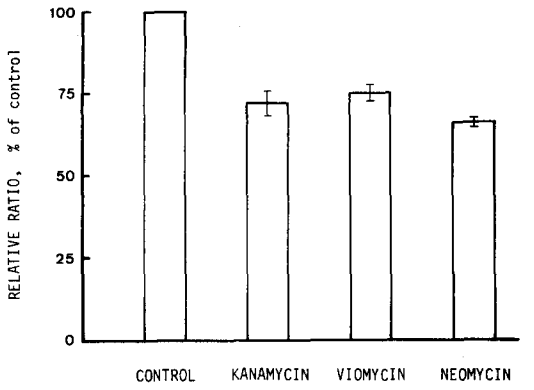


Fig. 5. Effects of nephrotoxic compounds on sulfamethizole uptake in isolated renal tubules. Relative ratio was calculated by tubule/medium concn ratio with nephrotoxins divided by the ratio without nephrotoxins. Data represent the mean ± S.D. of four experiments. Nephrotoxins used were 1% (w/v).

SMZ as a model drug for renal tubular transport to assess the effects of mercuric chloride ($HgCl_2$), NM, VM and KM, which are nephrotoxins and are known to produce histological changes mainly to proximal tubular epithelial cells [18–21], on tubular uptake of SMZ. As shown in Fig. 4, the concn dependency of SMZ uptake almost completely disappeared with the coexistence of $HgCl_2$, and the reduction of uptake was not competitive. These results confirmed that the inhibition of active transport by proximal tubular damage is involved in renal impairment in the presence of $HgCl_2$. With respect to the antibiotics present with SMZ in the medium, NM demonstrated the most potent inhibition of uptake as can be seen by the relative ratio to the control shown in Fig. 5. When the results of NM were analyzed by the double reciprocal plot, a tendency for non-competitive inhibition was noted and a reduction of $V_{max}$ of active uptake was observed (Fig. 6). VM and KM also showed similar inhibitions of SMZ uptake. This indicates that functional impairment of the renal proximal tubules plays an important role in renal damage induced by these nephrotoxins. The extent of inhibitory effects of this preparation, also, corresponds to that of the clinical toxicity to the kidneys caused by these compounds [22, 23]. These results suggested that the isolated renal tubule preparation can be a useful tool for the detection of drugs that induce proximal tubular damage.

Comparison of this preparation with another *in vitro* system was performed using renal cortical slices (unpublished data). With some nephrotoxins, a longer interval was needed to reach the intercellular space of the renal cortical slice. The isolated renal tubules differ from the slice in that there is no outermost tissue layer. That is, (1) the innermost cells are in direct contact with the bathing medium, (2) intercellular transport can ignored, (3) equilibrium can be attained rapidly between tissue and medium, and (4) the effect of binding to tissue protein can be excluded. These are probably the reasons for the inhibitory effects of antibiotics within a comparatively short period of time (30 min) in the isolated renal tubules. Therefore, this preparation is better than slices for the detection of drug nephrotoxicity *per se*.

We have also studied the effects of $HgCl_2$ and NM on renal handling of SMZ in dogs with renal insufficiency using the *in vivo* clearance technique [9]. Decreased clearance, and a reduction in $V_{max}$, as well as little or no changes in $K_m$ of secretion followed by aggravation of renal function were observed with both nephrotoxins. This is in agreement with the non-competitive inhibition of uptake and the reduction of $V_{max}$ of SMZ with $HgCl_2$ and NM, as demonstrated in the present experiment. Thus, these results also supported our findings that the isolated renal tubule preparation is a simple method for detection of nephrotoxicity of drugs.

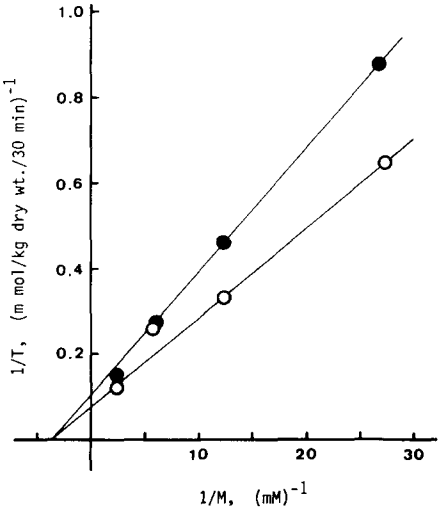


Fig. 6. Lineweaver–Burk plot of sulfamethizole (SMZ) uptake in isolated renal tubules with (●) and without (○) neomycin. T and M represent SMZ uptake in tubules and unbound SMZ concn in medium. Neomycin used was 1% (w/v).

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