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SUSTAINED URINARY EXCRETION OF SULFAMETHIZOLE FOLLOWING ORAL ADMINISTRATION OF ENTERIC COATED MICROCAPSULES IN HUMANS

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SUMMARY

Microcapsules containing sulfamethizole were prepared by coacervation of carboxymethylethylcellulose, an enteric coating material, from ethyl acetate solution in the presence of polylactic acid. Release rates of the drug from the microcapsules in vitro were much slower than dissolution rates of the drug from tablets. The urinary excretion of the drug following oral administration of the microcapsules in humans was sustained over that of tablets. Pharmacokinetic analyses indicated sustained absorption following the administration of the microcapsules. The extent of bioavailability was only slightly decreased following administration of the microcapsules.

INTRODUCTION

Polymer membranes and matrices have been used extensively for dosage form design (Nakano, 1979). One of the general methods of achieving sustained release of therapeutic agents has been through the use of coating of tablets, pellets, or granules by mechanical means such as pan coating, fluidized-bed coating, etc. Microencapsulation, a technique of encapsulating drug powders through coacervation, spray drying, etc. has been developed to achieve the sustained release of drugs (Bakan and Sloan, 1972). Poorly water-soluble polymers such as ethylcellulose have been utilized for this purpose (Jalsenjak et al., 1976).

Enteric coating materials such as cellulose acetate phthalate have also been examined for their ability to effect sustained release (Merkle and Speiser, 1973). The usefulness of enteric coated granules for sustaining blood levels of cephalexin has been demonstrated recently (Maekawa et al., 1977). Unlike enteric coated tablets, enteric coated microcapsules would be gradually emptied from the stomach, thereby achieving the sustained release of the encapsulated drug in the intestine. Since enteric coated microcapsules

emptied into the intestine are expected to release the encapsulated drug within a few minutes, the release rates depend on the emptying rates of the microcapsules from the stomach. Although the rates of stomach emptying vary among individuals, the emptying patterns of stomach contents usually follow first-order kinetics (Clements et al., 1978).

Carboxymethylethylcellulose (Tsujino, 1974), an enteric coating material which lacks ester groups, has been introduced. This material is not expected to undergo changes in dissolution characteristics even if it is stored in high humidity environments since it is not susceptible to hydrolysis.

This paper reports the release patterns of sulfamethizole, a urinary tract disinfectant, from carboxymethylethylcellulose microcapsules in vitro and demonstrates the usefulness of such microcapsules for achieving sustained plasma levels and, therefore, sustained urinary excretion due to sustained release in vitro. Since sulfamethizole is excreted into urine mostly unmetabolized and its plasma half-life is short (Triggs et al., 1975), the drug is used exclusively as a urinary tract disinfectant. If sustained excretion is achieved by means of sustained release in the gastrointestinal tract, the dosing interval may be extended.

MATERIALS AND METHODS

Materials

Carboxymethylethylcellulose (CMEC) with a degree of substitution of 0.65 in the carboxymethyl group and 2.1 in the ethoxy group was generously supplied by Freund, Tokyo. Polylactic acid was synthesized following the reported procedure (Kulkarni et al., 1971). The average molecular weight of the polylactic acid sample measured by a vapor pressure osmometer (Knauer, Germany) was 2000. Sulfamethizole of JP IX grade (lot XC 14GG) and sulfamethizole tablets (250 mg of drug content, lot D17GCE) were products of Eisai, Tokyo. A sulfamethizole powder for the preparation of microcapsules was ground in a mortar to reduce the particle size to 10.3 ± 7.2 μm in Green diameter (arithmetic mean) as measured by optical microscopy (Olympus BH microscope) using an eye-piece fitted with a micrometer. All other chemicals and solvents were of reagent grade and solvents were distilled when necessary.

Preparation of microcapsules

The following procedures were followed: (1) polylactic acid corresponding to 40% (w/w) fo CMEC was completely dissolved in 5% CMEC solution in ethyl acetate; (2) sulfamethizole corresponding to 40% (w/w) of CMEC was suspended in the solution and the suspension was stirred for several hours at a rate of 300 rpm and then the drug powders were dispersed finely by ultrasonification (Tomy, UR-200, 100W, 2 min); (3) ethyl ether was added dropwise to this suspension at a rate of 0.5–0.8 ml/min until complete phase separation occurred and embryonic microcapsules were obtained; (4) the microcapsules were washed twice with ethyl ether by decantation and then collected on a filter paper; and finally (5) the microcapsules were dried in vacuo for 24–48 h at ambient temperature.

Measurements of drug, CMEC and polylactic acid contents

Weighed amounts (250 mg) of the microcapsules were added to 50 ml of 1 N HCl

and shaken with 50 ml of benzene for 24 h. On standing, the mixture separated into two layers, with solid particles floating at the phase boundary. The benzene layer was separated and the aqueous phase and the solid particles were extracted with two 20 ml portions of benzene. The benzene extracts were pooled and the solvent was evaporated under vacuum. The residue was dried under vacuum, weighed and identified to be polylactic acid by infrared spectroscopy (Japan Spectroscopic, IR A-1). A 1 ml portion of the aqueous layer was sampled for spectrophotometric determination (Hitachi 200-20 spectrophotometer) of the drug content at 270 nm. The remaining aqueous layer was filtered through a sintered-glass disc to collect the solid particles. The solid particles were washed with three 20 ml portions of 1 N HCl followed by the same quantity of benzene. The residue was dried under vacuum, weighed and characterized by infrared spectroscopy to be CMEC.

Observation of microcapsules by a scanning electron microscope

The dried microcapsules were observed by scanning electron microscope (Hitachi, S-430) to examine their shape and surface characteristics.

Release studies

A release profile of the drug from microcapsules in JP IX disintegration medium no. 1 at pH 1.2 was obtained by a modification of the beaker method. An Erlenmeyer flask containing 200 ml of the test fluid was placed in a constant-temperature water bath maintained at 37.0 ± 0.2°C which was placed on top of a constant torque magnetic stirrer (Mitamura Riken). A weighed quantity (200 mg with drug content of 84 mg) of the microcapsules was suspended in the test fluid and the suspension was stirred by a magnetic stirring bar (3 cm long) at a rate of 200 rpm. One ml samples were withdrawn at predetermined time intervals. Then 1 ml of the fresh test solution was added to the flask to maintain the original volume. After diluting the sample solution with the same test fluid, the drug concentrations were analyzed spectrophotometrically at 270 nm. The dissolution of the drug from the tablet was similarly determined.

Urinary excretion studies

The sulfamethizole tablet or the microcapsules containing the same amount (250 mg) of the drug was orally administered to 4 normal male subjects. Following an overnight fast, these preparations were placed on top of the tongue of each subject and swallowed (avoiding chewing) with 50 ml of water. Immediately before administration of the preparations, the bladded was emptied completely to obtain the blank urine. In order to obtain a sufficient rate of urinary output during the first hours post-administration, about 100 ml of water was taken 1 h and 2 h post-administration. No food was taken for 4 h post-administration. Urine was thereafter collected at appropriate intervals for up to 12 h. For each urine sample obtained, the exact time and volumes of urine excreted were recorded. The cross-over design was used and the minimum interval of one week was allowed between trials. Amounts of sulfamethizole in urine samples were assayed colorimetrically employing diazo reaction using N-(β-diethylaminoethyl)-α-naphthylamine oxalate (Koizumi et al., 1964).

RESULTS AND DISCUSSION

Examinations of microencapsulation conditions

The microencapsulation procedures described in the method section were employed because of the following reasons. Although CMEC is soluble in ethyl acetate, acetone, ethanol and mixtures of an alcohol and a halogenated hydrocarbon, ethyl acetate was selected because of the lower solubility of the drug in this solvent than in others and because of toxicological considerations (Smyth et al., 1962). Two concentrations (5 and 10%) of CMEC were examined for effective encapsulation. The 10% solutions tended to give the coagulated polymer in the bottom of the flask upon phase separation whereas no such coagulation was observed in the 5% solutions. Therefore, the 5% solution was used for the preparation of the microcapsules. The addition of polylactic acid to the CMEC solution resulted in microcapsules with improved release characteristics of the drug. Polylactic acid seemed to be incorporated into the capsular wall serving presumably as a plasticizer. The proportion of polylactic acid adopted in the present preparations was selected on the basis of the following findings. For up to 40% of the amount of CMEC, the release sustaining effect was proportionally increased with the amounts of the polymer. However, the greater amounts of the polymer tended to give aggregated microcapsules. Longer stirring time before addition of ether for phase separation tended to give microcapsules with a slower release rate. An application of ultrasonic vibration helped aggregated drug particles to disperse in the medium. Ethyl ether was selected among poor solvents of CMEC such as *n*-hexane, *n*-pentane, and ethyl ether as a phase-separating agent because of its easy removal from the microcapsules after preparation and because of toxicological considerations.

Contents and characteristics of microcapsules

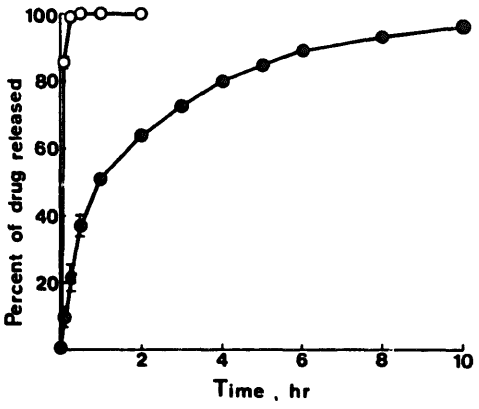
The drug content of the microcapsules was 40.0% whereas CMEC and polylactic acid contents were 51.4 and 8.6% (average of two measurements), respectively. Scanning electron microscopic observation revealed that the surfaces of drug powders are all covered by polymeric materials and few pores were observed. The size of the microcapsules was determined to be 16.4 ± 1.4 μm in Green diameter as measured by optical microscopy.

Release patterns

In Fig. 1, the release pattern of the drug from the microcapsules is compared with the dissolution pattern of the drug from the tablet in the acid solution. Sustained release from the microcapsules extended to 10 h whereas the drug in the tablet completely dissolved within 15 min. The release of the drug from the microcapsules in a buffer, pH 7.5 was complete in 20 min due to dissolution of the enteric capsular membrane at the intestinal pH. Although the release of the drug from the enteric microcapsules is observed even in the acidic medium, the rate is much less than that in the medium of neutral pH.

Urinary excretion patterns

Urinary excretion rates of the drug following oral administration of the microcapsules are compared with those of the tablet in Fig. 2. The fate of sulfamethizole following oral



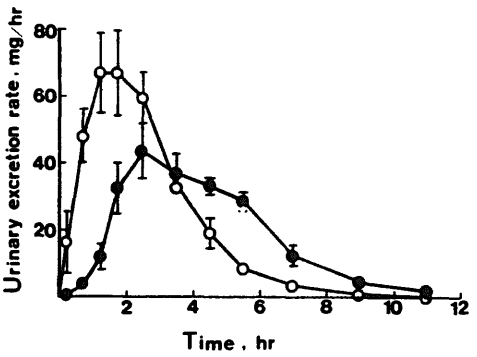
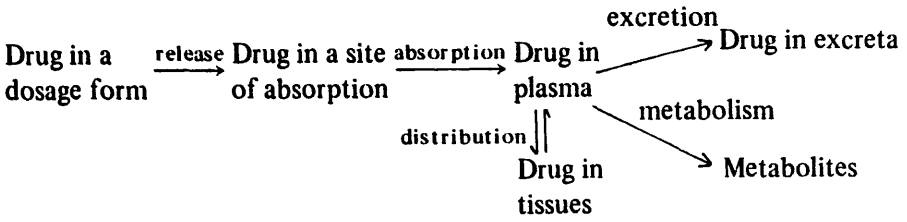


Fig. 1. Comparison between the release pattern of the drug from the microcapsules (•) and the dissolution pattern of the drug from the tablet (○). Average of 5 experimental runs with S.E. shown by vertical bars.

Fig. 2. Excretion rate of the drug following administration of 250 mg of the drug in the tablet (○) or the microcapsules (•). Average of 4 volunteers with S.E. shown by vertical bars.

administration of pharmaceutical preparations is depicted in the following fashion (Scheme 1). Since the tissue and central compartment could not be separated pharmacokinetically, based on the urine data, they were treated as a single compartment for both preparations.

Scheme 1



Drugs are absorbed into blood after release or dissolution from pharmaceutical preparations and are then excreted into urine. The excretion rate of an unmetabolized drug is expected to be proportional to the plasma level of the drug according to the following relationship,

$$\frac{dA_u}{dt} = k_{ex} V_d C_p$$

where dA_u/dt = excretion rate, k_{ex} = urinary excretion rate constant, V_d = volume of distribution, and C_p = concentration of drug in plasma.

Since the plasma level of the unmetabolized drug is expressed by the following equation,

$$C_p = \frac{k_a f D_0}{V_d(k_a - k_{el})} (e^{-k_{el}t} - e^{-k_a t})$$

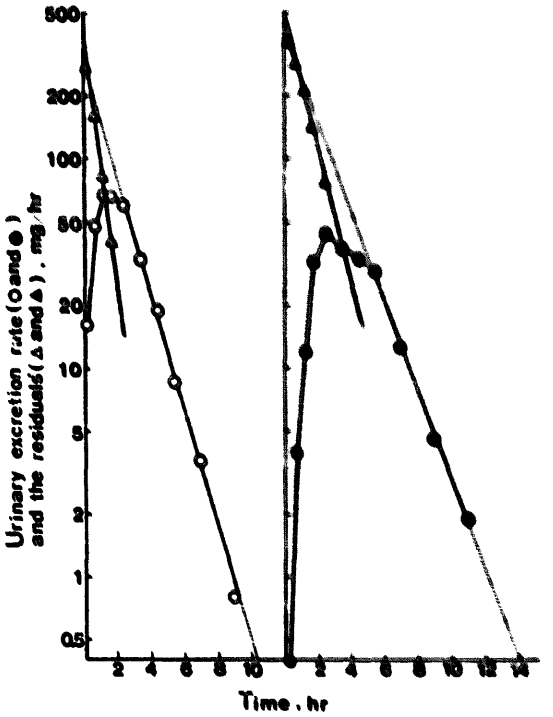


Fig. 3. Pharmacokinetic analysis of absorption and elimination patterns of the drug following administration of the drug in the tablet (open symbols) or the microcapsules (closed symbols). Average of 4 volunteers.

where k_a = absorption rate constant, f = fraction of dose absorbed, D_0 = dose, and k_{el} = elimination rate constant, the excretion rate is expressed by the following equation:

$$\frac{dA_u}{dt} = \frac{k_{ex}k_a f D_0}{k_a - k_{el}} (e^{-k_{el}t} - e^{-k_a t})$$

Fig. 2 indicates that excretion of the drug following oral administration of the microcapsules is sustained in comparison with that of the tablet. Sustained excretion is a reflection of the sustained blood level profile due to slow absorption, which in turn is attributed to the sustained release from the microcapsules. Therefore sustained excretion is the result of sustained release in vivo.

In order to quantitatively compare the absorption and excretion behavior of the drug following administration of two dosage forms, pharmacokinetic analysis was made to obtain the graphs shown in Fig. 3. Apparent elimination rate constants were obtained from the elimination phase of log excretion rate vs time plot which is expressed by

$$\log\left(\frac{dA_u}{dt}\right)_{el} = \log \frac{k_{ex}k_a f D_0}{k_a - k_{el}} - \frac{k_{el}}{2.303} t$$

whereas apparent absorption rate constants were obtained from the plot of residuals in

TABLE 1

APPARENT ABSORPTION AND ELIMINATION RATE CONSTANTS OF SULFAMETHIZOLE FOLLOWING ORAL ADMINISTRATION OF TWO PREPARATIONS TO 4 VOLUNTEERS

Volunteer no.	Absorption rate constant, h^{-1}		Elimination rate constant, h^{-1}	
	Tablet	Microcapsules	Tablet	Microcapsules
1	1.21	0.514	0.638	0.389
2	0.888	1.02	0.679	0.678
3	1.19	0.747	0.927	0.417
4	3.07	0.816	0.560	0.587
Mean ± S.E.M.	1.59 ± 0.50	0.774 ± 0.104	0.701 ± 0.079	0.518 ± 0.069

log excretion rate vs time plot which are expressed by

$$\log\left(\frac{dA_u}{dt}\right)_{res} = \log \frac{k_{ex}k_a f D_0}{k_a - k_{el}} - \frac{k_a}{2.303} t$$

The elimination and absorption rate constants thus obtained are tabulated in Table 1. Since the above procedures used for calculating rate constants are not valid for calculation of the rate constants following the administration of sustained release preparations, it should be kept in mind that the absorption and elimination rate constants calculated for the microcapsules are very coarse estimates and only approximate the true values. Thus the absorption rate constants for the microcapsules are indicative of sustained absorption but the values are only poor estimates and have no absolute meaning. It nevertheless indicates that the smaller apparent absorption rate constants following administration of the microcapsules than those of the tablet is a reflection of slower absorption due to sustained release from the microcapsules. Smaller apparent excretion rate constants following administration of the microcapsules most likely resulted from the sustained supply of the drug into the plasma due to sustained absorption.

Since reduced bioavailability of drugs has often been noted following administration of sustained release preparations supposedly due to incomplete release of drugs from preparations during the transit through the gastrointestinal tract, bioavailability of the drug following administration of the microcapsules was compared with that of the tablet. Cumulative amounts of the intact drug excreted following administration of the microcapsules amounted to 82% of the dose at 12 h post-administration, which is slightly smaller than that of the tablet (91%). Since the excretion process seems incomplete at 12 h following administration of the microcapsules as can be seen in Figs. 2 and 3, an expected amount of the drug to be excreted from the last sampling time to infinity was calculated by extrapolating the straight line in the elimination phase in log excretion rate vs time plot (Fig. 3) to time infinity. Thus the calculated 0–∞ h bioavailability following administration of the microcapsules amounted to 86%, which was only slightly smaller than the 0–∞ h bioavailability from the tablet, which was 91% since little drug was expected to be excreted after 12 h following administration of the tablet. Therefore,

only a slight reduction in bioavailability resulted following administration of the microcapsules.

The present in vivo data indicate the usefulness of enteric microcapsules prepared from enteric coating material in sustaining release of therapeutic agents in vivo and sustaining urinary excretion of the urinary tract disinfectant. Although only sulfamethizole was used in the present study, other drugs with short elimination half-lives may be microencapsulated similarly. Thus even drugs with short elimination half-lives can be made to maintain blood levels and urinary levels for a longer period by enteric microcapsulation.

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