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CHLOROTHIAZIDE-POLYVINYLPYRROLIDONE (PVP) INTERACTIONS : INFLUENCE ON MEMBRANE PERMEATION (EVERTED RAT INTESTINE) AND DISSOLUTION *

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ABSTRACT

The permeation of chlorothiazide through the in vitro everted rat intestine was investigated from dilute, saturated and supersaturated solutions in the presence and absence of PVP. Although chlorothiazide clearance was lower than that of either hydrochlorothiazide or salicylic acid, the high dose to solubility ratio rather than excessive membrane impermeability, seemed the primary factor limiting chlorothiazide absorption. Reduced chlorothiazide membrane transport from dilute solutions containing PVP and enhanced dissolution from a drug:PVP (1:2) mechanical mixure were observed and could be explained by the formation of a soluble complex in solution. Chlorothiazide transport rates, from systems supersaturated relative to crystalline chlorothiazide,

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were enhanced in the presence of PVP. This effect together with increased dissolution from a chlorothiazide:PVP(1:2) coprecipitate were consistent with the ability of PVP to inhibit drug crystallization.

INTRODUCTION

The diuretic chlorothiazide is incompletely absorbed following oral administration[1,2,3]. In addition absorption has been shown to be dose dependent in man following administration in solution[3], and in dogs[4], the proportion absorbed decreased as the dose increased. Reasons proposed for the limited absorption of chlorothiazide include: poor drug partitioning properties[5] resulting in low permeability of the gastrointestinal membrane to the drug[2], dependence of absorption on a site specific and apparently saturable absorption process[4] and, in the case of solid dosage forms, slow dissolution of the drug due to a high oral dose to solubility ratio at the intestinal absorption site[4], or in the case of solutions precipitation of the drug[2]. If the poor absorption of chlorothiazide is solubility related then agents which increase drug dissolution or inhibit crystal growth should enhance drug absorption. The water soluble polymer polyvinylpyrrolidone (PVP) can inhibit drug crystal growth[6,7,8] and crystallization[9]. It can also enhance dissolution both by forming high energy drug forms in coprecipitate systems[10,11] and by forming soluble complexes in solution. Furthermore, enhanced drug membrane transport using such high energy drug forms has

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been reported[12]. The ability to form complexes may however have an inhibitory effect on membrane transport and hence retard drug absorption[13]. The purpose of the present investigation was to examine the absorption of chlorothiazide and the possible influence of PVP on its biopharmaceutical properties using the everted rat intestine preparation.

METHODOLOGY

The preparation of the PVP-chlorothiazide coprecipitate and mechanical mix systems and the methods of analyses employed were similar to those previously outlined[11], chlorothiazide dissolution being determined by ultraviolet spectroscopy at 279 nm.

Everted Rat Intestine Preparation

Male Sprague-Dawley rats weighing about 200g were starved 24h prior to the experiment but allowed free access to water. The rats were sacrificed with sodium pentobarbitone, a method reported to prolong the structural integrity of the intestine[14]. The entire small intestine was removed via a midline incision of the abdomen and placed in an isotonic phosphate buffer, pH 7.4. For each section 10cm segments were cut, sleeved onto a glass rod, everted, and washed in the buffer solution according to the method of Wilson and Wiseman[15]. The perfusion apparatus used was similar to that of McElnay et al[16] and Blanchard and Straussner[17].

Each everted intestinal segment was ligated on both ends to glass cannulas and then placed in the test tube

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containing 150ml of the mucosal drug solution. The entire apparatus was placed in a water bath at 37°C. The mucosal solution was continuously gassed with 95% O_2 and 5% CO_2. A syringe containing the serosal solution was mounted on an infusion pump (B.Braun Perfusor IV) connected by a glass preheating coil to the everted segment, and thus 10ml samples could be removed at 10 min intervals for 1 hr.

Preparation of Mucosal Drug Solutions

The drugs used were salicylic acid,chlorothiazide and hydrochlorothiazide of B.P. quality.

Drugs were dissolved in an isotonic sodium phosphate buffer pH 7.4 of high buffering capacity[18].

Saturated and supersaturated drug systems were prepared using the method of Charnicki et al[19] for sodium chlorothiazide, the resulting solution was in every case adjusted back to pH 7.4 using hydrochloric acid. When PVP (plasdone-C 15, molecular wt 10,000) was included in the system it was added prior to adjusting the pH. Saturated solutions of chlorothiazide generally contained 11.7mg ml^{-1} of drug corresponding to the upper dose level of chlorothiazide (1.75g. 70kg^{-1} body wt.) used in a previous study[3].

Assay Procedures

Chlorothiazide and hydrochlorothiazide intestinal transport was estimated using the modified Bratton-Marshall assay procedure as outlined by Baer et al[20]. Salicylic Acid was assayed using the spectrofluorimetric procedure of Turner et al[18].

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THEORY

The diffusional mass transfer of drug in the steady state, across the everted rat gut, may be represented as follows:

$$J = A.P_{app}(C_D - C_R) = Cl(C_D - C_R) \qquad \text{Eq.1.}$$

where J is the flux (MT^{-1}), A is the effective surface area, P_{app} is the apparent permeability coefficient (LT^{-1}), C_D,C_R are the total drug concentrations in the donor and receiver compartments respectively, and the product $A.P_{app}$ is the clearance (Cl). If the change in ($C_D - C_R$) during the experiment is negligible and constant hydrodynamics are maintained between experiments then Eq.1 may be written as

$$J = Cl.C_D. \qquad \text{Eq.2.}$$

When drug is present in bound and unbound form then equation 2 becomes

$$J = \frac{Cl.C_D}{1 + KC_p}. \qquad \text{Eq.3.}$$

where C_p is the polymer concentration and K is the binding constant of drug to polymer.

RESULTS AND DISCUSSION

Permeability data for chlorothiazide,hydrochlorothiazide and salicylic acid, through the rat everted intestine, are summarized in Table 1. Clearance values of similar order of magnitude were obtained for the three compounds,salicylic acid, a drug known to be well absorbed[21], having the highest and chlorothiazide the lowest clearance. The salicylic acid value is in good

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TABLE 1

Drug Transfer Rates and Clearances (pH7.4) Obtained Using the Everted Rat Intestine

Drug	Mucosal concentration mg ml^{-1}	Transfer Rate $\pm$ SD (mg min^{-1}) x 10^2	Clearance (ml min^{-1}) x 10^2
Salicylic Acid (n=11)	1.0	2.26 $\pm$ 0.768	2.26
Hydrochlorothiazide (n=4)	0.335	0.724 $\pm$ 0.235	2.16
Chlorothiazide (n=5)	2.01	2.63 $\pm$ 0.522	1.31*
Chlorothiazide (n=9)	0.276	0.477 $\pm$ 0.223	1.73

* Level of significance vs salicylic acid and hydrochlorothiazide: $P < 0.05$

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agreement with the 1.8 x 10^{-2}mg min^{-1} reported by Blanchard and Straussner[17]. Chlorothiazide clearance tended to decrease with increasing concentration i.e. on increasing the drug concentration over an eightfold range, up to saturation, a decrease in clearance of the order of 35% was observed, this difference being almost significant at the 5% level. One of the characteristics of active absorption is transport against a concentration gradient. An experiment[22] was therefore carried out using equal concentrations of chlorothiazide in the mucosal and serosal fluids and incubating the preparation for 60 min. At the end of this period the ratio of the serosal to mucosal concentration was less than unity, this result does not support the presence of active absorption.

Kaplan & Cotler[23] have used the everted rat intestine as a screening technique to evaluate in vivo drug absorbability. Of 16 compounds investigated, 13 had clearance values in the range 1-4 x 10^{-2} and exhibited no in vivo permeability related absorption problems when administered in solution. Although the apparatus and technique of Kaplan and Cotler [23] is somewhat different to that of Blanchard and Straussner[17], preliminary studies in our laboratories indicate that the two methods give comparable results. It seems unlikely therefore that saturation of an active absorption site or excessive membrane impermeability are major limiting factors in chlorothiazide absorption. The higher clearance of

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TABLE 2

The Influence of PVP (10,000) on the Transport of Chlorothiazide in Dilute Solution (i.e. 16% saturated)Across the Everted Rat Intestine

PVP ($mg\ ml^{-1}$)	Chlorothiazide Clearance ($mg\ ml^{-1}$) x 10^2	Relative Rate Actual	Predicted
0.0(n=9)	1.73	1.00	1.00
10.0(n=7)	1.11	0.64	0.79
50.0(n=5)	0.74*	0.43	0.43

* $P < 0.02$

hydrochlorothiazide compared to chlorothiazide is consistent with published comparative partition data[5] and human absorption data[1] but is of a much smaller magnitude.

The ability of PVP to reduce the absorption rate of chlorothiazide in dilute solution is evident from Table 2. The presence of 50mg ml^{-1} PVP in solution reduced the transfer rate of a 0.3mg ml^{-1} chlorothiazide solution by over 50%.

The solubility of chlorothiazide increased in the presence of PVP indicating the formation of a soluble complex in solution. The binding constant of the complex K can be estimated from solubility data using equation 4.

$$C_d = C_s(1 + KC_p) \qquad \text{Eq.4.}$$

where C_d is the total drug in solution (bound and free), and where C_s is the drug solubility and C_p the PVP concentration[24]. A value of 2.64×10^{-2} ml mg^{-1} for K was obtained from solubility data and used in Eq.3 to estimate the expected reduction in transport due to complex formation. Reasonable agreement between the theoretical and actual values is evident, suggesting that the reduced drug transport is primarily due to complex formation.

The transport rates obtained using saturated and supersaturated chlorothiazide systems in the presence and absence of PVP are summarized in Table 3. Chlorothiazide precipitated from its sodium salt solution under physiological pH conditions resulting in a drug transport

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TABLE 3

The Influence of PVP on the Transport of Chlorothiazide, from Saturated and Supersaturated Systems (pH7.4), Across the Everted Rat Intestine

Mucosal fluid constituents	Transfer rate ± SD (mg min^{-1}) x 10^2	Relative rate Observed	Predicted
Saturated drug solution (n = 5)	2.63 ± 0.52	1.00	1.00
Drug suspension 11.7 mg ml^{-1} (n = 4)	4.33 ± 1.10	-	-
Drug[1] 11.7 mg ml^{-1} (n = 4)	5.87 ± 0.82	2.23	5.82
Drug[1] 11.7 mg ml^{-1} in PVP 1.0 mg ml^{-1} (n=2)	10.00 ± 0.94	3.80	5.67
Drug[1] 11.7 mg ml^{-1} in PVP 10.0 mg ml^{-1} (n=4)	13.13 ± 1.23	5.00	4.60
Drug[1] 11.7 mg ml^{-1} in PVP 50.0 mg ml^{-1} (n=3)	6.31 ± 0.72	2.40	2.51
Drug[1] 23.4 mg ml^{-1} in PVP 50.0 mg ml^{-1} (n=4)	9.14 ± 1.25	3.48	5.02

[1] Prepared from sodium salt.

rate only slightly (i.e. 1.36 fold ($P > 0.05$)) greater than that obtained from a chlorothiazide suspension of equivalent drug content. The inclusion of PVP enhanced drug transport rate, relative to the saturated solution, and inhibited drug crystallisation. In systems with a supersaturation ratio (relative to pure chlorothiazide) of 5.8 fold, a maximum rate 5 times greater than that for a saturated solution was observed in the presence of 10mg ml^{-1} PVP ($P < 0.001$). Increasing the PVP concentration to 50mg ml^{-1} gave a reduction ($P < 0.001$) in the relative enhancement which however was still 2.4 times greater than that observed with the saturated solution ($P < 0.001$). Systems with PVP concentrations below 10.0mg ml^{-1} readily became cloudy on preparation, those containing 10.0mg ml^{-1} PVP were metastable, while systems containing 50mg ml^{-1} PVP remained clear. When the supersaturation ratio was increased to approximately 12 fold the 50mg ml^{-1} PVP system also became cloudy and gave a membrane transport rate 3.48 times that of the saturated solution. These results suggest that the enhanced transport rates from PVP containing systems are due to the ability of PVP to retard and/or inhibit crystal growth. This inhibiting ability has been shown to be dependent on the relative rates of diffusion of drug and PVP to the crystal surface and therefore on the PVP concentration and supersaturation ratio[6]. Assuming no recrystallization were to occur (i.e. the supersaturated solutions remained stable) the relative transport rates in the presence of different PVP concentrations can be estimated from Eq.5

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$$\frac{J_{ss}}{J_s} = \frac{C_{Dss}}{C_{Ds}(1 + KC_p)} \qquad \text{Eq.5.}$$

where J_s and J_{ss} are the rates for the saturated and supersaturated systems respectively, C_{Ds} and C_{Dss} are the total drug concentrations in the respective donor compartments for the saturated and supersaturated systems respectively and the other terms have been defined previously. The relative enhancements predicted by equation 5 are included in Table 3. Below a PVP concentration of 10mg ml^{-1}, in systems containing 11.7mg ml^{-1} chlorothiazide, the experimental values are lower than predicted since insufficient PVP is present to prevent drug crystallization. The decrease in relative rate observed when the PVP concentration was increased to 50mg ml^{-1} reflects a decrease in the effective free drug concentration (due to binding) which occurred even though the system was stable to crystallization. Doubling the concentration at the latter PVP concentration caused precipitation of drug resulting in a rate lower than the predicted value.

A 1:2 chlorothiazide PVP coprecipitate was prepared to investigate the possibility that the enhanced membrane transport was related to the formation of a high energy form of the drug. In contrast to an equivalent mechanical mixture of the two components, X-ray diffraction peaks were absent from scans of the coprecipitate, suggesting the existence of drug in a non-crystalline (amorphous) state in the latter system.

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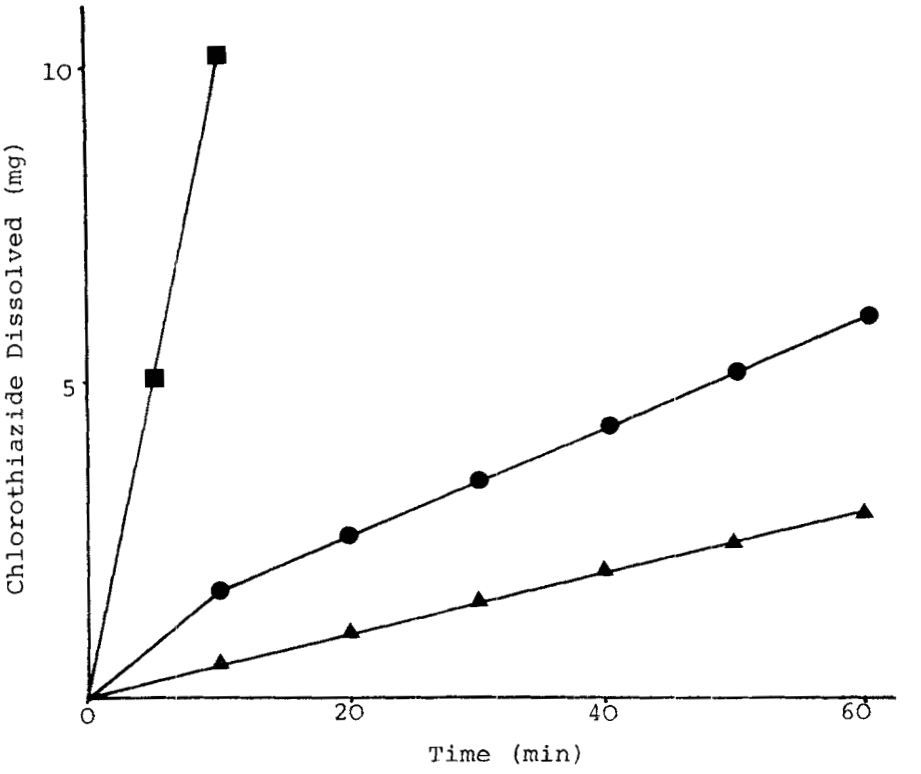

FIGURE I

Dissolution Profiles for Chlorothiazide ▲, Chlorothiazide-PVP (1:2) Mechanical Mixture ● and Coprecipitate ■, from 1.3 cm diameter discs using the modified beaker method.

Dissolution profiles for these systems together with that of pure chlorothiazide are shown in Fig.1. Coprecipitation brought about an 18.9 fold enhancement in the initial dissolution rate of chlorothiazide, while the mechanical mix system initially dissolved 2.84 times more rapidly than the pure drug. A relative enhancement in dissolution rate for the mechanical mix is to be

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expected due to the formation of soluble complexes in solution between the two components. In quantitative terms this initial dissolution rate G_T should be given by Eq.6.

$$G_T = \frac{C_{sd}D_d + KC_{sp}C_{sd}D_{d.p}}{h} \qquad \text{Eq.6.}$$

where D_d and $D_{d.p}$ are the diffusion coefficients of the drug and complex respectively, C_{sd} and C_{sp} are the solubilities of the drug and PVP respectively, h the diffusion layer thickness and the other terms are as previously defined[25]. Expressed in terms of the relative enhancement in initial dissolution rate G_T/G_o, Eq.6 becomes

$$\frac{G_T}{G_o} = 1 + \frac{D_{d.p}C_{sp}K}{D_d} \qquad \text{Eq.7.}$$

where G_o is the initial dissolution rate of pure chlorothiazide. In order to apply equation 7 to the current data, a value for C_{sp} the solubility (or effective surface concentration) of PVP during PVP dissolution is required. An estimate of C_{sp} was obtained from the dissolution rate for pure PVP (G_{PVP}) assuming $C_{sp} = G_{PVP}h/D_{PVP}$ taking the diffusion coefficient of PVP (D_{PVP}) as 1.55×10^{-6} from Simonelli et al[6]. The expected relative enhancement in chlorothiazide dissolution from the mechanical mix system thus obtained using Eq.7 was 2.7. This estimate is in good agreement with the actual value of 2.84 and

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consistent with a soluble complex explanation for the observed enhanced dissolution from the mechanical mix system. It is evident, from a consideration of equation 6, that for two different forms of the same drug, combined with PVP the ratio of the initial dissolution rates will be equal to the ratio of the solubilities of the two forms i.e. for the coprecipitate and mechanical mixture

$$\frac{G_{cop}}{G_{mm}} = \frac{C_{sh}}{C_{sd}}$$

where G_{cop} and G_{mm} are the initial dissolution rates observed for the coprecipitate and mechanical mixture respectively and C_{sd},C_{sh} the solubilities of the drug form in mechanical mixture and coprecipitate respectfully. The observed initial dissolution rate ratio was 6.64 suggesting that a high energy form of chlorothiazide, possibly amorphous,with a 6-7 fold greater solubility than that of crystalline chlorothiazide is formed and is stable in PVP on coprecipitation. The amorphous phase solubility is of a similar magnitude to the maximum enhancement in membrane transport observed from the supersaturated solutions stabilized with PVP. This coincidence may be fortuitous or may indicate that the degree of supersaturation which can be sustained by PVP may be dependent on the solubility of a stabilized amorphous form.

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