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Influence of advanced age on the disposition of acetazolamide

D. J. CHAPRON[1]*, K. R. SWEENEY[1], P. U. FEIG,[2] & P. A. KRAMER[1]
[1]School of Pharmacy, University of Connecticut, Storrs, and [2]Department of Nephrology, School of Medicine, University of Connecticut Health Centre, Farmington, Connecticut, USA

1 The disposition kinetics of acetazolamide (AZ) has been studied in four young and four elderly healthy volunteers, each of whom received an intravenous bolus dose of 5 mg/kg. The concentration time profile of AZ was determined in plasma, plasma ultrafiltrate, erythrocytes and urine.

2 While the mean area under unbound plasma concentration-time curves was 81% higher in elderly subjects, areas based on total drug concentrations were similar in both groups. The mean renal plasma clearance was similar in both groups. The mean renal plasma clearance was similar between young and old for total AZ, but was significantly lower in the elderly for unbound drug (8.88 ml min^{-1} kg^{-1} *vs* 15.7 ml min^{-1} kg^{-1}). Renal clearance of unbound AZ correlated well with creatinine clearance ($r = 0.846$, $P < 0.01$).

3 Peak erythrocyte levels were 45% higher in the elderly group (37.2 μg/ml *vs* 25.3 μg/ml) and were paralleled by a 46% increase in the mean area under the erythrocyte concentration-time curve for this age group. The unbound fraction of AZ in plasma was significantly greater in elderly than younger subjects (6.9 *vs* 4.1%, $P < 0.05$). Integrated AZ erythrocyte concentrations correlated positively with AZ free fraction in plasma and inversely with its unbound renal clearance. These observed differences in AZ disposition between elderly and young have served to clarify host factors which may importantly influence susceptibility to adverse effects.

Keywords acetazolamide disposition elderly

Introduction

Chronic primary open-angle glaucoma is the most common type of glaucoma and its management is usually medical. While many different topically-applied medications are available, oral agents are limited to carbonic-anhydrase inhibitors (CAI) of which acetazolamide (AZ) is the most extensively investigated and clinically tested. Unfortunately the potential therapeutic value of AZ as well as other CAI has never been fully realized because of an unacceptably high incidence of adverse effects associated with their long-term use. Nearly 50% of patients receiving CAI complain of a disabling symptom complex of malaise, fatigue, weight loss, depression, anorexia and loss of libido (Epstein & Grant, 1977). Excessive serum levels of AZ were implicated as a potential cause of these effects in some instances. Recently high erythrocytic levels of AZ in patients have been associated with significant toxicity (Inui *et al.*, 1982). The resultant inhibition of erythrocytic carbonic anhydrase, an enzyme that greatly facilitates carbon dioxide exchange and transport within capillary beds,

Correspondence: Mr D. J. Chapron, Pharmacokinetics Laboratory, University of Connecticut Health Centre, Farmington, Connecticut, 06032 USA

may produce toxicity secondary to increased carbon dioxide retention (Woodbury & Kemp, 1982).

Because the prevalence of glaucoma increases dramatically with advanced age and because AZ is highly bound to plasma proteins and entirely excreted unchanged by the kidney, its use in the elderly may be problematic (Maren, 1967). We decided to comprehensively characterize the disposition kinetics of AZ in both elderly and young normal subjects. In particular, we analyzed the pharmacokinetic factors influencing erythrocyte uptake and sequestration of AZ since these could provide insight into mechanisms controlling the delivery of drug to this potentially important site of toxicity.

Methods

Our study population was comprised of four elderly and four young volunteers. All were healthy as ascertained by physical examination and had normal hepatic and renal function as assessed by standard clinical laboratory tests. Elderly subjects were fully ambulatory and living independently. Pertinent subject characteristics are listed in Table 1. All volunteers were non-smokers and non-drinkers and only one subject (elderly) was taking medication chronically (0.125 mg of digoxin daily).

The study protocol was approved by our Institutional Review Board and written, informed consent was obtained from each subject prior to participation. Subjects fasted overnight and for up to 4 h following early morning administration of the test dose of AZ. To minimize the local trauma of multiple venepunctures, a flexible catheter with a heparin lock was placed in a superficial arm vein for the initial 12 h sampling period. The lock was maintained with a very dilute heparinized saline solution (10 units/ml) and special care was taken not to flush the lock with more than 0.5 ml of solution between samplings.

An AZ solution for injection was prepared from a commercially available parenteral dosage form reconstituted with sterile water (Diamox®, Lederle). A small sample of this solution was removed from the vial and saved frozen for later determination of its exact concentration. AZ (approximately 5 mg/kg, the dose rounded to the nearest 50 mg) was administered by intravenous bolus injection into an arm vein contralateral to the heparin lock. Because it was necessary to aspirate each vein before and during injection in order to avoid perivascular injection, the dead volume of the syringe was determined and the administered dose appropriately corrected.

Blood samples (6 ml) were collected at approximately 0, 5, 10, 20, 40, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, 600 and 720 min via the heparin lock and at 24, 32, 48, 56 and 72 h by venepuncture using a plastic syringe. Whole blood was collected into heparinized tubes and immediately divided into two aliquots. One aliquot was immediately centrifuged (Fisher Micro-centrifuge, Model 235A) for 30 s and the plasma quickly separated from the cellular elements and stored frozen. From the other aliquot two capillary tubes of blood were drawn and a microhaematocrit determined in duplicate (IEC Model MB Microhaematocrit Centrifuge). The haematocrit was corrected by assuming that the trapped plasma represented 1.5% of the packed erythrocyte column (Garby & Vuille, 1961).

Total urine collections were made approximately every 2 h for the first 12 h post-injection and then at least every 8–12 h for 72 h. Immediately after each collection the urine volume was measured and an aliquot stored frozen. In some younger patients more frequent blood and urine collections were taken. None of the volunteers had a history of urinary tract dysfunction and extra care was taken to ensure that all patients complied with the collection protocol. During the study, none of the volunteers participated in recreation that required

Table 1 Subject characteristics

Elderly	*Sex*	*Age (years)*	*Weight (kg)*	*Creatinine clearance (ml/min)*
AD	F	76	66.4	47.8
OH	F	82	65.9	75.5
VS	F	78	52.7	74.4
CS	F	78	80.0	71.4
Young				
SC	F	32	60.0	139.5
SM	F	27	53.6	106.9
PH	M	44	70.0	127.6
PF	M	36	84.5	119.3

physical activity; they spent most of their time sitting.

Protein binding

Plasma protein binding of AZ was determined at 37°C using ultrafiltration (Amicon Micropartition System MPS-1 with a YM-30B membrane). Using plasma ultrafiltrate no absorptive losses of AZ to the ultrafilter could be demonstrated. The filter retained greater than 99.9% of applied plasma protein. Approximately 40% of the applied sample volume was actually collected as ultrafiltrate. Ultrafiltrates were collected in duplicate as two consecutive cuts. Binding determinations were performed on six plasma samples during the first 24 h of AZ administration.

Analytical methods

AZ was quantitated in plasma, plasma ultrafiltrate, whole blood, and urine by a recently developed reverse-phase high pressure liquid chromatographic technique (Chapron & White, 1984). Briefly, this involved spiking each sample (0.2–0.5 ml) with internal standard (sulphadiazine) and then adding one ml of a 50% ammonium sulphamate solution. Whole blood samples were heated in boiling water for 25 s and then quickly cooled in a cold water bath. All samples were extracted with ethyl acetate. A phosphate buffer (pH 8.0) was used to wash the ethyl acetate extract. Drug and internal standard were back-extracted into a glycine buffer (pH 10.0) which was then washed with ethyl ether. Separation of AZ and internal standard from other biological constituents was achieved on a 10 micron C-18 reverse-phase column using a mobile phase containing acetonitrile, methanol and acetate buffer (pH 4.0; 3:2:95). The eluent was monitored at 254 nm. Plasma and urinary creatinine concentrations were measured on an ASTRA-8 (Beckman Instruments, Palo Alto, CA).

Kinetic analysis

Concentrations of AZ in erythrocytes were estimated indirectly from Eq. 1

$$C_{RBC} = \frac{C_{WB} - (1 - H)\, C_p}{H} \qquad (1)$$

where C_{RBC}, C_{WB}, and C_p represent AZ concentrations in red blood cells, whole blood and plasma, respectively, while H is the haematocrit.

Concentration-time profiles of AZ in plasma, ultrafiltrate, erythrocytes, and whole blood were computer-fitted to polyexponential equations using a nonlinear regression analysis technique (Metzler, 1974). Data were weighted by factors proportional to the reciprocal of their variance with the variance considered to be proportional to the magnitude of the dependent variable. Exponential terms were added until a significant reduction in the sum of weighted squared residuals did not occur upon the addition of further terms. Plasma clearance of total and unbound AZ were calculated for each subject using the model-independent equations,

$$CL_T = D/AUC_t \qquad (2)$$

$$CL_u = D/AUC_u \qquad (3)$$

where CL_T and CL_u are plasma clearances for total and unbound drug, respectively, D is the administered intravenous dose and AUC_t and AUC_u represent the area under the plasma total and unbound AZ concentration-time curve respectively including its extrapolation to infinity. This method of calculation provides a time-averaged plasma clearance of AZ which should be identical to its renal plasma clearance since this diuretic is reported to be excreted by humans entirely as unchanged drug in the urine (Maren, 1967).

All AUC values were calculated manually from measured AZ levels using the log trapezoidal rule and extrapolated to infinity by dividing the last measured AZ level by the terminal elimination rate constant which was obtained from the polyexponential equation best describing the concentration-time data. For statistical analysis, the unpaired Student's *t*-test was used, $P < 0.05$ considered a minimum level of statistical significance.

Results

Mean plasma AZ concentration-time profiles were nearly identical in young and elderly subjects (Figure 1). A very rapid initial decline was followed by a progressively more gradual decline such that plasma levels were still detectable at 72 h. The mean concentration-time profile for unbound AZ in plasma reflected higher levels in the elderly for about 8 h post drug administration (Figure 2). Thus, while no difference was noted between age groups in the mean AUC for total AZ concentrations, the mean AUC for unbound drug was 81% higher in the elderly (elderly: 11.08 $\mu g\ ml^{-1}\ h$; young: 6.12 $\mu g\ ml^{-1}\ h$; $P < 0.05$).

Urinary excretion accounted for a considerable portion of the initial decline in plasma levels. In the four young subjects an average of 73.8% of the injected dose was excreted into

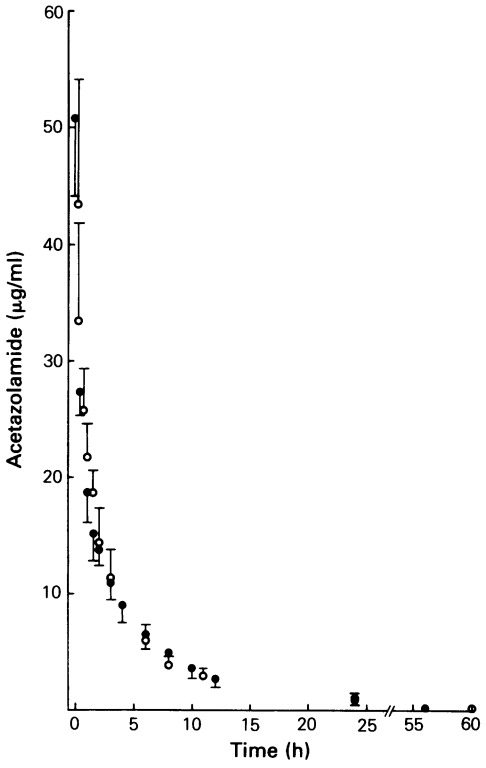

Figure 1 Mean plasma concentrations (± s.d.) of acetazolamide in elderly (○) and young (●) volunteers after intravenous injection of acetazolamide, 5 mg/kg.

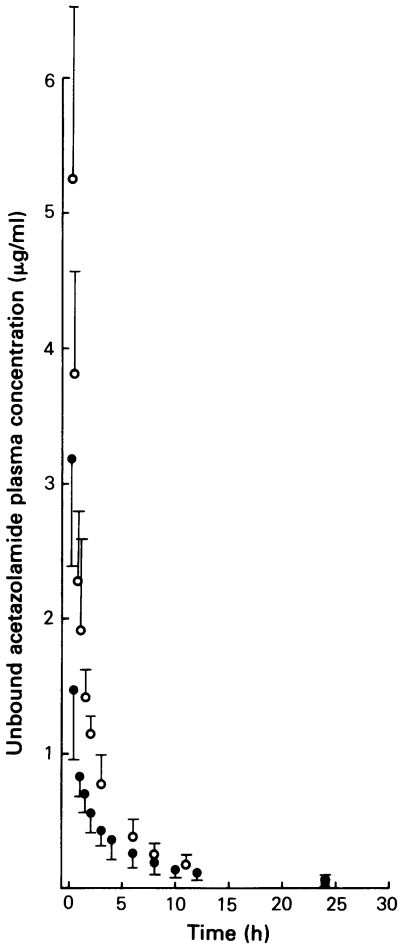

Figure 2 Mean plasma concentrations (± s.d.) of unbound acetazolamide in elderly (○) and young (●) volunteers after intravenous injection of acetazolamide, 5 mg/kg.

the urine during the first 8 h and 40.1% of the dose appeared there as soon as 2 h after injection. Interestingly, mean data for three elderly subjects (one elderly subject missed an early collection) showed a cumulative urinary AZ excretion percentage that closely approximated that seen in younger subjects. Furthermore, urinary excretion rate plots for young and elderly showed a similar and overlapping profile (Figure 3).

Recovery of unchanged AZ was nearly complete, exceeding 90% in seven of eight and 99% in four subjects (see Table 2). Nonrenal elimination was, therefore, considered to be negligible and plasma clearance equated to renal clearance. Time-averaged clearances of both total and unbound AZ were estimated. Although mean values for total drug did not differ between groups (old *vs* young), the unbound clearances were signicantly lower in the elderly subjects (8.9 ml min^{-1} kg^{-1} *vs* 15.7 ml min^{-1} kg^{-1}, $P < 0.05$). The unbound clearance of AZ was considerably greater than glomerular filtration, indicating that active tubular secretion is the major process contributing to the renal clearance of AZ in man. The unbound clearance of AZ correlated positively with measured creatinine clearance (Figure 4).

Erythrocyte concentration-time profiles for AZ exhibited an uptake phase which peaked between 0.45 and 3.0 h and gradually declined at a rate closely approximating the whole blood decline. At later times nearly all the AZ in whole blood resides in the erythrocyte. The mean time to achieve maximum erythrocytic AZ levels was similar for both age groups, 1.17 and 1.16 h for old and young, respectively. Peak erythrocyte concentrations, however, were about 45% higher in the elderly compared to young controls (37.2 ± 3.62 μg/ml *vs* 25.3 ± 4.40 μg/ml, $P < 0.01$), and the mean AUC

for erythrocytic AZ in elderly subjects was 46% higher than that observed in younger subjects (see Figure 5 and Table 2).

Plasma protein binding of AZ was determined over a wide range of drug concentrations. It was found to be concentration-dependent, but only when AZ exceeded a threshold concentration. In elderly and young subjects the mean concentrations at which protein binding was observed to become concentration-dependent were 21.5 and 17.9 μg/ml, respectively. The unbound fraction of AZ in plasma in the concentration independent range was found to be significantly greater in the elderly than younger subjects (6.4% *vs* 4.0% $P < 0.05$, see Table 2).

Log concentration-time profiles for plasma, plasma ultrafiltrate, whole blood, and post-peak erythrocytic data were concave at all time points. Although a polyexponential equation fits the data very well (eg., three terms for plasma and four terms for plasma ultrafiltrate with Corr and r^2 values greater than 0.974 and 0.981, respectively) residual plots showed systematic deviations. Presently we are in the process of developing a non-linear mathematical model to describe AZ disposition.

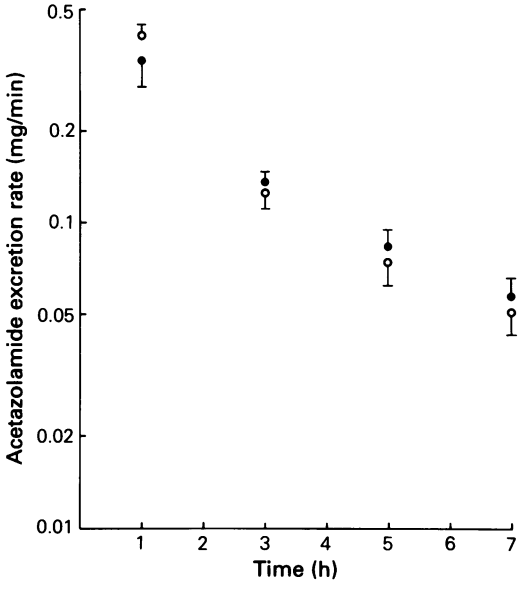

Figure 3 Mean urinary excretion rate (± s.d.) of acetazolamide in elderly (○) and young (●) volunteers after intravenous injection of acetazolamide, 5 mg/kg.

Table 2 Pharmacokinetic parameters for acetazolamide following an intravenous injection of drug (5 mg/kg)

	% dose recovered	*Plasma free fraction*	*Total clearance* (*ml min^{-1} kg^{-1}*)	*Unbound clearance* (*ml min^{-1} kg^{-1}*)	AUC_f	AUC_t (*μg ml^{-1} h*)	AUC_{rbc}
Elderly							
AD	95.0	0.077	0.69	8.21	11.17	132.8	962.1
OH	105.0	0.057	0.76	10.18	9.07	121.1	773.6
VS	91.9	0.054	0.75	11.45	8.88	135.8	736.9
CS	68.7	0.068	0.45	5.69	15.19	193.6	1053.3
Mean (± s.d.)		0.064 (0.011)	0.66 (0.15)	8.88 (2.51)	11.08 (2.93)	145.8 (32.5)	881.5 (151.2)
Young							
SC	99.5	0.032	0.78	21.35	4.15	114.3	600.4
SM	99.3	0.035	0.69	16.11	5.46	128.1	593.0
PF	100.0	0.035	0.54	15.21	5.65	158.5	506.2
PH	95.0	0.057	0.57	10.04	9.20	162.4	711.1
Mean (± s.d.)		0.040 (0.012)	0.65 (0.11)	15.68 (4.63)	6.12 (2.16)	140.8 (23.4)	602.7 (84.0)
P value		$P < 0.05$	NS	$P < 0.05$	$P < 0.05$	NS	$P < 0.02$

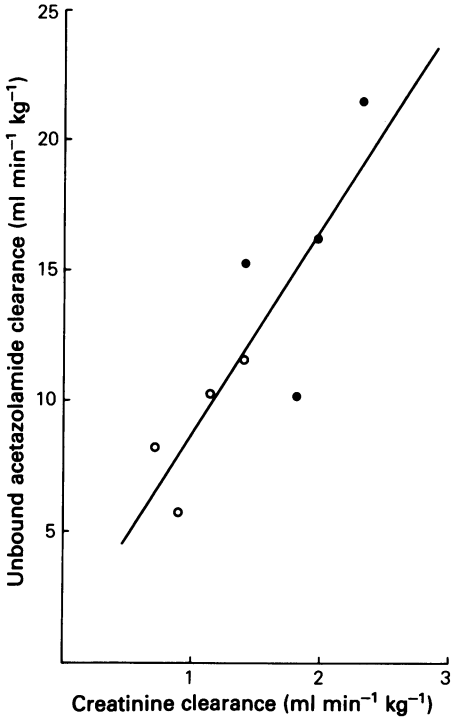

Figure 4 Correlation between plasma clearance of unbound acetazolamide and creatinine clearance ($r = 0.846$; $P < 0.01$).

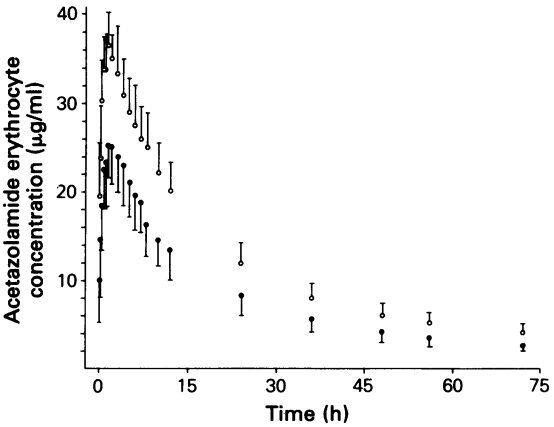

Figure 5 Mean erythrocyte concentrations (± s.d.) of acetazolamide in elderly (○) and young (●) volunteers after intravenous injection of acetazolamide, 5 mg/kg.

Discussion

AZ is the standard inhibitor of carbonic anhydrase (CA) that is widely employed in biological studies on the functional significance of this enzyme. It is used in humans to treat a wide variety of disorders including glaucoma, epilepsy, fluid retention, acute mountain sickness, periodic paralysis, hydrocephalus and chronic respiratory disease associated with metabolic alkalosis. Despite its diverse use in medicine, comprehensive studies on its disposition kinetics in humans are lacking. Since this drug finds its greatest use in the elderly for the treatment of glaucoma, kinetic studies in this population are of particular relevance.

The results of our investigation show that AZ disposition is very complex and cannot be described by standard linear compartmental models. Similar observations were made by Kunka & Mattocks (1979) in their study of AZ dispositon in the rabbit. Given the physiological relevance of measured plasma, erythrocyte and urine levels of AZ and the potential for non-uniqueness in parameter values obtained when fitting complex non-linear models to such data, this report focuses on model-independent parameters derived from the observed data.

The total clearance of AZ was found to be low and independent of age. Unbound clearances, however, were significantly lower in elderly than young subjects emphasizing the desirability of correcting clearances for the degree of plasma protein binding. By comparison, Maren & Robinson (1960) measured the unbound clearance of AZ in two hydrocephalic and one nonhydrocephalic child and found it approximated 17 ml min^{-1} kg^{-1}. A significant relationship between the total clearance of AZ (corrected for creatinine clearance) and its plasma free fraction was observed (Figure 6). Furthermore, urinary excretion rate plots for AZ very closely paralleled its decline in plasma ultrafiltrate (Figure 7). Both these findings indicate a dependence of clearance on plasma protein binding. For a drug which has a high tubular secretory component to its clearance, AZ is unusual in that this secretory pathway appears restricted to the unbound fraction in plasma.

Unbound AZ clearance correlated positively with creatinine clearance (Figure 4). The y-intercept approximates zero as predicted for a clearance process which is mediated entirely via renal mechanisms. Since creatinine clearance primarily reflects glomerular filtration while AZ clearance reflects primarily tubular secretion, such a correlation is consistent with earlier studies that found parallel decrements in GFR and tubular secretory function with advancing age (Davies & Shock, 1950). Such changes are considered consistent with the loss of intact nephrons with advancing age (Barrows, 1969; Papper, 1973). The positive linear relationship

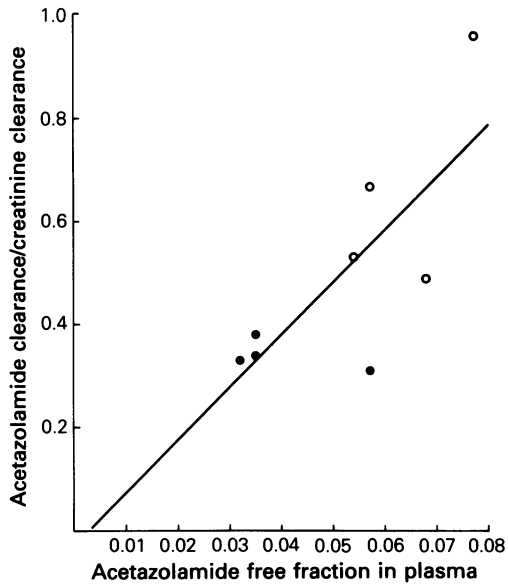

Figure 6 Correlation between the ratio of acetazolamide plasma clearance to creatinine clearance and the free fraction of acetazolamide in plasma ($r = 0.764$; $P < 0.05$).

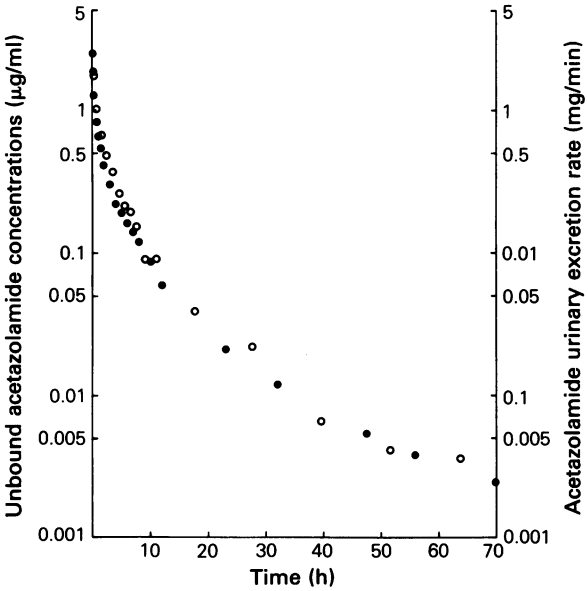

Figure 7 Unbound plasma concentrations (●) and urinary excretion rates (○) of acetazolamide in a representative subject (SC) after intravenous injection of acetazolamide 5 mg/kg.

between the clearance of AZ and that of creatinine over a wide range indicates that their ratio is constant and independent of age. This finding contrasts that of Reidenberg and colleagues (1980) who found that the ratio of procainamide clearance to creatinine clearance declined with advancing age. Inclusion of children in their study or the confounding influence of concurrent heart disease could account for the discrepancy. Previous studies from our laboratory have shown that the ratio of renal drug clearance to creatinine clearance is similar between young and old adults for theophylline (Antal *et al.*, 1981) and lithium (Chapron *et al.*, 1982).

The rate of excretion of an intravenous dose of AZ was nearly identical for old and young (Figure 3). The reduction in unbound renal clearance and the reduction in plasma protein binding with old age occur in parallel and offset each other such that the product of the unbound clearance and unbound plasma concentration, urinary excretion rate, is nearly independent of age. Thus, despite the diminished unbound renal clearance of AZ in the elderly, the rate of delivery of AZ to the tubular lumen was similar in both age groups. This observation, a reduced clearance yet an equivalent urinary excretion rate, is to our knowledge unique in the geriatric clinical pharmacology literature.

Many glaucoma patients find the side effects of long-term AZ administration intolerable. Epstein & Grant (1977) reported that elevated total plasma levels of AZ were associated with adverse effects in some, but not a majority of patients. They postulated that additional factors, in particular, the degree of inhibition of erythrocytic carbonic anhydrase induced by AZ, may be involved (Epstein & Grant, 1979). This postulate has been strengthened by recent observations in epileptic patients showing that high erythrocytic AZ levels correlate with a variety of side effects (Inui *et al.*, 1982). Woodbury & Kemp (1982) postulated that these side effects result from carbon dioxide retention in tissue as a result of inhibition of carbon dioxide exchange across the erythrocyte membrane.

Numerous studies including our own have shown that AZ accumulates and persists in the erythrocyte (Maren, 1967). Comparisons of plasma *vs* erythrocyte concentration-time profiles of AZ show a non-parallel rate of decline. The rate of decline of plasma levels exceeds that for erythrocytes. This results in a significant increase over time in the erythrocyte: plasma concentration ratio. While peak erythrocyte concentrations occurred at nearly identical times, peak levels were significantly higher in the elderly, suggesting that a greater fraction of the plasma AZ was available to the erythrocytes of the elderly individuals. Since AZ

concentration-time profiles for total drug were nearly identical between young and old, the free fraction of AZ in plasma at the time of peak was used as an index of its availability to erythrocytes. Peak erythrocyte levels of AZ correlated linearly with plasma free fraction (Figure 8). It should be noted that a reduced red cell mass in the elderly does not appear to be responsible for the increased peak red cell levels observed in this group. The mean haematocrit was similar between old and young (elderly 0.414 ± 0.035 *vs* young 0.418 ± 0.022) and the dose of AZ administered was based on body weight.

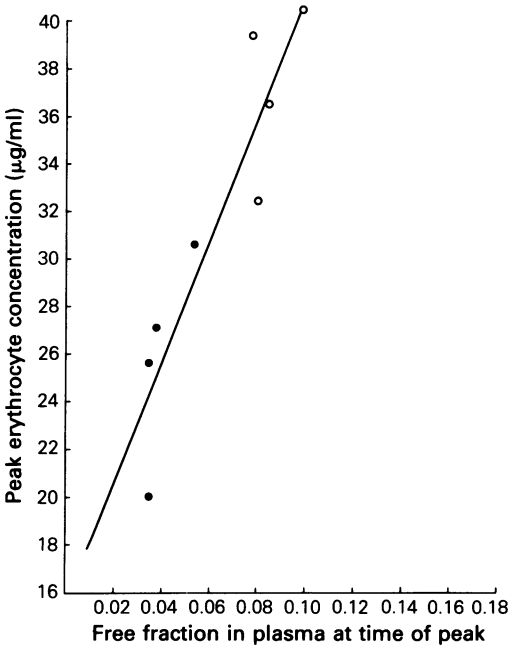

Figure 8 Correlation between peak erythrocyte concentrations of acetazolamide and corresponding plasma free fraction ($r = 0.919$; $P < 0.01$).

Mean area under the erythrocyte concentration-time curve was 46.3% greater in the elderly compared to young subjects, correlated linearly with the area under the unbound plasma level time curve (Figure 9) and therefore inversely with clearance of unbound drug from the plasma. Thus the unbound fraction of AZ in plasma governs the concentration of AZ that is freely diffusable and, hence, potentially available to the erythrocyte, while the renal clearance of unbound AZ may be viewed as a competing process limiting the availability of AZ to the erythrocyte by removing drug from the body. Plasma protein binding and renal clearance of unbound AZ affect peak and

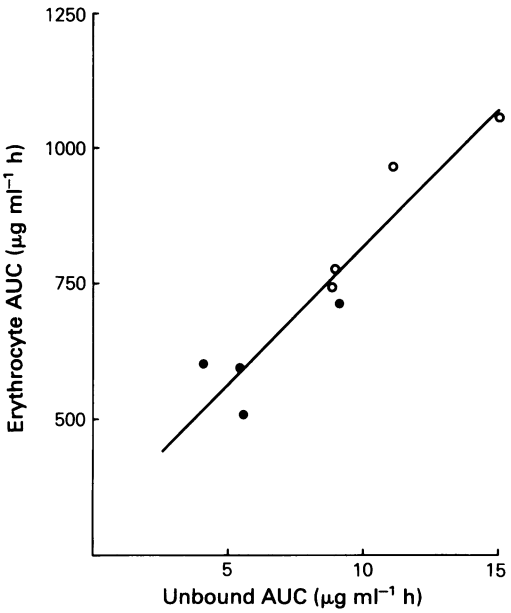

Figure 9 Correlation between areas under erythrocyte and unbound plasma acetazolamide concentration versus time curves ($r = 0.943$; $P < 0.01$).

integrated erythrocyte AZ concentrations and may importantly influence the degree of inhibition of erythrocyte carbonic anhydrase. These factors may prove useful for the identification of those patients most susceptible to the common side effects associated with AZ administration. Additional studies are needed to determine the relationship between erythrocyte AZ concentrations and the drug's side effect profile.

In summary, while these single dose studies may not predict the situation of chronic dosing, several important therapeutic points are raised by our results. They include (1) the elderly have a reduced capacity to clear unbound AZ from plasma and this correlates with their age-related reduction in creatinine clearance, (2) the elderly also have a reduced plasma protein binding and this offsets the reduced unbound clearance of AZ resulting in similar excretory rates, (3) the above factors predispose this group to enhanced accumulation of AZ in the erythrocyte, (4) dosage reductions for AZ seem appropriate in the elderly, but need to be confirmed by efficacy studies, and (5) the disposition of AZ is governed by its binding to plasma proteins and its secretion by renal tubular cells, both of which are frequent loci for drug-drug interactions.

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