Relationship of Pharmacokinetics to Pharmacological Response for Acetazolamide

ROBERT L. KUNKA * **and ALBERT M. MATTOCKS** ˣ

Received March 20, 1978, from the *School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication August 31, 1978. *Present address: School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.

Abstract □ Acetazolamide concentration values derived from a nonlinear model system were related to two pharmacological responses in the rabbit. Kidney response was measured by monitoring urine flow and sodium elimination. Ocular response was followed using an applanation tonometer. Maximum urine flow and sodium elimination occurring immediately after injection correlated with log dose. Urine flow dropped below control values along with a rise in osmolality, suggesting the involvement of antidiuretic hormone. Sodium elimination was correlated with plasma levels. Urine pH is thought to be involved in reducing accessibility of drug to carbonic anhydrase in the kidney. Maximum ocular response also was correlated with log dose. Ocular response was related to a protein fraction, which is believed to be mainly carbonic anhydrase. However, the duration of ocular response was related to the red blood cell protein fraction. Thus, drug activity could conceivably be regulated by monitoring a tissue that is not the site of action and can be sampled readily.

Keyphrases □ Acetazolamide—relationship of pharmacokinetics to pharmacological response, kidney and ocular responses □ Pharmacokinetics—acetazolamide, relationship to two pharmacological responses □ Carbonic anhydrase inhibitors—acetazolamide, relationship of pharmacokinetics to pharmacological responses

Although pharmacokinetic research has been mainly concerned with the time course of a drug and its metabolites in a biological system, the relationship of pharmacokinetics to pharmacological response is now being emphasized. An earlier report (1) described the pharmacokinetics of acetazolamide using a nonlinear model system. This model proposed a single compartment of distribution containing two drug-binding tissues, T_1 and T_2, where T_2 represents binding to red blood cells and T_1 represents binding to other tissues. Binding in the tissues is believed to consist of attachment to carbonic anhydrase (2, 3) following the general concepts of enzyme inhibition. Such a model, incorporating the known mechanism of action of acetazolamide, was thought to offer the most promise for correlation of drug distribution with physiological effect.

The purpose of this study was to determine whether the drug distribution proposed by the model can be related to the measured physiological response of acetazolamide. The drug elicits two major types of response involving well-separated organs, the kidney and the eye, *via* carbonic anhydrase inhibition.

EXPERIMENTAL

At the same time that samples were being collected for plasma, red blood cell, and urine drug level measurements as described previously (1), physiological response was being measured. Additional animals were given acetazolamide, and only physiological response was measured. The New Zealand White rabbit was the experimental animal, and the drug was injected intravenously at several levels.

To evaluate the effect of the drug on renal function, urine samples were examined for flow rate, osmolality, and sodium elimination. Following catheterization of the bladder, urine was collected at 5–15-min intervals at first and then at 1-hr intervals and flow rates were calculated. Osmolality was measured directly with an electronic freezing-point osmometer[1]. Sodium content in urine was measured with a specific ion electrode[2]. This procedure consisted of diluting 1 ml of urine to 100 ml, adding 2 ml of ionic strength adjuster solution (25% ammonium chloride and 4% ammonium hydroxide), mixing, and reading with the sodium electrode. The reading was referred to a curve prepared by reading a series of sodium chloride standards ranging from 10^{-1} to 10^{-5} *M*.

To evaluate the effect of the drug on ocular function, intraocular pressure readings were taken at frequent intervals throughout the experiment using a recording applanation tonometer[3]. Only readings that reflected accurate measurement of intraocular pressure as judged by the shape of the applanation curve were accepted. In most cases, the average of three readings was made. A drop of procaine solution was given at the beginning of the experiment and, if needed, during the experiment.

For 1–2 hr before injection, measurements of urine flow and intraocular pressure were made to assure relatively constant control values. Two rabbits did not respond to acetazolamide and were not used.

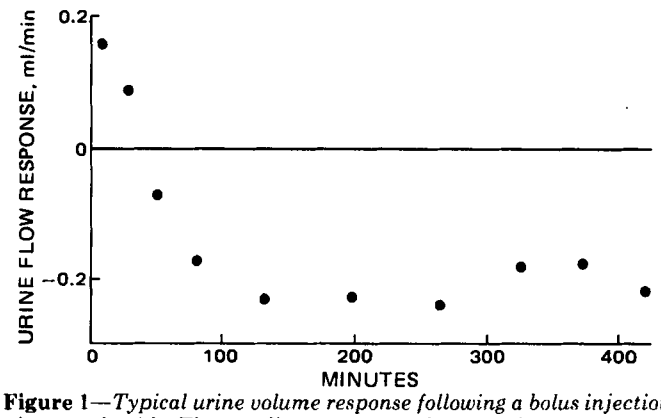

Figure 1—*Typical urine volume response following a bolus injection of acetazolamide. The zero line represents the control rate.*

RESULTS AND DISCUSSION

Kidney Response—Urine flow rate response was calculated as the flow rate, in milliliters per minute, minus the control rate. The urine excretion rate increased dramatically immediately after injection (Fig. 1). This increase was followed by a sharp drop to levels below control

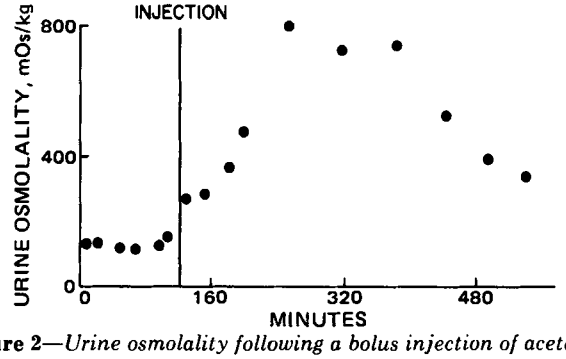

Figure 2—*Urine osmolality following a bolus injection of acetazolamide.*

[1] DigiMatic, Advanced Instruments, Needham Heights, Mass.
[2] Orion 94-11A sodium-ion electrode and 90-01-00 reference electrode, Orion Research, Cambridge, Mass.
[3] Applanation pneumatonograph, Alcon Laboratories, Fort Worth, Tex.

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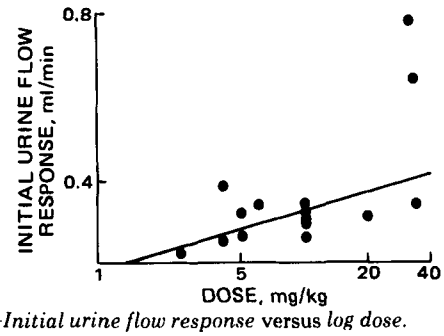

Figure 3—*Initial urine flow response* versus *log dose.*

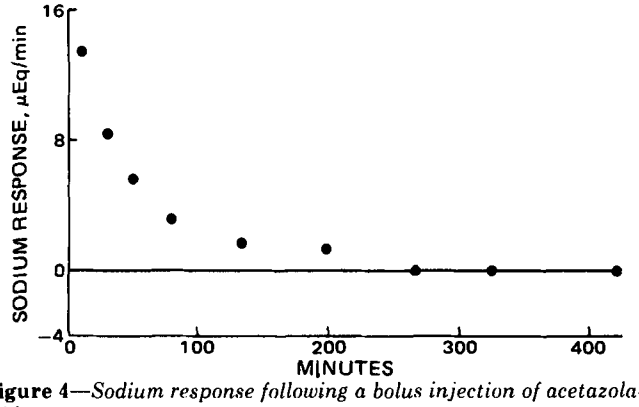

Figure 4—*Sodium response following a bolus injection of acetazolamide.*

values. These subnormal rates were usually maintained for the duration of the experiment.

Urine osmolality increased immediately after drug administration, probably due to the increased sodium and bicarbonate elimination. However, a dramatic rise in osmolality occurred a short time afterward (Fig. 2). High osmolality was maintained for several hours but usually returned to normal during the experimental period. This dramatic increase in osmolality was not correlated with sodium elimination and thus appeared to be due entirely to increased water resorption and concentration of the urine. This effect would most likely result from release of antidiuretic hormone (ADH).

Thus, the drug effect on urine flow seems to be confounded by compensatory mechanisms, and attempts at correlation of flow to acetazolamide concentrations in body compartments are not justified. The earlier responses (points 1 and 2) show a sharp decrease following drug administration and appear to reflect minimum compensatory activity (Fig. 2). This response suggests that urine flow increase is correlated with plasma concentration and not with red cell or general tissue levels since the latter decreased quite slowly. Initial flow increases did show the common log dose relationship (Fig. 3).

Sodium response was calculated as the sodium elimination rate, in microequivalents per minute, minus the control rate. Sodium elimination increased sharply following the bolus injection of acetazolamide (Fig. 4). Maximum sodium elimination occurred within 20 min of injection in

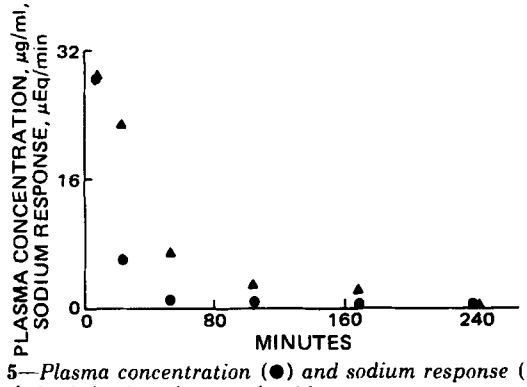

Figure 5—*Plasma concentration* (●) *and sodium response* (▲) *following a bolus injection of acetazolamide.*

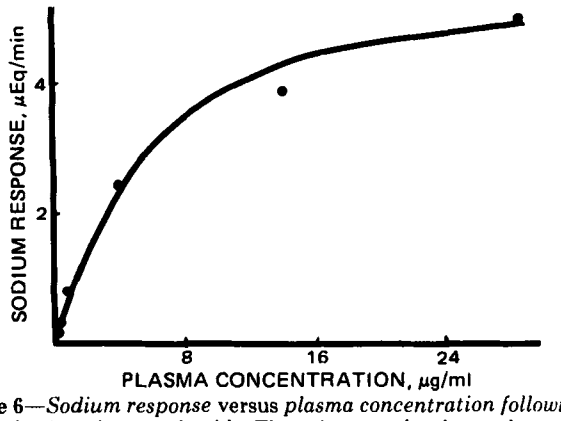

Figure 6—*Sodium response* versus *plasma concentration following a bolus injection of acetazolamide. The points are the observed response. The fitted curve was obtained from the equation: sodium response = 5.98*(C)/(5.60 + C).

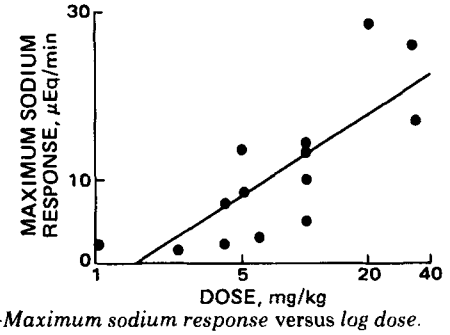

Figure 7—*Maximum sodium response* versus *log dose.*

virtually all experiments. A sharp fall followed, with the level dropping to less than 10% of maximum and remaining at approximately this level for the duration of the experiment. Since the effect of acetazolamide depends on its blocking carbonic anhydrase in the kidney, one might expect this drug action to be related to tissue concentration; but tissue concentrations peak immediately after injection and decrease quite slowly (1), obviously not correlating with kidney response. However, a definite relationship between sodium response and plasma level was seen (Fig. 5).

When response is plotted *versus* plasma concentration, a Langmuir-type curve results (Fig. 6); the response appears to be a linear function of plasma level at low concentrations, to be nonlinear in the intermediate range, and to approach a maximum response at high levels. Eight data sets could be plotted in this manner. Other sets were not suitable since sodium excretion dropped below control levels at later points. Of the eight, seven yielded Langmuir-type curves; the eighth, involving a small dose of 4 mg/kg, was linear throughout. This result was thought to be due to not having achieved high enough plasma levels to demonstrate nonlinearity. A linear relationship was seen between log dose and sodium elimination (Fig. 7).

Closer consideration of the environment in which kidney carbonic anhydrase operates reveals some interesting concepts. Although carbonic anhydrase appears in both the proximal and distal renal tubules, reab-

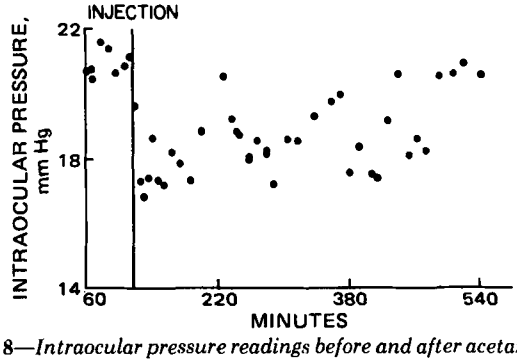

Figure 8—*Intraocular pressure readings before and after acetazolamide injection.*

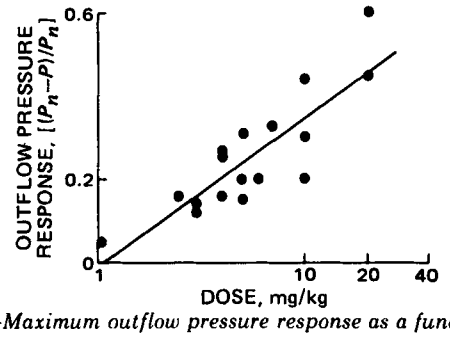

Figure 9—*Maximum outflow pressure response as a function of log dose.*

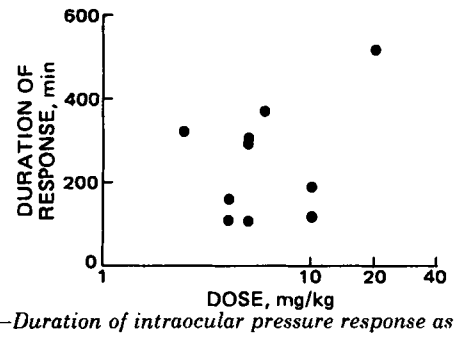

Figure 10—*Duration of intraocular pressure response as a function of log dose.*

sorption of sodium *via* the action of carbonic anhydrase occurs primarily in the luminal membrane of the tubule cell (4). The unique location of enzyme adjacent to a fluid prone to considerable variations in pH may expose these cells to substantial variations in drug transfer not encountered in other regions. Control readings of urine pH in the animals studied ranged from 6.16 to 8.86 with an average value of 7.89. At this pH, acetazolamide, with a pKa of 7.4, would be 24% undissociated. Within 30 min after dosing, urine pH rose to 7.76–8.82 with an average of 8.24, at which pH the drug would be 13% undissociated.

If it is assumed that only the undissociated form of the drug may diffuse across the cell wall, the effective drug concentration within the region of the enzyme may be greatly reduced by the change in urine pH. The effective drug concentration drops to about one-fourth. Thus, the action of the drug to increase the pH of the tubular fluid probably increases its own removal from the site of action, hence decreasing its duration of activity. These assumptions also suggest that the dynamics of drug activity in the kidney will differ from those in other regions.

Ocular Response—Shortly after an intravenous dose of acetazolamide, the intraocular pressure began to drop. It reached a minimum in 15–20 min, cycled up to nearly normal and back down to the minimum, and eventually returned to normal where it remained. The wave-like variation in intraocular pressure, which was more pronounced during drug activity, may reflect a muscular resistance to outflow of intraocular fluid.

The variation in pressure (Fig. 8) makes assignment of a single value to drug response difficult. It was decided to characterize effect as the average drop, $\Delta P/P_n$, where ΔP is the change in outflow pressure and P_n is the normal outflow pressure. Outflow pressure was taken in the usual manner as the intraocular pressure minus 9 mm Hg (5–7). As well as can be determined by experimental measurement, each dose of acetazolamide gave an early minimum pressure (maximum response), which did not change significantly until it returned to normal.

Obviously, the ocular response shows no correlation with plasma level. Data from all animals studied, however, indicated that the ocular response terminates before the tissue-bound concentration, T_1, approaches 99% of maximum. This finding agrees with the theoretical concept that more than 99% of the carbonic anhydrase in the ciliary body must be inhibited for physiological response to occur. When the enzyme of the ciliary body is considered to be a part of a large mass of tissue called T_1, a drop to 99% of the maximum bound value would be expected to result in loss of activity. Thus, the pharmacokinetic model apparently does predict the effective drug concentration, T_1, with reasonable accuracy whereas a predicted plasma level, except for the peak concentration, would be of no use in assessing drug effect.

Correlation of the results of this study with those of the kinetic report (1) indicated that the acetazolamide is rapidly taken up by the binding tissue, T_1, at which time maximum intraocular pressure response occurs. Beyond this point, the tissue concentration is practically independent of plasma level, being primarily dependent upon drug–tissue dissociation.

The magnitude of intraocular response was a function of T_1 and correlated with log dose (Fig. 9). However, no correlation was found between the duration of response and dose (Fig. 10). This finding suggests that duration of activity is dependent on the dissociation rate of the drug from its tissue attachment. Response terminated when the red cell concentration fell to 6–9 μg/ml. In four animals, intraocular pressure did not return to normal during the experiment; in three of these animals, the red cell drug level did not fall below 6 μg/ml.

It has been reported that metabolic and respiratory acidosis lowers intraocular pressure (8, 9). Therefore, acetazolamide might offset, to some degree, intraocular pressure *via* action on erythrocyte enzymes in this manner. This concept suggests that red cell drug concentrations may be used as a means of selecting intervals for drug administration.

The common concepts of maximum drug action *via* enzyme inhibition are that the enzyme is present in limited quantity and that the drug is given in quantities sufficient to saturate all enzyme. Thus, a maximum effect is obtained and then decreases as drug slowly dissociates from the enzyme–drug complex. In this case, the enzyme is present throughout the body, and the amount of drug is not sufficient to saturate all enzyme. Yet, the enzyme in a given organ, such as the eye, may be nearly saturated, causing a drug response. Moreover, because of the large amount of enzyme present in the body, a substantially larger dose would be distributed to a variety of organs and the eye would only receive a small fraction of the additional drug, thus giving a higher response but not yet its maximum response. This concept explains how each dose above 1 mg/kg gave an ocular response, yet a maximum response was not elicited at 20 mg/kg. From literature reports (3, 10), 10–20 mg/kg is expected to be near the level yielding maximum effect.

The results of this study and the previous report (1) demonstrate the need to use an unusual model system to describe the kinetics of a drug in a manner suitable for correlation with pharmacological response. Since acetazolamide acts by enzyme inhibition, it is representative of a rather large class of therapeutic agents, so a much wider application of this tissue-binding model is anticipated. Advantages of this model are that it takes into account the mechanism of drug action and simultaneously describes the concentrations in different tissues. Ultimately, as the technique is refined, one of these tissues may be the site of action.

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