Pharmacokinetics of acetazolamide after intravenous and oral administration in horses

Michael K. Alberts, DVM; Cyril R. Clarke, BVSc, PhD; Charles G. MacAllister, DVM; Lynne M. Homer, DVM

Objective—To determine the pharmacokinetics of acetazolamide administered IV and orally to horses.

Animals—6 clinically normal adult horses.

Procedure—Horses received 2 doses of acetazolamide (4 mg/kg of body weight, IV; 8 mg/kg, PO), and blood samples were collected at regular intervals before and after administration. Samples were assayed for acetazolamide concentration by high-performance liquid chromatography, and concentration-time data were analyzed.

Results—After IV administration of acetazolamide, data analysis revealed a median mean residence time of 1.71 ± 0.90 hours and median total body clearance of 263 ± 38 ml/kg/h. Median steady-state volume of distribution was 433 ± 218 ml/kg. After oral administration, mean peak plasma concentration was 1.90 ± 1.09 μg/ml. Mean time to peak plasma concentration was 1.61 ± 1.24 hours. Median oral bioavailability was 25 ± 6%.

Conclusions and Clinical Relevance—Oral pharmacokinetic disposition of acetazolamide in horses was characterized by rapid absorption, low bioavailability, and slower elimination than observed initially after IV administration. Pharmacokinetic data generated by this study should facilitate estimation of appropriate dosages for acetazolamide use in horses with hyperkalemic periodic paralysis. (*Am J Vet Res* 2000;61:965–968)

Acetazolamide (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide), a sulfanilamide, is a carbonic anhydrase inhibitor used in human and veterinary medicine. Introduced to human medicine in the early 1950s, acetazolamide was initially used as a diuretic for treatment of congestive heart failure.[1] Since then, use of acetazolamide in human medicine has extended to the treatment of glaucoma,[2] prophylaxis of several myopathies (including acetazolamide responsive myotonia congenita[3] and hyperkalemic periodic paralysis[4]), treatment of seizures,[5] and prevention of acute mountain sickness.[6] In veterinary medicine, acetazolamide has not been used as widely as in human medicine. Small animal ophthalmologists have used acetazolamide for the treatment of glaucoma in dogs.[7] In cattle, acetazolamide is administered to control udder edema.[8,9] Acetazolamide has also been recommended for prophylactic treatment of horses affected by the heritable muscle disease **hyperkalemic periodic paralysis (HPP)**.[10-15]

Despite the use of acetazolamide in veterinary therapeutics, especially for treatment of HPP, the pharmacokinetics of acetazolamide in various animals have not been studied extensively. In humans, acetazolamide is absorbed rapidly and undergoes biphasic elimination.[16,17] Limited pharmacokinetic data derived from a study of the effects of acetazolamide on exercise-induced metabolic and respiratory responses in horses revealed a mean unbound plasma acetazolamide concentration of 1.38 ± 0.64 μg/ml approximately 14 hours after 3 consecutive days of oral administration (30 mg/kg of body weight, q 12 h).[18] The purposes of the study reported here were to develop a suitable **high-performance liquid chromatography (HPLC)** assay for measuring acetazolamide plasma concentrations and to use this assay to study the pharmacokinetics of acetazolamide after IV and oral administration in horses.

Materials and Methods

Horses—Six adult horses (413 to 673 kg; 2 Thoroughbreds and 4 Quarter Horse-type) were housed in individual 4 × 4 m box stalls and fed a ration of grain twice daily and prairie hay ad libitum. Two horses were geldings; the others were mares. Horses ranged in age from 3 to 11 years. Before initiation of the study, all horses were acclimated for at least 14 days and were determined to be clinically normal on the basis of results of physical examination, CBC, and serum biochemical analyses. Horses also were dewormed by oral administration of fenbendazole.[a]

Experimental design—Each horse received the following 2 treatments: 1 dose of acetazolamide,[b] administered IV at 4 mg/kg, and 1 dose of acetazolamide,[c] administered PO at 8 mg/kg. Treatments were separated by a washout period of a minimum of 14 days. To minimize any possible effects of treatment sequence, horses were randomly assigned to the initial treatment group, and a crossover design was used.

Drug formulation and administration—Physical examinations (including rectal body temperature, pulse rate, and respiratory rate) were performed immediately prior to each administration of acetazolamide. For IV administration, acetazolamide dry powder was reconstituted with sterile saline (0.9% NaCl) solution to provide a concentration of 100 mg/ml. Acetazolamide was injected into the right jugular vein, and blood samples were collected from the left jugular vein at time 0 (before administration), 5, 10, 15, 25, 35, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after administration. For oral administration, food was withheld immediately before acetazolamide was administered as crushed tablets containing 250 mg of drug/tablet, mixed with a small volume (approx 10 ml) of corn syrup.[d] Blood samples were collected from the right jugular vein at time 0 (before administration), 10, 20, and 45 minutes and 1, 2, 3,

Received Feb 23, 1999.
Accepted Jul 22, 1999.
From the Departments of Veterinary Clinical Sciences (Alberts, MacAllister) and Physiological Sciences (Clarke, Homer), College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078. Dr. Alberts' present address is Cedar Creek Equine, PO Box 1865, Poulsbo, WA 98370.
Address correspondence to Dr. Clarke.

4, 6, 8, 12, and 24 hours after administration. All blood samples (10 ml) obtained by jugular venipuncture were collected into sterile lithium heparinized vacuum tubes.[e] Samples were centrifuged at 600 × g for 15 minutes, and plasma was obtained and stored at –20 C until assays were performed.

Acetazolamide assay—Plasma acetazolamide concentrations were determined by HPLC after extraction of acetazolamide with ethyl acetate, using modifications of a reported assay.[16] Briefly, 50 µl of internal standard (20 µg of chlorothiazide[f]/ml of deionized H_2O), 0.5 ml of 0.05 *M* sodium acetate buffer, and 10 ml of ethyl acetate were added to 1 ml of sample in a glass culture tube.[g] The tube was capped, and the combination was vortexed for 3 minutes, then centrifuged at 1,000 × g for 10 minutes. Organic supernatant was transferred to a clean test tube and evaporated to dryness under N_2. Precipitate was redissolved in 200 µl of mobile phase solution (95% 0.05 *M* sodium acetate, 3% acetonitrile, and 2% methanol). Acetazolamide[h] standards (0.19, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 µg/ml) were prepared by adding appropriate volumes of acetazolamide stock solutions (2.5, 25, 125, and 250 µg/ml prepared in ethanol) to blank equine plasma. These test standards were subjected to the same analytic procedures as the test samples. Extracted samples were filtered through a 0.45-µm filter,[i] and 50 µl of each extract was injected into an HPLC system that consisted of a gradient programmer,[j] pump,[k] variable wavelength UV absorbance detector[l] set at 254 nm, and an integrator.[m] A 150-mm, 4.6-mm ID reverse-phase (C-18; 100-A particle size) column[n] provided adequate separation of peaks. Chromatography was performed at 20 C.

Intra-assay precision was determined by assaying 5 replicates of each of 2 plasma concentration standards (0.39 and 25 µg/ml) together with standards used to construct a calibration curve. Accuracy was estimated by addition of known amounts of acetazolamide to blank plasma and comparison of the added concentrations with concentrations calculated by use of standards. The relationship between standard concentrations and the ratio of acetazolamide to internal standard peak areas was best described by use of a power function ($R^2 > 0.95$). The lowest standard concentration was detectable, thereby providing adequate sensitivity to satisfy the study objectives. Coefficients of variability describing intra-assay precision were low (7.22% at 0.39 µg/ml and 3.79% at 25 µg/ml). Concentrations calculated by use of the standard curve deviated < 8.22% from known values, which indicated a satisfactory level of accuracy.

Pharmacokinetic analyses—Initial estimates of coefficients and exponents of polyexponential equations describing acetazolamide disposition after IV administration were obtained by subjecting mean data to analysis, using iterative least-squares regression analysis.[19] Individual concentration-time data were then analyzed by use of a computer program for nonlinear weighted least-squares regression.[20] Minimal sums of weighted residuals were approached by use of the Simplex fitting algorithm. Choice of appropriate pharmacokinetic model was based on lowest weighted sum of squares, F test, and lowest Akaike information criterion value for individual data. **Areas under the IV and PO disposition curves** (AUC_{IV} and AUC_{PO}, respectively) were calculated, using trapezoidal approximations between time of drug administration and collection of the last blood sample. **Steady-state volume of distribution** (Vd_{ss}) and **total body clearance** (Cl_B) were calculated, using values generated by noncompartmental analysis (**area under the first moment curve [AUMC]**, **mean residence time [MRT]**, and AUC_{IV}). Equations used were as follows:

$$MRT = AUMC/AUC_{IV};\ Cl_B = Dose/AUC_{IV};\ \text{and}\ Vd_{ss} = MRT \times Cl_B$$

Bioavailability was determined using the ratio of the areas under the individual IV and PO curves. Medians and median deviations were used as estimates of central tendency and dispersion, respectively, for pharmacokinetic values that represented ratios of observed values and usually do not follow a normal distribution.

Results

Disposition of acetazolamide in plasma after IV administration was best described by the 3-compartment open pharmacokinetic model,[21] using the following equation:

$$C_p = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is plasma concentration at time t, e is the base of the natural logarithm, P, A, and B are zero-time plasma concentration intercepts of the triphasic disposition curve, and π, α, and β are hybrid rate constants related to the slopes of the distribution and elimination phases (**Fig 1**).

Rate of decrease of acetazolamide during the first 8 hours after administration was rapid (median **half-life** [$t_{1/2(\alpha)}$], 1.01 ± 0.19 hours, consistent with a low MRT [median MRT, 1.71 ± 0.90 hours] and high Cl_B [median Cl_B, 263 ± 38 ml/kg/h]; **Table 1**). Median steady-state Vd_{ss} was 433 ± 218 ml/kg.

Oral administration of 8 mg/kg of acetazolamide resulted in detection of the drug within 10 minutes in 3 of 6 horses and within 20 minutes in 5 of 6 horses (acetazolamide was not detected until 1 hour after administration in the 6th horse). Mean **time to peak concentration** (T_{MAX}) was 1.61 ± 1.24 hours, with a mean peak plasma concentration of 1.90 ± 1.09 µg/ml. Median MRT for oral administration was 6.77 ± 1.40 hours, which was substantially larger than the corresponding value for IV administration, thereby suggesting a flip-flop model of drug absorption. Median drug bioavailability was 25 ± 6%.

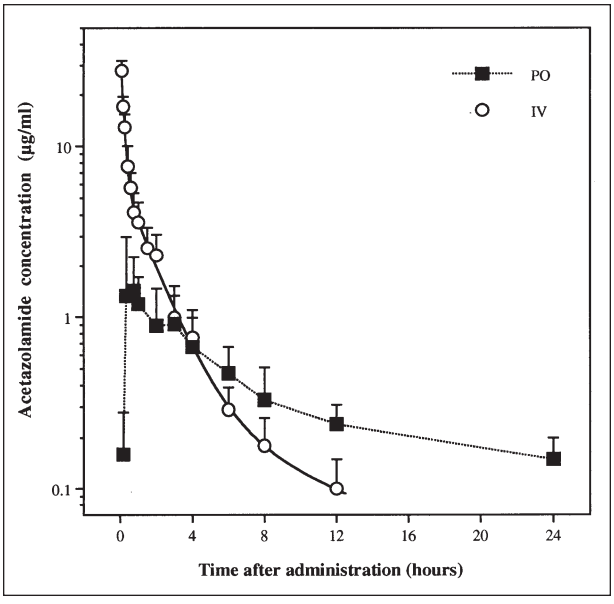

Figure 1—Disposition of acetazolamide in plasma after IV administration (4 mg/kg of body weight) and oral administration (8 mg/kg) in 6 clinically normal adult horses. Fitted curve is based on the 3-compartment fit to mean (± SD) data.

Table 1—Pharmacokinetic values derived from analysis of individual disposition curves for IV and oral administration of acetazolamide in 6 clinically normal adult horses

Determinant	Value
IV Administration	
P (μg/ml)	38.22 ± 9.16
A (μg/ml)	6.65 ± 1.87
B (μg/ml)	0.41 ± 0.25
π ($hour^{-1}$)	7.100 ± 1.494
α ($hour^{-1}$)	0.722 ± 0.172
β ($hour^{-1}$)	0.123 ± 0.079
$t_{1/2}(\pi)$(hour)*	0.10 ± 0.02
$t_{1/2}(\alpha)$(hour)*	1.01 ± 0.19
$t_{1/2}(\beta)$(hour)*	7.62 ± 2.20
AUC (0–24)	15.41 ± 2.85
AUMC (0–24)	33.74 ± 19.06
MRT (hour)*	1.71 ± 0.90
Cl_B (ml/kg/h)*	263 ± 38
Vd_{ss} (ml/kg)*	433 ± 218
Oral Administration	
AUC (0–24)	8.26 ± 3.28
AUMC (0–24)	58.97 ± 27.27
MRT (hour)*	6.77 ± 1.40
T_{MAX} (hour)	1.61 ± 1.24
C_{MAX} (μg/ml)	1.90 ± 1.09
F (%)*	25.0 ± 6.0

Data are expressed as mean ± SD unless indicated otherwise.
*Values represent median ± median deviation.
P, A, B 5 Zero time plasma concentration intercepts of the triphasic disposition curve. p, a, b 5 Hybrid rate constants related to the slopes of the distribution and elimination phases. $t_{1/2(p)}$, $t_{1/2(a)}$, $t_{1/2(b)}$ 5 Half-lives of distribution and elimination corresponding to each of the hybrid rate constants. Vd_{ss} 5 Steady state volume of distribution. Cl_B 5 Total body clearance. AUC (0–24) 5 Area under the plasma drug versus time curve for 0 to 24 h. AUMC (0–24) 5 Area under the moment curve for 0 to 24 h. MRT 5 Mean residence time. T_{MAX} 5 Time necessary to achieve maximal plasma concentration (C_{MAX}). F 5 % Bioavailability.

Discussion

Acetazolamide is used extensively in equine veterinary medicine to control the effects of hyperkalemia in horses with HPP. Hyperkalemic periodic paralysis of horses is an autosomal dominant genetic condition in which a replacement of phenylalanine with leucine causes a defect in sodium ion channels of muscle cells.[22] The mechanism of action of acetazolamide in relation to this condition is poorly understood but may relate to altered potassium homeostasis via pharmacologic actions induced by acetazolamide in the renal system. Inhibition of carbonic anhydrase in the proximal convoluted tubules of the kidney decreases reabsorption of sodium ions through indirect inactivation of luminal membrane-bound H^+-Na^+ antiporters. Increased delivery of sodium to the distal convoluted tubule then increases electrochemical gradients and aldosterone concentrations in the distal tubules, which enhance sodium reabsorption and potassium excretion.[23] Resolution of clinical signs may result from lowering of extracellular potassium and a permissive effect on muscle membrane physiologic factors, which return affected cells to a more normal condition. Alternatively, acetazolamide may stabilize muscle membranes by prevention of sudden potassium losses from intracellular stores through the potassium channel defect, by induction of glycogenolysis and an increase in insulin concentration, which should potentiate movement of potassium in an intracellular direction[4] by decreasing the exchange of potassium across RBC membranes and establishing a steady state,[10] or by an unknown mechanism, possibly relating to changes in acid-base status.[24,25]

Bioavailability of acetazolamide in humans is reported to be extremely high and may approach 100%.[23,26] This is in considerable contrast to the relatively low bioavailability of 25 ± 6% in the horses of our study. Peak plasma concentration after oral administration of acetazolamide in humans is achieved within 60 to 120 minutes.[27,28] This value is comparable with the mean value observed in the horses of our study (T_{MAX} = 1.61 ± 1.24 hours). The median Vd_{ss} of 433 ± 218 ml/kg indicated drug distribution that exceeded extracellular fluid volume and approached that of total body water.[26] This value is higher than the Vd_{ss} of 200 ml/kg[29] reported for humans, which suggests there may be a wider distribution of acetazolamide in horses. The reason for this difference is unknown but may relate to extensive plasma protein binding in humans (93%).[29]

In humans, acetazolamide is reported to have a $t_{1/2(\beta)}$ of 3.5 hours,[29] compared with a median $t_{1/2(\beta)}$ of 7.62 ± 2.20 hours in the horses in this study. However, this slow elimination rate developed late in the disposition profile. During the first 8 hours after administration, plasma acetazolamide concentrations decreased rapidly, with a median Cl_B value of 263 ± 38 ml/kg/h. It is not clear what the relative contributions of drug distribution and renal excretion are during the period of rapid decrease of plasma concentration.[26,30]

Studies evaluating the effectiveness of single doses of acetazolamide in humans with glaucoma to decrease intraocular pressure suggest that serum concentrations > 6 μg/ml (dose of 63 mg, PO)[31] and plasma concentrations ≥ 4.2 μg/ml (dose of 125 mg, PO)[28] are effective. Effective prophylaxis of acute mountain sickness,[6] Isaacs' syndrome,[32] hypokalemic periodic paralysis,[33] and HPP[4] has been reported in humans, using doses of acetazolamide that would induce plasma drug concentrations in excess of 4.2 μg/ml. Beech et al[13] reported that horses with HPP were effectively protected from clinical signs of disease when 2.2 to 4.4 mg/kg of acetazolamide was administered PO twice daily for 1 week prior to challenge with orally administered potassium. Assuming that acetazolamide plasma concentrations increase linearly with increased doses in horses, as reported in humans,[28] the corresponding peak plasma drug concentrations in the horses of this study would be approximately 0.52 and 1.04 μg/ml for the 2.2 mg/kg and 4.4 mg/kg doses, respectively. These values suggest that much lower plasma concentrations may be required for effective prophylaxis in horses than in humans. The reason for this apparent difference is unknown, yet fortunate, considering the low bioavailability of acetazolamide in horses. Clearly, further pharmacodynamic studies are needed to elucidate the relationship between acetazolamide plasma concentration and therapeutic efficacy in horses with HPP.

[a]Panacur, Hoechst, Somerville, NJ.
[b]Acetazolamide, Bedford Laboratories, Bedford, Ohio.
[c]Acetazolamide, Schein Pharmaceutical, Florham Park, NJ.

[d]Karo, CPC International, Englewood Cliffs, NJ.
[e]Monoject, Sherwood Medical, St Louis, Mo.
[f]Chlorothiazide, Sigma-Aldrich Inc, St Louis, Mo.
[g]Pyrex, Corning Glass Works, Corning, NY.
[h]Acetazolamide, Sigma-Aldrich Inc, St Louis, Mo.
[i]SI, Westboro, Mass.
[j]Model 2360, Isco Inc, Lincoln, Neb.
[k]Model 2350, Isco Inc, Lincoln, Neb.
[l]Model V4, Isco Inc, Lincoln, Neb.
[m]Model SP 4290, Spectra-Physics, San Jose, Calif.
[n]Penomenex, Torrance, Calif.

References

1. Leaf AL, Schwartz WB, Relman AS. Oral administration of a potent carbonic anhydrase inhibitor ("Diamox") I. Changes in electrolyte and acid–base balance. *N Engl J Med* 1954;250: 759–763.

2. Spaeth GL. Potassium, acetazolamide, and intraocular pressure. *Arch Ophthalmol* 1967;78:578–582.

3. Trudell RG, Kaiser KK, Griggs RC. Acetazolamide responsive myotonia congenita. *Neurology* 1987;37:488–491.

4. Hoskins B. Studies on the mechanism of action of acetazolamide in the prophylaxis of hyperkalemic periodic paralysis. *Life Sci* 1977;20:343–349.

5. Reiss WG, Oles KS. Acetazolamide in the treatment of seizures. *Ann Pharmacother* 1996;30:514–519.

6. Larson EB, Roach RC, Schoene RB, et al. Acute mountain sickness and acetazolamide. Clinical efficacy and effect on ventilation. *JAMA* 1982;248:328–332.

7. Regnier A, Toutain PL. Ocular pharmacology and therapeutic modalities. In: Gelatt KN, ed. *Veterinary ophthalmology*. 2nd ed. Philadelphia: Lea & Febiger, 1991;162–194.

8. Gouge HE, Shor AL, Johnson WP. Control of udder edema in dairy cows: clinical evaluation of acetazolamide sodium. *Vet Med (Praha)* 1959;54:343–345.

9. Vestweber JGE, Al-Ani FK, Johnson DE. Udder edema in cattle: effects of diuretics (furosemide, hydrochlorothiazide, acetazolamide, and 50% dextrose) on serum and urine electrolytes. *Am J Vet Res* 1989;50:1323–1328.

10. Cox JH. An episodic weakness in four horses associated with intermittent serum hyperkalemia and the similarity of the disease to hyperkalemic periodic paralysis in man. *Proc Am Assoc Equine Pract* 1985;31:383–391.

11. Spier SJ, Carlson GP, Holliday TA, et al. Hyperkalemic periodic paralysis in horses. *J Am Vet Med Assoc* 1990;197:1009–1016.

12. Cox JH, DeBowes RM. Episodic weakness caused by hyperkalemic periodic paralysis in horses. *Compend Contin Educ Pract Vet* 1990;12:83–88.

13. Beech J, Lindborg S. Prophylactic efficacy of phenytoin, acetazolamide and hydrochlorothiazide in horses with hyperkalaemic periodic paralysis. *Res Vet Sci* 1995;59:95–101.

14. Guglick MA, MacAllister CG, Breazile JE. Laryngospasm, dysphagia, and emaciation associated with hyperkalemic periodic paralysis in a horse. *J Am Vet Med Assoc* 1996;209:115–117.

15. Carr EA, Spier SJ, Kortz GD, et al. Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. *J Am Vet Med Assoc* 1996;209:798–803.

16. Hossie RD, Mousseau N, Sved S, et al. Quantitation of acetazolamide in plasma by high performance liquid chromatography. *J Pharm Sci* 1980;69:348–349.

17. Hartley R, Lucock M, Becker M, et al. Solid-phase extraction of acetazolamide from biological fluids and subsequent analysis by high–performance liquid chromatography. *J Chromatogr* 1986;377:295–305.

18. Rose RJ, Hodgson DR, Kelso TB, et al. Effects of acetazolamide on metabolic and respiratory responses to exercise at maximal O_2 uptake. *J Appl Physiol* 1990;68:617–626.

19. Brown RD, Manno JE. ESTRIP, a BASIC computer program for obtaining initial polyexponential parameter estimates. *J Pharm Sci* 1978;67:1687–1691.

20. Bourne DW. BOOMER, a simulation and modeling program for pharmacokinetic and pharmacodynamic data analysis. *Comput Methods Programs Biomed* 1989;29:191–195.

21. Baggot JD. Principles of pharmacokinetics. In: *Priniciples of drug disposition in domestic animals. The basis of veterinary clinical pharmacology*. Philadelphia: WB Saunders Co, 1977;144–189.

22. Hanna WJ, Tsushima RG, Sah R, et al. The equine periodic paralysis Na^+ channel mutation alters molecular transitions between the open and inactivated states. *J Physiol* 1996;497:349–364.

23. Jackson EK. Diuretics. In: Hardman JG, Limbird LE, Molinoff PB, et al, eds. *Goodman & Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill Book Co, 1996; 691–695.

24. Haskins SC, Munger RJ, Helphrey MG, et al. Effect of acetazolamide on blood acid-base and electrolyte values in dogs. *J Am Vet Med Assoc* 1981;179:792–796.

25. Heller I, Halevy J, Cohen S, et al. Significant metabolic acidosis induced by acetazolamide. Not a rare complication. *Arch Intern Med* 1985;145:1815–1817.

26. Martinez MN. Use of pharmacokinetics in veterinary medicine. Article II: volume, clearance, and half-life. *J Am Vet Med Assoc* 1998;213:1122–1127.

27. Yakatan GJ, Frome EL, Leonard RG, et al. Bioavailability of acetazolamide tablets. *J Pharm Sci* 1978;67:252–256.

28. Friedland BR, Mallonee J, Anderson DR. Short-term dose response characteristics of acetazolamide in man. *Arch Ophthalmol* 1977;95:1809–1812.

29. Ritschel WA, Paulos C, Arancibia A, et al. Pharmacokinetics of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude. *J Clin Pharmacol* 1998;38:533–539.

30. Maren TH, Mayer E, Wadsworth BC. Carbonic anhydrase inhibition I: the pharmacology of Diamox 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide. *Bull Johns Hopkins Hosp* 1954;95:199–243.

31. Ellis PP, Price PK, Kelmenson R, et al. Effectiveness of generic acetazolamide. *Arch Ophthalmol* 1982;100:1920–1922.

32. Celebisoy N, Colakoglu Z, Akbaba Y, et al. Continuous muscle fibre activity: a case treated with acetazolamide. *J Neurol Neurosurg Psychiatry* 1998;64:256–258.

33. Vroom FW, Jarrell MA, Maren TH. Acetazolamide treatment of hypokalemic periodic paralysis. Probable mechanism of action. *Arch Neurol* 1975;32:385–392.