Effects of high altitude exposure on the pharmacokinetics of furosemide in healthy volunteers

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Abstract. Introduction: A cascade of pathophysiological events occurs with the ascension to high altitude (H). We have performed studies on the effects of exposure to H on the pharmacokinetics of drugs. The hypothesis behind these studies has been that the exposure to H, which produces marked physiological changes in the body, may alter pharmacokinetics, and consequently, pharmacodynamics. Our previous studies suggest that drugs highly bound to plasma proteins are most likely to exhibit altered disposition. Objective: In continuation of our research, we selected furosemide which is about 98% bound to plasma proteins, renally excreted and has low binding to red blood cells. Subjects, materials and methods: Furosemide (40 mg) was administered orally to 3 groups of young healthy volunteers. One group who had been residing at sea level (group L), the same group after 15 hours of exposure to high altitude (3,600 m, group HA) and a group of volunteers living at H for at least 6 months (group HC). Results: Our results are in accordance with the most recent pharmacokinetic studies on furosemide in which a terminal half-life of approximately 20 – 30 h was reported. Total proteins were 9.3% and 12.7% higher in groups HA and HC, respectively, than in group L. Albumin in group HC was 8.2% higher than group L. Bilirubin increased 17.7% and 41.2% in groups HA and HC, respectively, in comparison with group L. A rapid disposition rate constant in groups HA and HC was the only pharmacokinetic parameter that was significantly different from those in group L. Concentration of furosemide in plasma water increased significantly after H exposure, thus, the binding diminished from 97.2% in group L to 95.1% and 91.1% in groups HA and HC, respectively. Conclusion: Exposure to H produces an increase in the free fraction of furosemide in humans, which could be of therapeutic importance.

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Introduction

A cascade of pathophysiological events occurs with the ascension to high altitude (H) [Hackett and Roach 2001, Houston 1992, Sutton 1992]. The term "high-altitude illness" is used to describe the cerebral and pulmonary syndromes that can develop in unacclimatized persons after ascent to H. In both the brain and the lungs, hypoxia elicits neurohumoral and hemodynamic responses that result in overperfusion of microvascular beds, elevated hydrostatic capillary pressure, capillary leakage, and consequent edema [Hackett and Roach 2001]. The hypoxia stimulates erythropoietin resulting in an increased red blood cell (RBC) count, and hematocrit and fluid accumulate in the membrane of the alveoli preventing oxygen uptake into the blood. Increased ventilation decreases carbon dioxide levels and raises the blood pH and, as a consequence, the kidneys remove bicarbonate to stabilize the blood pH. Failure of the sodium pump at H results in loss of potassium, disturbances in the electrolyte balance, and thus edema. There is an increase in plasma protein concentration after reaching H [Surks 1966].

Although the problem of medical treatment [Hackett and Roach 2001, Harris et al. 1998] and the efficacy of drugs at high altitude have been studied [Albrecht and Albrecht 1969, Aslam and Kahn 1996], there are only a few reports dealing with pharmacological changes and hardly any on pharmacokinetics.

Our group has performed studies on the effects of exposure to high altitude on the pharmacokinetics of drugs. The hypothesis behind these studies has been that exposure

to H, which produces many physiological changes, may alter drug pharmacokinetics, and consequently, pharmacodynamics. The problem is relevant from a public health point of view, because an increasing number of people travel to high-altitude locations for sport, as tourists or for military and other reasons. Drug pharmacokinetic changes as a consequence of high altitude exposure could lead to modifications in dosage regimens in order to maintain efficacy or prevent toxicity.

Our previous pharmacokinetic studies with meperidine and acetazolamide at H, indicated altered drug disposition due to increased erythrocyte binding and decreased protein binding [Ritschel et al. 1996a,b, 1998a,b] and indicated that further pharmacokinetic research in this field with other drugs was required. Protein binding of both acetazolamide and meperidine was decreased after H exposure and this was particularly marked with meperidine. These findings suggest that H is most likely to exhibit altered drug disposition in those drugs which are highly protein bound and renally excreted. Furosemide inhibits the active reabsorption of chloride ion in the thick ascending limb of the loop of Henle by binding to one of the Cl^- binding sites of the $Na^+/2Cl^- K^+$ cotransport system. It is a potent loop diuretic used in the treatment of edematous states associated with cardiac, renal and hepatic failure, and for the treatment of hypertension. It is a weak acid with a pKa of 4.7 [Boles and Schoenwald 1990], is more than 80% ionized under physiological pH conditions [Ritschel and Kearns 1999] and about 98% protein-bound. For these reasons, we selected this drug to continue our research on the effect of H on the pharmacokinetics of drugs.

Subjects, materials and methods

Subjects and study design

The study was carried out in 3 groups of 12 healthy volunteers. The Institutional Review Board of the University of Chile, Santiago, Chile, and the Chilean Armed Forces approved the protocol. All of the volunteers were men recruited from the Chilean Army. Inclusion parameters included a minimum of 6 months of residence at sea level or H (3,600 m), completion of physical examination, urinalysis and blood chemistry tests. Residence of 6 months was considered to be sufficient for acclimatization to high altitude, which usually occurs over a period of a few days to a few months. Exclusion parameters included any results outside the established normal range for urine and blood chemistry tests, any previous gastrointestinal, hepatic, cardiovascular, pulmonary, or renal disease, previous severe mountain sickness and use of any drug in the 10 days preceding the study. Group L consisted of volunteers living at sea level (military base at Arica, the northernmost city in Chile). Group HA were the same subjects as group L, but after short-term exposure to high altitude 1 week after the first phase of the study. This involved traveling 160 km by bus to the military base at Putre in the Andes of North Chile which is at a height of 3,600 m. Subjects arrived in the afternoon and the study was performed the following morning. The other group HC comprised subjects who had resided for at least 6 months at the study site at Putre.

The mean (± SD) height of the subjects was 1.69 ± 0.08 m for groups L and H, and 1.71 ± 0.06 m for group HC. Body weight was 62.9 ± 8.5 kg for group L and H, and 67.3 ± 9.0 kg for group HC. All the volunteers were 19 years old.

For all groups, the treatment was the same: after an overnight fast with water allowed ad libitum, a dose of 40 mg of furosemide was given to each volunteer in a immediate release tablet (Lasix) along with 250 ml of water. Participants were given a standard breakfast 2 hours after dosing. Blood samples were collected via intravenous catheter or individual needle puncture at appropriate intervals over 72 hours.

An aliquot of each blood sample was lyzed for analysis of drug in whole blood. Heparinized blood was centrifuged and plasma separated. Half of the plasma samples obtained at 0.5, 1.5, 2, and 3 hours after administration were filtered (Micropartition System MPS-1, Amicon Division, Danvers, MA, USA) to obtain plasma water. The samples collected at sea level were immediately frozen after separation. At high altitude, samples were frozen and packed in dry ice for transportation to the laboratory for analysis.

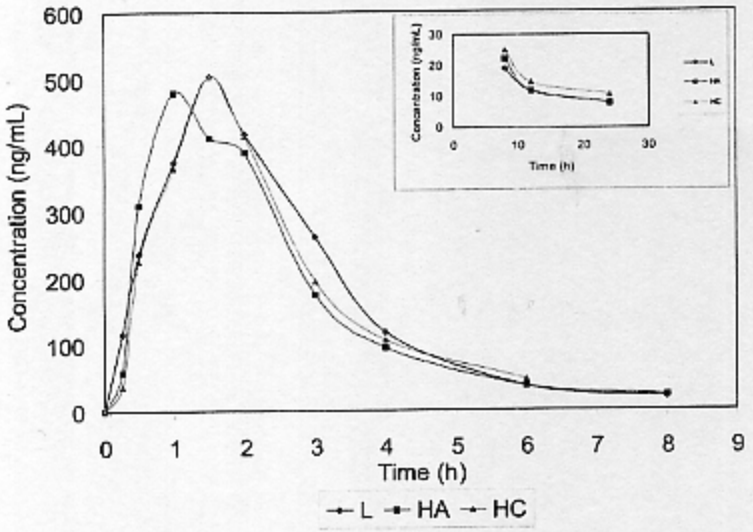

Figure 1. Mean whole blood concentration-time profiles for furosemide in healthy volunteers at low altitude (L), after short-term exposure to high altitude (HA), and after long-term exposure to high altitude (HC).

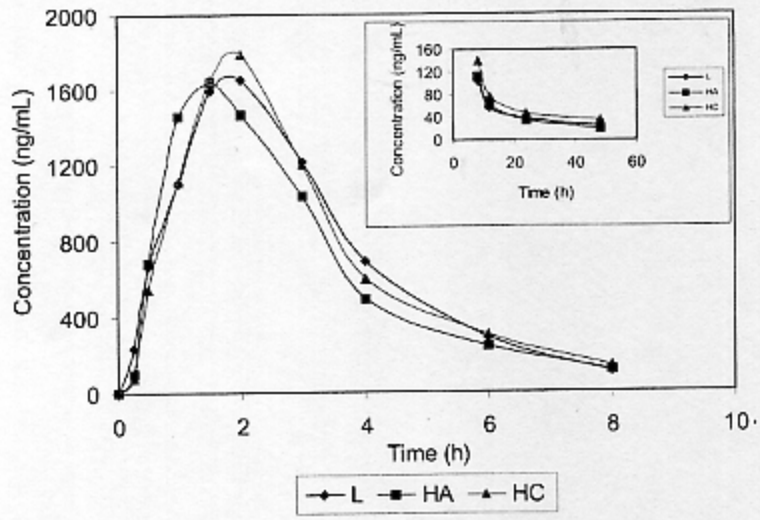

Figure 2. Mean plasma concentration-time profiles for furosemide in healthy volunteers at low altitude (L), after short-term exposure to high altitude (HA), and after long-term exposure to high altitude (HC).

Analytical procedure

All samples were analyzed for furosemide by a validated high-pressure liquid chromatography method using an L-6200 (Merck Hitachi) gradient pump, F-1080 (Merck Hitachi) fluorometric detector ($\lambda_{ex} = 345$, $\lambda_{em} = 405$), L-7350 (Merck Hitachi), oven column at 30 °C, and L-7250 (Merck Hitachi) autosampler. A C18 chromatographic column (Chromolith performance Rp 18e 100 – 4.6 mm) was used. The mobile phase was a lineal gradient of acetonitrile : orthophosphoric acid pH 2.1 = 5 : 95 (t = 0), 41 : 59 (t = 7 min).

Pharmacokinetic analysis

The concentration-time data for furosemide were analyzed using compartmental analysis and the RESID computer program [Ritschel 1975]. The extent of protein binding was determined by the following equation:

$$EPB = \frac{C_p - C_w}{C_p} \times 100$$

where C_p, and C_w are the concentrations in plasma and plasma water, respectively. Binding to erythrocytes was calculated according to the following equation [Ritschel 1992]:

$$C_E = \frac{C_b - C_p(1-H)}{C_b} \times 100$$

where C_E is binding to erythrocytes, and C_b and C_p are concentrations in blood and plasma, respectively, and H is the hematocrit.

Statistical analysis

Pharmacokinetic parameters were subjected to 1-way analysis of variance. A level of $p < 0.05$ was considered to be statistically significant.

Results

Both, blood clinical tests and urinalysis were within normal range for all volunteers. Hematocrit and hemoglobin were 10% and 11.3% higher, respectively, in the group HC in comparison with group L. The values for total proteins in groups HA and HC were 9.3% and 12.7% higher, respectively. For albumin, there was no difference between groups L and HA, but the values in HC group were 8.2% higher than in group L. The concentration of bilirubin increased 17.7% and 41.2% in groups HA and HC, respectively, in comparison with group L (Table 1).

Table 1. Blood parameters of healthy volunteers at low altitude (L), after short-term exposure to high altitude (HA) and after long-term exposure to high altitude (HC). The values in parenthesis are the SD.

	L	HA	HC
Hematocrit (%)	44.58 (2.46)	–	49.00 (1.59)
Hemoglobin (g/dl)	14.78 (0.97)	–	16.45 (0.57)
Total protein (g/dl)	7.17 (0.38)	7.84 (0.41)	8.08 (0.46)
Albumin (g/dl)	3.80 (0.54)	3.75 (0.22)	4.11 (0.31)
Bilirubin (mg/dl)	0.51 (0.21)	0.60 (0.19)	0.72 (0.27)

Whole blood and plasma

Figures 1 and 2 show the concentration-time profiles for furosemide in whole blood and plasma. Data were best fitted by a 2-compartment open model. Mean pharmacokinetic parameters in blood and plasma are listed in Table 2. The concentration-time profiles in whole blood in the 3 situations studied, L, HA and HC have similar shapes. The curve peaks earlier in group HA and the absorption rate constant is higher than in the other 2 cases, however, the differences are not statistically significant. The slow disposition half-life (h) was 18.47 ± 4.45, 16.09 ± 3.16, and 20.54 ± 11.44 in whole blood, and 19.41 ± 5.07, 16.30 ± 2.71, and 21.68 ± 11.56 in

Table 2. Pharmacokinetic parameters of furosemide in healthy volunteers in plasma and whole blood at low altitude (L), after short-term exposure to high altitude (HA) and after long-term exposure to high altitude (HC). The values in parenthesis are the SD.

	Plasma			Blood		
	L	HA	HC	L	HA	HC
λ_z (h^{-1})	0.0380 (0.0100)	0.0436 (0.0069)	0.0398 (0.0173)	0.0396 (0.0099)	0.0445 (0.0083)	0.0419 (0.0176)
α (h^{-1})	0.82 (0.26)	0.71 (0.27)	0.57 (0.16)	0.76 (0.24)	0.70 (0.20)	0.61 (0.17)
Ka (h^{-1})	1.69 (0.94)	1.70 (0.81)	1.31 (0.52)	1.28 (0.43)	1.70 (0.74)	1.35 (0.34)
$t_{1/2a}$ (h)	19.41 (5.07)	16.30 (2.71)	21.68 (11.56)	18.47 (4.45)	16.09 (3.16)	20.54 (11.44)
$t_{1/2\alpha}$ (h)	0.90 (0.20)	1.13 (0.47)	1.32 (0.52)	0.97 (0.22)	1.07 (0.29)	1.24 (0.45)
t1/2a (h)	0.55 (0.34)	0.49 (0.20)	0.61 (0.24)	0.60 (0.19)	0.46 (0.13)	0.55 (0.18)
t_{max} (h)	1.58 (0.64)	1.38 (0.57)	1.58 (0.79)	1.54 (0.66)	1.38 (0.57)	1.58 (0.79)
C_{max} (µmol/l)	3.93 (1.39)	3.54 (1.35)	3.70 (1.82)	2.29 (0.91)	1.89 (0.72)	1.89 (0.92)
AUC (µmol/l × h)	10.18 (2.39)	9.23 (1.91)	11.22 (4.52)	5.22 (1.26)	4.96 (0.99)	5.56 (2.35)
MRT (h)	10.65 (4.44)	9.62 (4.08)	13.89 (10.37)	9.47 (4.12)	8.61 (4.16)	12.97 (12.00)
Cl_{total}/F (l/h//kg)	0.18 (0.04)	0.20 (0.05)	0.19 (0.11)	0.39 (0.09)	0.41 (0.08)	0.42 (0.30)
Vd_{area}/F (l/kg)	5.05 (1.89)	4.60 (1.01)	5.18 (2.27)	10.44 (3.70)	9.28 (1.87)	10.50 (5.18)

Table 3. Furosemide bound to plasma protein in healthy volunteers at low altitude (L), after short-term exposure to high altitude (HA), and after long-term exposure to high altitude (HC). The values in parenthesis are the SD.

Time (h)	L (%)	HA (%)	HC (%)
0.5	97.57 (0.90)	94.96 (1.00)	90.41 (1.90)
1.5	97.56 (1.20)	95.17 (0.50)	91.70 (1.30)
2.0	97.26 (1.00)	95.34 (0.60)	91.79 (1.20)
3.0	96.39 (1.90)	94.81 (0.50)	90.12 (1.90)
Mean ± SD	97.20 (0.40)	95.07 (0.20)	91.00 (0.40)

Table 4. Pharmacokinetic parameters of furosemide in healthy volunteers in urine at low altitude (L), after short-term exposure to high altitude (HA), and after long-term exposure to high altitude (HC). The values in parenthesis are the SD.

	L	HA	HC
λ_z (h^{-1})	0.0398 (0.01)	0.0348 (0.01)	0.0344 (0.01)
$t_{1/2}$ (h)	18.21 (4.10)	21.51 (6.10)	21.75 (6.20)
Xu∞ (mg)	12.02 (0.50)	14.98 (0.50)	11.29 (0.30)
fu/F (%)	30.04 (3.20)	37.45 (3.30)	28.21 (1.70)

plasma for groups L, HA and HC, respectively. MRT tends to increase with chronic exposure to high altitude in both whole blood and plasma. The values for the L and HA groups were very similar (approximately 10 hours) and the values for group HC were 12.97 h and 13.89 h for whole blood and plasma, respectively, but the differences were not statistically significant. All volunteers exposed to H exhibited a rapid disposition constant measured both with whole blood and plasma. There were no statistically significant differences in the other pharmacokinetic parameters between the 3 groups studied and this was true for data on both whole blood and plasma.

Plasma water

Furosemide in plasma water increased after exposure to H (Table 3). There were significant differences between data obtained at sea level and those from the HA and HC groups at all time points measured. The concentration data were pooled to obtain a value for the protein binding of furosemide in the 3 situations studied (L, HA and HC) and these data showed a decrease in binding after exposure to H. The value of 97.2 ± 0.40% at sea level dropped to 95.07 ± 0.20% after short-term exposure and 91.00 ± 0.40% after long-term exposure. The differences were statistically significant.

Binding to erythrocytes

The binding to erythrocytes also diminished in the group exposed to H for at least 6 months. The binding was 5.4% in the group at the sea level and 0.70% in the group after chronic exposure to high altitude.

Urinary excretion

Table 4 contains the pharmacokinetic parameters calculated from urinary data. Excretion rate constants and $t_{1/2}$ were calculated from the slopes of the urinary excretion rate-versus time plots at the middle of the collection interval. The mean ± SD values of $t_{1/2}$ (h) were 18.21 ± 4.10, 21.51 ± 6.10 and 21.75 ± 6.20 for groups L, HA and HC, respectively. The differences are statistically not significant.

The cumulative amounts excreted uncorrected for bioavailability fu/F were 30.04 ± 3.20%, 37.45 ± 3.30% and 28.21 ± 1.70% for groups L, HA and HC, respectively. The difference between HA and the other 2 situations is statistically significant.

Discussion

The results of this study are in accordance with the most recent pharmacokinetic studies

of furosemide [Vree et al. 1995, Vree and van Der Ven 1999] in which a terminal half-life of approximately 20 – 30 h has been reported. During this slow elimination phase, only 4.6 ± 1.5% of the administered dose are excreted, which is 14% of the amount excreted during the first 15 h. The terminal half-life in our study was approximately of 20 h, both in plasma and whole blood. Volume of distribution, clearance and mean residence time are also in accord with this earlier study.

In the case of $t_{1/2}$, although the differences are not statistically significant, we noted a faster elimination in group HC compared with groups HA and L, both in plasma and whole blood. The volume of distribution, although not statistically significant, is decreased in group HA, which is consistent with the physiological findings of reduced plasma volume in subjects 24 hours after arrival at high altitude [Poulsen et al. 1999, Sawaka et al. 1996]. This reduction is stabilized with long-term exposure to high altitude and V_d tends to be approximately the same as at sea level.

Comparison of the fast disposition rate constant obtained in plasma in the 3 groups of this study shows that α decreases from 0.82 ± 0.26 h^{-1} to 0.71 ± 0.27 h^{-1} (–13%) after short-term exposure to high altitude and to 0.57 ± 0.16 h^{-1} (–30%) after chronic exposure to high altitude; this kind of decrease in α is also observed in whole blood. We also observed an increase in MRT when comparing chronic exposure to high altitude and at sea level. This increase, although not statistically significant, was 30.7% and 37.0% in plasma and whole blood, respectively. This increase in MRT after chronic exposure to high altitude is not consistent with the faster elimination half-lives noted and could be related to the rapid disposition rate constant on exposure to H.

The half-life of furosemide obtained from urine data in the 3 situations of this study (18.21 ± 4.10 h) was comparable to those obtained with plasma and whole blood in volunteers at sea level but this value increased significantly after exposure to H being 21.51 ± 6.10 h and 21.75 ± 6.20 h for groups HA and HC, respectively. The fraction excreted in the urine uncorrected for bioavailability was 24.7% higher in group HA than in the volunteers at sea level and 32.8% higher than in group HC.

The erythrocyte uptake of furosemide was 5.4% in the volunteers at sea level and this value decreased to 0.7% on chronic exposure at high altitude in spite of the fact that red blood count is known to be higher in subjects exposed to high altitude and that hematocrit was higher in group HC compared to the group at sea level. Erythrocyte experiments indicate some changes during the process of acclimatization to the altitude. An expansion of the erythrocyte volume has been described [Sanchez et al. 1970] which would be mediated by erythropoietin. A more recent study has shown that erythrocyte volume does not change during the first 13 days. However, the time course and magnitude of erythrocyte volume adaptation during altitude acclimatization is still not well understood. It is not known at the present time if these alterations in erythrocytes on exposure to altitude affect the binding of drugs in general or of furosemide in particular. Furosemide is highly bound to protein and almost exclusively to albumin [Boles and Schoenwald 1990]. Increases in plasma concentrations of total protein, thyroxine binding globulin, and ceruloplasmin and a depression in prealbumin have been detected during the first 2 weeks at high altitude [Surks 1966]. High altitude exposure caused an increase in the synthesis of albumin and fibrinogen in human volunteers [Imoberdorf et al. 2001]. In our study, we found a 9.3% and 12.7% increase in total proteins in groups HA and HC, respectively, and an increase of 8.2% in albumin in group HC compared to the group at sea level. Nevertheless, we found an increase in the concentrations in plasma water of furosemide in the volunteers exposed to altitude with the percentage bound to proteins equal to 97.2%, 95.1%, and 91.1% for groups L, HA and HC, respectively (Table 3). The free fraction remained relatively constant 0.5 h – 3.0 h after administration when the determinations were performed. Furosemide has been classified in the group of drugs susceptible to displacement from human serum albumin by bilirubin [Maruyama et al. 1984]. We observed higher concentrations of bilirubin as a consequence of exposure to altitude, this increase was 20% for group HA and 44% for group HC. The increases in bilirubin plasma concentration might explain in part the increase in free fraction of furosemide. From the pharmacokinetic parameters obtained in

this study, it is not possible to draw conclusions regarding changes in excretion or metabolism as a consequence of the exposure to high altitude. However, the finding that the free fraction of furosemide is increased could be of therapeutic importance since it could affect the intensity and duration of the effects.

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References

Albrecht E, Albrecht H 1969 Metabolism and hematology at high altitude and the effect of drugs on acclimatization. Fed Proc *28:* 1118-1123

Aslam M, Khan MZ 1996 The role of drugs in high altitude disorders. J Pakistan Med Assoc *46:* 90-92

Boles LL, Schoenwald RD 1990 Furosemide, a pharmacokinetic/pharmacodynamic review (part I). Clin Pharmacokinet *18:* 381-408

Hackett PH, Roach,RC 2001 High-altitude illness. N Engl J Med *345:* 107-114

Harris MD, Terrio JM, Miser WF, Yetter JF III 1998 High-altitude medicine. Am Fam Physician *57:* 1924-1926

Houston CS 1992 Mountain sickness. Sci Am *267:* 58-62

Imoberdorf R, Garlick PJ, McNurlan MA, Casella GA, Peheim E, Turgay M, Bartsch P, Ballmer PE 2001 Enhanced synthesis of albumin and fibrinogen at high altitude. J Appl Physiol *90:* 528-37

Jürgens G, Christensen HR, Brosen K, Somne J, Loft S, Olsen NV 2002 Acute hypoxia and cytochrome P450-mediated hepatic drug metabolism in humans. Clin Pharmacol Ther *71: 214-220*

Maruyama K, Harada S, Nishigori H, Iwatsuru M 1984 Classification of drugs on the basis of bilirubin-displacing effect on human serum albumin. Chem Pharm Bull (Tokyo) *32:* 2414-20

Poulsen TD, Klausen T, Richalet JP, Kunstrup I L, Fogh-Andersen N, Olse NV 1999 Plasma volume in acute hypoxia: comparison of a carbon monoxide rebreathing method and dye dilution with Evans' blue. Eur J Appl Physiol *77:* 457-461

Ritschel WA 1975 A curve fitting program to generate pharmacokinetic parameters. International Symposium Clinical Pharmacokinetics, Salzgitter Rigelheim, Germany, July. 20 21

Ritschel WA 1992 Binding of drugs to biological material. In: *Hamilton IL (ed)* Handbook of Basic Pharmacokinetics (4th ed). American Pharmacists Association, Washington, DC pp 138-155

Ritschel WA, Schapiro-Jacobson M, Akileswaran R, Wetzelsberger KM, Lücker PW 1995 Meperidine uptake and binding to human blood ex vivo. Methods Find Exp Clin Pharmacol *17:* 477-481

Ritschel WA, Paulos C, Arancibia A, Pezzani M, Agrawal BS, Wetzelsberger KM, Lücker PW 1996a Pharmacokinetics of meperidine in healthy volunteers after short- and long-term exposure to high altitude. J Clin Pharmacol *36:* 610-616

Ritschel WA, Paulos C, Arancibia A, Pezzani M, Agrawal MA, Wetzelsberger KM, Lücker PW 1996b Urinary excretion of meperidine and normeperidine in man upon acute and chronic exposure to high altitude. Methods Find Exp Clin Pharmacol *18:* 49-53

Ritschel WA, Paulos C, Arancibia A, Pezzani M, Agrawal MA, Wetzelsberger KM, Lücker PW 1998a Pharmacokinetics of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude. J Clin Pharmacol *38:* 533-539

Ritschel WA, Paulos C, Arancibia A, Pezzani M 1998b Urinary excretion of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude. Methods Find Exp Clin Pharmacol *20:* 133-137

Ritschel WA, Kearns G 1999 Handbook of Basic Pharmacokinetics Including Clinical Applications (5th ed). American Pharmaceutical Association, Washington, DC, pp 66-69, 129-132, 193-209

Sanchez C, Merino C, Figallo L 1970 Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude. J Appl Physiol *28:* 775-778

Sawka MN, Andrew JY, Rock PB, Lyon TP, Boushel R, Freund BJ, Muza SR, Cymerman A, Dennis RC, Pandolf KB, Valeri CR 1996 Altitude acclimatization and blood volume: effects of exogenous erythrocyte expansion. J Appl Physiol *81:* 639-642

Starks MI 1966 Metabolism of human serum albumin in man during acute exposure to high altitude (14,000 ft). J Clin Invest *45:* 1442-1451

Sutton JR 1992 Mountain sickness. Neurol Clin *10:* 1015-1030

Vree TB, Van Den Biggelaar-Martea M, Verwey Van Wissen V 1995 Furosemide and its acyl glucuronide show a short and a long phase in elimination kinetics and pharmacodynamic effect in man. J Pharm Pharmacol *47:* 964-969

Vree TB, Van Der Ven AJ 1999 Clinical consequences of the biphasic elimination kinetics for the diuretic effect of furosemide and its acyl glucoronide in humans. J Pharm Pharmacol *51:* 239-248