JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 1389–1398

Received March 19, 2004
Accepted July 16, 2004
DOI 10.1211/0022357044535
ISSN 0022-3573

A pharmacokinetic and pharmacodynamic study of desmopressin: evaluating sex differences and the effect of pre-treatment with piroxicam, and further validation of an indirect response model

Johanna Mercke Odeberg, Torbjörn Callréus, Stefan Lundin, E. Bodil Roth and Peter Höglund

Abstract

Desmopressin is a synthetic vasopressin analogue mainly used in treatment of diabetes insipidus and nocturia. Studies in rats have revealed a sex difference in the response to a vasopressin infusion, which was diminished after treatment with an NSAID. This study was performed in man to investigate the influence of sex and concomitant treatment of piroxicam on the pharmacokinetics and dynamics of desmopressin, and to validate a previously described indirect response model. Eight healthy males and eight healthy females participated in the trial, which was conducted in a pharmacokinetic (PK) part followed by a pharmacodynamic (PD) part. Desmopressin was administered intravenously as a single dose (PK = dose 2 μg, PD = dose 0.2 μg). Piroxicam was administered to achieve steady state. The pharmacokinetic parameters of desmopressin were estimated and calculated by means of two-compartmental analysis. In the dynamic part a study design based on an oral hydration model was used. Parameters for urine flow and urine osmolality were estimated. Individual estimates of the pharmacokinetic parameters served as input to the indirect response model that subsequently was fitted to urine osmolality data. The pharmacokinetics of desmopressin after a fixed bolus injection was neither influenced by piroxicam nor sex of the subject. The pharmacodynamics of desmopressin showed a sex difference where females exhibited a more pronounced antidiuretic effect than males, which was statistically significant when the effects were submaximal (>4.5 h after dose). The sex differences were diminished after pre-treatment with piroxicam, indicating a prostaglandin PGE_2-mediated mechanism. The indirect response model was confirmed, although the modelling could not distinguish a sex difference, indicating a limitation of this model compared with traditional descriptive statistics.

Department of Clinical and Experimental Pharmacology, University Hospital Lund, Sweden

Johanna Mercke Odeberg, Torbjörn Callréus, Stefan Lundin, Peter Höglund

Hospital Pharmacy, Malmö University Hospital, Malmö, Sweden

E. Bodil Roth

Correspondence: P. Höglund, Department of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden. E-mail: Peter.Hoglund@skane.se

Acknowledgement: We thank Birgitta Håkansson, Gertrud Lundkvist and Stina Ragnarsson, Department of Clinical Pharmacology, University Hospital of Lund, Sweden, for clinical assistance during the trial.

Introduction

Desmopressin, (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analogue of the antidiuretic hormone vasopressin, which is involved in the regulation of body fluid osmolality. The antidiuretic effect of vasopressin is mediated by its interaction with G-protein coupled receptors (V2 receptors) of the cells in the renal collecting duct, resulting in increased water permeability. Also urea and Na^+ transport into the renal medulla is enhanced, augmenting the antidiuretic effect. Desmopressin acts almost exclusively on these V2 receptors, with negligible effect on extrarenal vasopressin receptors (V1 receptors). Desmopressin is mainly used in the treatment of central diabetes insipidus and nocturnal enuresis, and its side effects are few. At much higher dosages desmopressin is also used in the treatment of bleeding disorders. The primary potential adverse effect associated with desmopressin is water intoxication (Robson et al 1996; Jackson 2001).

There are a number of examples of sex differences in drug pharmacokinetics and pharmacodynamics. With regard to pharmacodynamics, sex differences have been observed in baseline characteristics as well as in drug response, which might be related to modulation of sex hormones. As for the pharmacokinetics, differences in physiological factors between females and males, such as body weight, organ size, percent of body fat,

glomerular filtration rate and gastric motility, seem to be of relevance, and the physiological factors can be influenced by hormonal changes as well. Sex-related differences in phase I and phase II metabolism have also been shown (Harris et al 1995; Beierle et al 1999). The clinical significance of sex differences is not always known. Historically women have been under-represented in clinical drug trials. For desmopressin, a drug used for decades, it seems unclear whether there is any sex difference in either the kinetics or the dynamics.

Prostaglandins inhibit the antidiuretic action of vasopressin. Prostaglandin inhibitors, NSAIDs (nonsteroidal anti-inflammatory drugs) have been shown to enhance the antidiuretic response to desmopressin (Dusing et al 1981; Moses et al 1981; Jackson Roberts & Morrow 2001; Agnoli et al 2002). In rats a sex difference has been observed during treatment with vasopressin, but this difference was not seen after treatment with indometacin. Indometacin significantly enhanced the antidiuretic response in both sexes but the magnitude was greater in female than in male rats. One possible mechanism for the sex difference might be an estrogen stimulation of the biosynthesis of prostaglandins in the kidney (Wang et al 1997).

The primary aim of this study was to evaluate whether the pharmacokinetics and pharmacodynamics of desmopressin in healthy subjects are influenced by sex or by concomitant treatment with the NSAID piroxicam.

To investigate the pharmacodynamics a study design based on a hydration model was used (Vilhardt & Lundin 1986; Callréus & Höglund 1998). The subjects were orally overhydrated by water, and the drug was given intravenously. Intake of fluid increases excretion of water from the kidneys, and the endogenous vasopressin secretion is suppressed (Geelen et al 1996). The effect on diuresis will then be due to exogenous desmopressin only. In the data analysis an integration of pharmacokinetic and pharmacodynamic modelling using mathematical models was also performed. In a previous study we have shown that an indirect response (IDR) model can successfully be used to describe the dynamics of desmopressin. Desmopressin increases reabsorption of water leading to an increase in urine osmolality, and is described by an indirect response model (Callréus et al 1999). In our previous IDR study mentioned above, only one dose level (0.4 μg) was tested. However, to validate the model, testing with other dose levels is desirable.

The secondary aim of this study was therefore to confirm the estimates given in our previously described IDR model evaluated with 0.4 μg desmopressin, by testing a 50% lower dose level (0.2 μg).

Materials and Methods

The study was conducted in two parts, since the pharmacokinetics and pharmacodynamics cannot satisfactorily be studied simultaneously due to the need of different dose levels of desmopressin. The dose level chosen as suitable for the dynamic part of the study would not give high enough plasma concentrations for a satisfactory analysis and further pharmacokinetic calculations.

Subjects

Eight male and eight female subjects, aged 22–46 years (mean 29), participated in the study, which consisted of four separate trial days. BMI (body mass index) was 20–29 kg m^{-2} (mean 23) for females and 21–24 kg m^{-2} (mean 23) for males. Body weight was 50–78 kg (mean 66) for females and 65–87 kg (mean 78) for males. All subjects were considered healthy according to medical history, physical examination and laboratory investigation. None of the subjects smoked or used any medications suspected to interfere with the outcome of the study. The University Ethics Committee approved the study. Written informed consent was obtained from each subject, before his or her entry into the study.

Sample size

Mean values for AUC after a dose of 4 μg are reported to be 503 mg h mL^{-1} with a standard deviation of 46 (Callréus & Höglund 1998). To be able to detect a 20% difference in AUC with 80% power and 5% risk (two-tailed alpha), 4 subjects were needed in each group. Because of limited information regarding the variability for the dynamic parameters we doubled the number of subjects in each group. With a total of 16 subjects we had a 98% power to be able to detect a 20% difference of AUC.

Part A: the pharmacokinetics of desmopressin, with and without piroxicam pre-treatment, after a dose of 2 μg

Study design

An open crossover design, with randomised selection to two different treatment sequences: intravenous desmopressin with or without pre-treatment with piroxicam, was used to study the pharmacokinetics. Between each treatment there was a wash-out period of at least 14 days. Tea, coffee and alcohol were not allowed 24 h before a study day. The subjects arrived at the clinic around 0700 h. Breakfast was eaten at home. One intravenous indwelling catheter was inserted in each arm; one for obtaining blood samples, the other for the injection of desmopressin. The last dose of piroxicam was taken orally and then immediately followed by the administration of desmopressin. Blood sampling was performed according to a predetermined schedule. Lunch was served 3 h after dose. The study day lasted approximately 12 h after the desmopressin dose, and thereafter a fluid restriction prevailed: the subjects were instructed not to drink more than 0.5 L until 0800 h the next day.

Study drug

Desmopressin (Minirin, Ferring) was given as a rapid infusion of 2 μg (diluted to 10 mL with isotonic saline) over < 60 s. Piroxicam (Piroxicam NM Pharma, NM Pharma) was given orally once daily for four days until steady state. On day 1 a higher loading dose (20 mg) was given to faster reach steady state. On days 2, 3 and 4 the dose was 10 mg. The dose choices were relevant for the clinical use of both drugs.

Blood sampling

Samples for desmopressin analysis were obtained pre-dose and 5 min, 10 min, 15 min, 30 min, 50 min, 1.17 h, 1.83 h, 2.83 h, 4.17 h, 5.83 h, 7.33 h, 10 h and 12 h after the dose. Samples for piroxicam analysis were obtained before the first dose on day 1, pre-dose on the study day (day 4) and then after drug administration at 2 h, 5 h and 7.33 h.

Laboratory methods

Blood samples (7 mL) for desmopressin analysis were collected in EDTA vacuum tubes. The samples were always centrifuged within 45 min. Plasma was stored at −20°C until assay. Before analysis all samples were extracted with acetone and petroleum ether. Plasma desmopressin levels were measured by a radioimmunoassay technique, using a specific antiserum raised in rabbits as described by Lundin et al (1985). The lower limit of quantification of the assay was determined to be 1 pg desmopressin in 1 mL plasma, and the recovery was 60%. The intra- and interassay coefficients of variation at 10 pg mL^{-1} were 6.0% and 7.1%, respectively. Blood samples for piroxicam analysis were collected in 5-mL heparin vacuum tubes. The samples were centrifuged within 1.25 h (20°C, 1000 *g*). Plasma was stored at −20°C until HPLC assay. To 0.25 mL plasma, 0.25 mL H_3PO_4, 50 μL internal standard (30 μg mL^{-1} tenoxicam in methanol) and 5.0 mL ethylacetate was added. Calibration samples, 0.8–6.4 μg mL^{-1}, were prepared in blank plasma from a stock solution of 10 or 20 μg mL^{-1} in methanol. The calibration samples were analysed in duplicates. The samples underwent mixing for 30 s followed by centrifugation (10 min, 1000 *g*). The organic phase was transferred to a new glass tube and evaporated to dryness with nitrogen. The residue was taken up into 50 μL mobile phase, and 20 μL was injected on the HPLC. The HPLC system used was an LDC/Milton Roy constraMetric III connected to an LDC/Spectra Monitor III UV-detector working at 315 nm. The analytes were separated on a LiChrosorb RP-18 (7 μm) 250 × 4 mm column. The mobile phase comprised of 60% phosphate buffer (0.4 M, pH 7.5) and 40% MeOH, and the flow rate was 1.0 mL min^{-1}.

Calculations and statistics

The pharmacokinetics of desmopressin can be described by a two-compartmental method (Fjellestad-Paulsen et al 1993; Callréus & Höglund 1998; Callréus et al 1999). The pharmacokinetic parameters, area under the curve (AUC), clearance (CL) and volume of distribution at steady state (V_{ss}) and central (V_c) (which were all normalised to the body weight of the subjects), half-life ($t_{1/2}^{\lambda}$) and rate constants k_{10}, k_{12} and k_{21}, were estimated and calculated by means of two-compartmental analysis, weighted by reciprocals of predicted values, with WinNonlin Professional version 3.3 (Pharsight corporation., USA). V_{ss} was used ($V_{ss} = D \times AUMC/(AUC^2)$) instead of V_β ($V_\beta = CL/\beta$), because it is independent of drug elimination and considered the most useful term to describe the apparent distribution space in a multicompartment system (Gilbaldi & Perrier 1982). The pharmacokinetic parameter, area under the curve at steady state $AUC_{(ss)}$ based on blood concentrations of piroxicam, was estimated and calculated by means of non-compartmental analysis, in the same version of WinNonlin. We have assumed that AUC has a normal distribution when log transformed.

Means standard deviations are used for descriptive statistics. For statistical analysis the MIXED procedure in SAS (version 8.2; SAS Institute, Cary, NC) was used. Two sets of analyses were performed: one for the modelling parameters and one for pharmacodynamics. The statistical model for the modelling parameters comprised sex, pre-treatment and interaction between pre-treatment and sex as fixed effects and subjects within sex as random effect. Least square means and 95% confidence intervals were calculated. Contrasts between sex, pre-treatment and interactions were formed as appropriate. The statistical model for pharmacodynamics comprised the trichotomised estradiol levels and male sex as a four level factor and interaction with pre-treatment as fixed effects and subject within sex as random effect. Least square means and 95% confidence intervals were calculated. Contrasts between sex, pre-treatment, different estradiol levels and interactions were formed as appropriate.

Paired *t*-test has been used for serum sodium (S-Na), potassium (S-K) and osmolality (S-Osm) with 95% confidence interval (CI), and performed in SPSS, version 11.0. The level of significance was set to $P = 0.05$ in all the statistical analyses.

Part B: pharmacodynamics of desmopressin with and without piroxicam pre-treatment after a dose of 0.2 μg

Study design

An open randomised two-period incomplete crossover design, with desmopressin intravenously with or without pre-treatment with piroxicam, was used to study the pharmacodynamics. The randomisation of females was also dependent on if they were low or high in estrogen, based on information of last day of their period. Between each treatment there was at least a washout period of fourteen days. The subjects arrived at the clinic around 0700 h. One intravenous indwelling catheter was inserted in each arm, as in part A. Thereafter a standardised breakfast and the last dose of piroxicam was given, and then blood samples for piroxicam analysis were drawn according to a special schedule. At the same time the hydration procedure was started.

Hydration procedure

To achieve over-hydration and a steady-state diuresis, the subjects drank a volume of tap water corresponding to 15 mL kg^{-1} body weight. The hydration procedure started approximately 2 h before the injection of desmopressin. Every 20 min during the whole study day, urine was voided and the volume was measured. To ensure continuous over-hydration, the subjects replaced their fluid loss with a volume of tap water equivalent to urine volume plus 10 mL extra (compensation for extra renal water loss). After 2 h, the steady state of diuresis was achieved and desmopressin was given as a bolus dose. Lunch was served 3 h after the dose. Samples for urine electrolytes, as

well as serum electrolytes, were collected according to a predetermined schedule. The study day lasted until approximately 8 h after the desmopressin dose, followed by the same fluid restrictions as in part A.

Study drugs

Desmopressin was given as a rapid injection of 0.2 μg (1 mL) over < 30 s. The same dosing as in part A was used for piroxicam.

Blood and urine sampling

Samples for analysis of S-Na, S-K and S-Osm (gel vacuum tubes 5 mL) were obtained −2 h and −10 min before desmopressin dose, and then 10 min, 1 h, 2.5 h, 4.33 h, 5.67 h and 7.17 h after dose. Samples for analysis of serum estradiol (S-estradiol) in females (gel vacuum tubes 5 mL) were obtained at the start of hydration (2 h before dose). Urine volumes were collected every 20 min starting at T − 2 h (T = time for desmopressin dose), and the volume was registered and the volumes were saved for pooling. Samples for analysis of urine sodium (U-Na), potassium (U-K) and osmolality (U-Osm) were obtained from urine volumes collected at intervals from 20 min to 1 h. If the intervals were longer than 20 min, the urine was pooled. The urine samples were taken 2 h pre-dose desmopressin and then 20 min, 40 min, 1.33 h, 2 h, 2.67 h, 3.33 h, 4 h, 4.67 h, 5.67 h and 6.67 h after the desmopressin dose. Piroxicam — see part A.

Laboratory analysis

Serum and urine samples were routinely analysed daily at the Clinical Chemistry lab., University Hospital, Lund, Sweden. Piroxicam – see part A.

Data analysis

For each individual urine flow at steady state, U-fl_{ss} (mean urine volume of portions ending at T = −20 and T = 0, expressed as mL min^{-1}), U-fl_{min} (mean value of the urine portions at time points 90–170 min), urine flow reduction in % ($100 \times (1 - (U\text{-}fl_{min}/U\text{-}fl_{ss})$) was calculated. RO (urine osmolality at baseline before dose), U-Osm_{max} (the maximum urine osmolality) and T_{max} (time for U-Osm_{max}) were estimated from the urine osmolality/time curve.

For urine osmolality the area under the curve (AUC_{osm}) was estimated at different intervals of the dose effect; 0–4 h (from start and during max. effect of drug), 0–6.67 h (the whole interval of measured drug effect), 1.33–2.67 h (max. effect of drug), 4–6.67 h (decreasing effect). For piroxicam, the same parameters as in part A were calculated. The estradiol values were categorised into three groups: low (< 200) (mean 141, s.d. 39), intermediate (201–350) (mean 258, s.d. 50) and high (351 <) (mean 542, s.d. 197). For the electrolytes and osmolality the intention was to calculate clearance.

IDR (indirect response) model

Physiological processes involved in the production or the elimination of a response can be either inhibited or stimulated by a drug. To describe these processes, four IDR models have been developed (Dayneka et al 1993). In this study, the measured response variable is the urine osmolality (mOsm kg^{-1}), which in the calculations has been equated to urine osmolarity (mOsm L^{-1}). The rate of change of response with no drug can be described by $dR/dt = k_{in} - k_{out} \times R$, where k_{in} represents the zero-order constant (mOsm L^{-1} h^{-1}) for production of the response, k_{out} defines the first-order rate constant (h^{-1}) for elimination of response and R is the response variable representing urine osmolarity (mOsm L^{-1}). According to this basic indirect response model, a drug-induced increase in response may be produced by either a stimulation of k_{in} or an inhibition of k_{out} by the drug. Desmopressin inhibits the urine flow rate by increasing the rate of reabsorption of water from the distal part of the renal tubular system. Based on the mechanism of action, desmopressin is considered to inhibit k_{out} in the IDR model according to an inhibition function I(C): $I(C) = 1 - (I_{max} \times C^{\gamma}/IC50^{\gamma} + C^{\gamma})$, where C is the concentration of desmopressin in plasma, I_{max} represents the maximum effect attributed to desmopressin ($0 < I_{max} \leq 1$) regardless of dose, IC50 represents the concentration producing 50% of I_{max} and γ is the sigmoidicity factor. Maximum inhibition is obtained when C >> IC50 causing I(C) to approach $1 - I_{max}$. The rate of change of response in the presence of desmopressin (drug) can then be described by: $dR/dt = k_{in} - k_{out} \times I(C) \times R$. A more detailed explanation for the model of choice for desmopressin is given in a previous paper (Callréus et al 1999). The individual estimates of the pharmacokinetic parameters V_c, k_{10}, k_{12} and k_{21} from part A served as input to the IDR model that subsequently were fitted to urine osmolarity data. Also in this second step, individual analysis was performed for each subject and each study day. The analyses were performed in WinNonlin Professional version 3.3 (Pharsight corporation, USA); the IDR model was written in an ASCII file. Weighting was chosen for the predicted data (to the power of 1). From the modelling procedure we obtained the estimates for I_{max}, IC50, γ, k_{in} and k_{out}. Statistical analyses were performed comparing the estimates between sex, piroxicam treatment and interaction sex/piroxicam treatment, and if any impact of estradiol, followed by a simulation of the estimates with different doses to see if the simulation provided a realistic fit. The estimates were then compared with the estimates (s.d.) from our previous study; $I_{max} = 0.8$ (0.09), $IC50 = 3.7$ (1.2) pg mL^{-1}, $\gamma = 13$ (3.5), $k_{in} = 1440$ (399) mOsm L^{-1} h^{-1} and $k_{out} = 8.39$ (3.7) h^{-1}, which were also used in simulations. In both simulation sets, the pharmacokinetics from this study served as input.

Results

Safety

All subjects completed both parts of the study. One subject had to finish the second trial day in part B after 5 h, due to fever. The most frequently reported adverse event was headache, and it was less frequent in part B, where the lower dose was administered. A total of 12 occasions of headache were reported, similarly distributed between the occasions with piroxicam treatment and without. One subject experienced fever and influenza-like symptoms during treatment with piroxicam in both part A and B, which was judged as a possible related adverse event. The

ongoing planned piroxicam treatment in B (second visit) was stopped, and the subject completed the study.

Part A

Pharmacokinetics of desmopressin

In Figure 1, the plasma concentration over time is shown graphically. The inter- and intra-individual variability was low irrespective of sex or pre-treatment with piroxicam. The concentrations over time were compatible with the proposed two-compartment model for the disposition of desmopressin. The pharmacokinetic parameters AUC, V_{ss}, CL, $t_{1/2}$, V_c, k_{10}, k_{12}, and k_{21} were subsequently estimated (Table 1) and the results analysed statistically. No differences were detected between sex, treatment with piroxicam or interaction of sex and treatment with piroxicam regarding the pharmacokinetic parameters of desmopressin. On two occasions the desmopressin was apparently partly administered subcutaneously and these data were excluded from the analysis of pharmacokinetics. On one occasion, for one subject, it was not possible to fit the data with a two-compartment model and the data was therefore excluded from this model analysis.

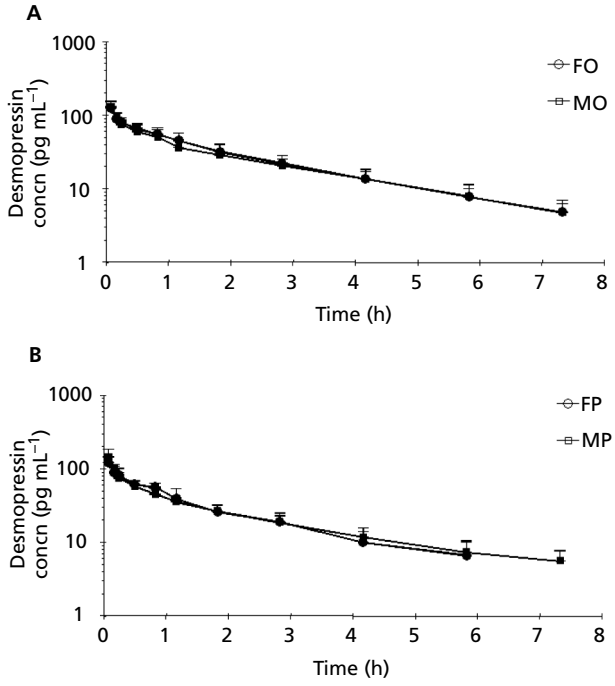

Figure 1 Desmopressin plasma concentration over time in females (FO) and males (MO) without pre-treatment (A) and in females (FP) and males (MP) pre-treated with piroxicam (B). Data are means ± s.d., n = 8.

Pharmacokinetics of piroxicam

There was no sex differences regarding the pharmacokinetics of piroxicam after a fixed dose: Female AUC_{ss}/Male $AUC_{ss} = 1.2$, 95% CI (0.9–1.5). Neither was there any difference when comparing the estimates of AUC_{ss} from part A, with estimates of AUC_{ss} for the subjects treated in part B (data not shown).

Part B

Urine volume and urine osmolality

Females and males were initially hydrated with (mean (s.d.)) 975 (131) mL and 1174 (105) mL, respectively. The data from the second visit of one subject was excluded from the analysis due to incomplete participation in the trial procedures. At the end of the hydration period, urine flow at steady state ($U\text{-}fl_{ss}$) was achieved and the high urine flow rate and low baseline osmolality (R_0) indicates over-hydration. After the administration of the desmopressin dose, the urine flow ($U\text{-}fl_{min}$) was quickly reduced to very low levels (mean value 1–1.1 mL min^{-1} for females and 1.7–1.8 mL min^{-1} for males), and the urine osmolality was thereby increased: maximum mean values in the range 746–782 mOsm kg^{-1} for females and 656–663 mOsm kg^{-1} for males (Table 2, Figures 2A and 2B). The maximal reduction of urine flow was approximately 90% in each group.

Table 1 Mean values and standard deviations of the pharmacokinetic parameters of desmopressin

	AUC (pg h mL^{-1})	V_{ss} (mL kg^{-1})	CL (mL h^{-1} kg^{-1})	$t_{1/2}$ (h)	V_c (mL kg^{-1})	k_{10} (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})
FO	209 ± 46	420 ± 88	155 ± 39	2.1 ± 0.8	195 ± 67	0.9 ± 0.5	4.0 ± 2.7	3.1 ± 1.9
MO	185 ± 34	365 ± 109	145 ± 57	1.9 ± 0.3	145 ± 57	1.1 ± 0.4	4.7 ± 3.3	2.6 ± 0.9
FP	197 ± 71	371 ± 101	172 ± 58	1.8 ± 0.8	183 ± 65	1.1 ± 0.5	5.2 ± 4.7	3.9 ± 2.9
MP	186 ± 23	377 ± 148	140 ± 21	2.2 ± 0.7	130 ± 84	1.4 ± 0.8	5.2 ± 4.3	1.9 ± 0.9
Female	204 ± 56	397 ± 94	163 ± 47	2.0 ± 0.8	190 ± 63	1.0 ± 0.5	4.5 ± 3.5	3.4 ± 2.3
Male	185 ± 28	371 ± 126	142 ± 26	2.1 ± 0.5	137 ± 70	1.3 ± 0.6	5.0 ± 3.7	2.3 ± 0.9
P	191 ± 47	374 ± 125	154 ± 42	2.0 ± 0.7	151 ± 79	1.3 ± 0.7	5.2 ± 4.3	2.7 ± 2.1
O	196 ± 41	391 ± 101	149 ± 35	2.1 ± 0.6	168 ± 65	1.0 ± 0.4	4.4 ± 2.9	2.8 ± 1.4
All	193 ± 43	383 ± 111	152 ± 38	2.0 ± 0.7	160 ± 71	1.2 ± 0.6	4.8 ± 3.6	2.8 ± 1.7

The kinetic parameters were calculated using a two compartmental model. F = female, M = male, O = without pre-treatment, P = with piroxicam treatment.

Table 2 Mean values and standard deviations for R_0 (baseline before dose), U-fl_{ss} (urine flow at steady state), U-Osm_{max} (maximum urine osmolality), T_{max} (time to reach U-Osm_{max}), U-fl_{min} (minimal urine flow) and U-$flow_{Maxred}$ (maximum reduction of urine flow)

Sex and treatment	R_0 (mOsm kg^{-1})	U-fl_{ss} (mL min^{-1})	U-Osm_{max} (mOsm kg^{-1})	T_{max} (h)	U-fl_{min} (mL min^{-1})	U-$flow_{maxred}$ (%)
FO	61.3 ± 10.3*	13.2 ± 2.8	774.5 ± 215.7	2.4 ± 1.1*	1.0 ± 0.9	92.3 ± 5.3
MO	75.3 ± 16.8	15.9 ± 4.5	632.8 ± 86.9	1.2 ± 0.6	1.8 ± 0.7	87.0 ± 7.1
FP	69.0 ± 6.2	11.9 ± 3.6	781.5 ± 213.8	2.3 ± 1.0	1.1 ± 0.5	91.1 ± 2.6
MP	63.4 ± 8.7	16.1 ± 4.8	655.5 ± 214.0	2.0 ± 0.6*	1.7 ± 1.0	90.3 ± 3.3

FO, MO = female, male without pre-treatment; FP, MP = female, male with piroxicam treatment. *$P < 0.05$ compared with MO in respective column.

There was no statistical difference detected between sex, treatment with piroxicam or interaction between sex and treatment with piroxicam for these parameters, apart from R_0 and time to reach maximal urine osmolality (T_{max}). R_0 was higher in males without pre-treatment (MO) compared with females without (FO), and T_{max} was shorter in MO compared with the other groups (Table 2).

A trend could be observed graphically (Figures 2A and 2B) for urine flow and urine osmolality for a higher antidiuretic effect in females compared with males when desmopressin expressed its maximum effect (0–4 h). The sex difference was statistically significant when the antidiuretic effect of desmopressin started to decrease from 4.5 h onwards, until the last time of measurement (Figure 2A). The sex difference was dependent on the category females without pre-treatment (FO) compared with males without pre-treatment (MO), but the sex difference was absent when both sexes were pre-treated with piroxicam (MP and FP). Concomitant piroxicam treatment seemed to mainly increase the diuresis in females but somewhat decrease it in males. Females without pre-treatment had the slowest recovery. A similar pattern was seen for the urine osmolality. The area under the curve for urine osmolality at different time intervals revealed no differences for $AUC_{(0–4\,h)}$, $AUC_{(0–6.67h)}$ or $AUC_{(1.33–2.67\,h)}$ (data not shown). When the desmopressin effect was diminishing, in the interval 4–6.67 h, there was a sex difference without pre-treatment (FO versus MO, $P < 0.05$), but not for females and males when they were pre-treated. S-estradiol, as a covariate, did not reach any statistically significant influence on any of the urine parameters.

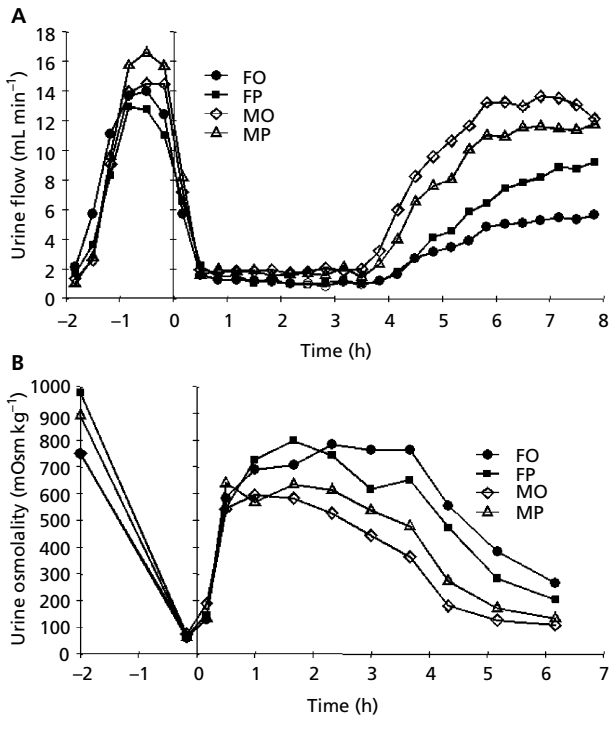

Figure 2 Urine flow (A) and urine osmolality (in each voiding) (B) over time for the four groups of subjects. F = female, M = male, O = without, P = with piroxicam. Time is given as the midpoint for the urine sampling period. Data are means (s.d. not shown for clarity), n = 8.

Serum electrolytes and osmolality

S-Na, S-Osm and S-K were all affected by a low dose of desmopressin in over-hydrated subjects. After the bolus injection of desmopressin there was a significant decrease during the first 2.5 h for S-Na and S-Osm (Table 3). The lower mean values for females were below or at the limit of the reference interval for S-Osm and S-Na, respectively. The next time point for measurements was at 4 h and by then the electrolytes had started to increase slightly again, but there was still a significant difference from baseline at the end of the trial day (data not shown). S-K in the hydrated subjects showed a different pattern (data not shown), with an immediately rise ($P < 0.05$) just after

Table 3 S-Na and S-Osm before and after (2.5 h) desmopressin

Parameter	Before dose	After 2.5 h
S-Osm (mOsm kg^{-1})	F = 288.4 ± 3.4	F = 278.1 ± 3.4*
	M = 293.3 ± 4.1	M = 285.0 ± 4.0*
S-Na (mmol L^{-1})	F = 138.1 ± 1.5	F = 133.1 ± 1.9*
	M = 138.8 ± 1.5	M = 135.1 ± 1.7*

*$P < 0.05$, paired sample *t*-test. F = female, M = male.

the dose, followed by a decrease ($P < 0.05$) observed at 2.5 h, which was similar to the other electrolytes, but the mean values for S-K at the different time points were all within the reference interval for S-K. The variance analysis revealed a sex difference ($P < 0.05$) for S-Osm, but not for the two electrolytes. Piroxicam did not seem to affect the electrolytes.

Urine electrolytes

For the electrolytes and osmoles our intention was to calculate clearances and excretion rates. In a previous study with heart failure patients (Juhlin et al 2004), the serum-electrolytes and osmolality did not change after hydration, and therefore we had a less frequent sampling schedule in this study. Because of the changes in serum electrolytes observed in this study clearance calculations were not possible, using the sparse data on serum electrolytes or osmolality. The urine sodium excretion is expressed graphically in Figure 3. After 4 h there was a trend for a sex difference, but piroxicam did not seem to have any influence on Na excretion in either sex.

IDR model

Predicted urine osmolarity using the IDR model was in accordance with observed urine osmolality for the subjects, as illustrated by one representative subject (Figure 4A).

The results estimated for desmopressin were $I_{max} = 0.95$ (0.04), $IC50 = 0.72$ (0.5) pg mL^{-1}, $\gamma = 2.9$ (2.3), $k_{in} = 702$ (239) mOsm L^{-1} h^{-1} and $k_{out} = 11.4$ (4.7) h^{-1}. There were no statistically significant differences between sexes nor was there any influence of estradiol or piroxicam, therefore the mean values were given for all the subjects. When comparing the estimates with the results in our previous study, we found statistically significant differences for all parameters except k_{out}. When simulating data using different dose levels and the two different sets of estimates (from the previous study and this study, respectively), the curve patterns differed (Figures 4B and 4C). The simulated curve from the previous study (Figure 4C) gave a poorer fitting, especially since it predicted a much shorter duration of the effect than actually observed; after 0.2 μg the time duration in this study was approximately 4 h (Figures 2A and 2B) and after 0.4 μg (the previous study) the time duration was approx 5–6 h (Callréus et al 1999).

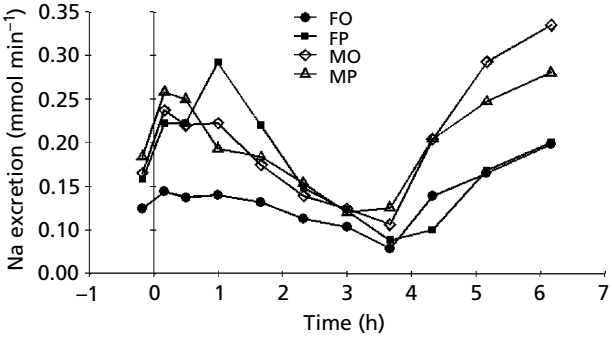

Figure 3 Sodium excretion over time for the four groups of subjects. F = female, M = male, O = without, P = with piroxicam. Data are means (s.d. not shown for clarity), n = 8.

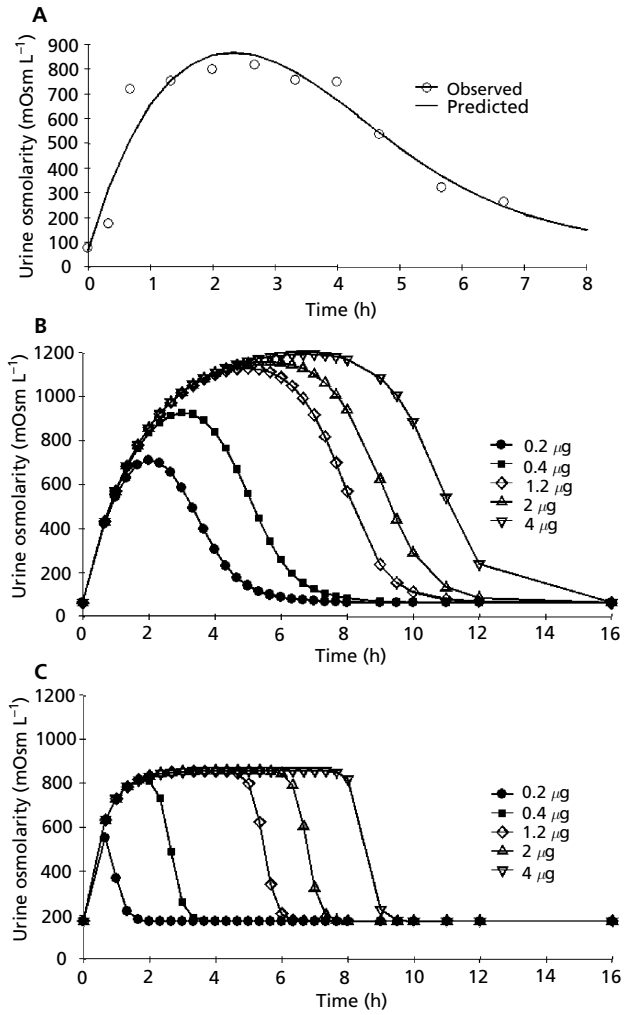

Figure 4 A. The observed and predicted values of osmolarity after using the IDR model for one representative subject (subject no 1). B, C. Simulated curves from the indirect response model with different dose levels of desmopressin (range 0.2–4 μg). B. Simulated curves with the estimates from this study: $I_{max} = 0.95$ (0.04); $IC50 = 0.72$ (0.5) pg mL^{-1}; $\gamma = 2.9$ (2.3); $k_{in} = 702$ (239) mOsm L^{-1} h^{-1} and $k_{out} = 11.4$ (4.7) h^{-1}. C. Simulated curves with the estimates from the previous study (Callréus et al 1999): $I_{max} = 0.8$ (0.09); $IC50 = 3.7$ (1.2) pg mL^{-1}, $\gamma = 13$ (3.5); $k_{in} = 1440$ (399) mOsm L^{-1} h^{-1} and $k_{out} = 8.39$ (3.7) h^{-1}.

Discussion

Pharmacokinetics

In our study there was no evidence for a pharmacokinetic interaction between piroxicam and desmopressin. Piroxicam is completely absorbed after oral administration and is extensively (99%) bound to plasma proteins (Jackson Roberts & Morrow 2001). It is not clear whether desmopressin is bound to plasma protein, but according to Dollery (1999) it seems

unlikely since there is little or no protein binding of endogenous vasopressin. A displacement interaction is therefore not likely to occur.

Piroxicam is extensively metabolised in man, predominately by an iso-enzyme of the CYP2C family, with only 5–10% excreted unchanged in the urine (Jackson Roberts & Morrow 2001; Olkkola et al 1994). Desmopressin has been reported to be excreted mainly unchanged in the urine (Fjellestad-Paulsen et al 1993).

The estimates we found for the pharmacokinetics of desmopressin (Table 1) are in accordance with previous findings, both in euhydrated and over-hydrated subjects. In a previous study we found that, after an intravenous dose of 4 μg in over-hydrated subjects (15 mL kg^{-1}), the mean values of area under the curve (AUC), half-life ($t_{1/2}$), clearance (CL) and volume of steady state (V_{ss}) for desmopressin were 502 ± 16 (s.e.m.) pg h mL^{-1}, 2.97 ± 0.24 h, 1.77 ± 0.10 mL min^{-1} kg^{-1} and 373 ± 30 mL kg^{-1}, respectively (Callréus & Höglund 1998). Oral over-hydration in combination with the effect of an antidiuretic drug induces an increase in plasma volume. The similarities of the parameters show that over-hydration of the subjects does not seem to have an important influence on pharmacokinetic parameters (e.g. the volume of distribution) that could tentatively be most influenced by over-hydration. This was also the finding in our earlier study where we compared the pharmacokinetics after different levels of over-hydration (Callréus et al 1999). Comparing AUCs after the two different doses (2 μg and 4 μg) also confirms linearity of desmopressin. In another study, after an intravenous dose of 2 μg, the half-life was reported to be 1.3 h and the clearance 1.71 mL min^{-1} kg^{-1} (Fjellestad-Paulsen et al 1993). However, in our previous study the pharmacokinetics were calculated after a very low dose of desmopressin (0.4 μg), and the terminal half-life of elimination was prolonged (range 2.76–8.37 h, mean 4.36 h), and the clearance was lower (CL = 1.34 (s.d. 0.35) mL min^{-1} kg^{-1}). At low doses the method of analysis has been shown to have limitations, and therefore the actual estimates for the pharmacokinetic parameters calculated after a 0.4-μg dose should be interpreted with caution and not be seen as representative. We did not see any sex differences regarding the pharmacokinetics for the two drugs. According to the product information supplied by Ferring AB, there is no sex difference regarding the kinetics of desmopressin, although there is no published data available. In a study by Richardson et al (1985) investigating age and sex influence on piroxicam disposition, higher concentrations were seen in women than in men after a single dose, although the differences in volume of distribution and clearance were eliminated by adjusting the parameters for weight. However, the predicted steady-state concentrations were similar between young females and males. Since the doses were fixed, we could have expected sex differences due to difference in body size.

Pharmacodynamics

Urine flow and urine osmolality

In our study we could observe a sex difference for the urine flow and urine osmolality after a bolus injection of desmopressin. Females exhibited a more pronounced antidiuretic effect with desmopressin. The sex difference was most obvious when the effect of desmopressin became submaximal after 4 h, and only then statistically significant.

The sex differences were not seen when females and males had been pre-treated with piroxicam for 4 days, which indicates that the difference might be mediated by prostaglandin-dependent mechanisms. In our study piroxicam pre-treatment did not influence sodium excretion in either males or females, indicating that the putative prostaglandin-dependent sex difference was mediated by differences in tubular cell sensitivity to desmopressin. In animals, as well as in man, cyclooxygenase inhibition has been shown to affect the formation of renal tubular aquaporines (u-AQP2), which mediates the desmopressin water retention (Pedersen et al 2001).

It has been shown in rats that the renal prostaglandin (PGE2) biosynthesis is significantly enhanced by estradiol treatment (Chang 1988), and Wang et al (1997) demonstrated a sex difference in antidiuretic response to vasopressin in rats, which was diminished by indometacin. Indometacin significantly enhanced the antidiuretic response in both sexes but the magnitude was greater in female than in male rats (Wang et al 1997). This pattern was not seen in our study. The reasons for the diminished sex difference after piroxicam treatment in our study are difficult to clarify since there was no statistically significant difference between the sexes (FO and FP vs MO and MP). From a graphical point of view (Figure 2A) there can be three possible explanations: a decreased diuresis in males; an increased diuresis in females; or a combination of the two alternatives.

As it has been emphasized in a recent review by Breyer & Breyer (2000), the renal effects of prostaglandins shows a complex picture. PGE_2 has dual opposing effects on several processes such as vascular tone, water and sodium absorption. Thus, water absorption is increased or decreased depending on the underlying experimental design (Breyer & Breyer 2000). The results in our study should be viewed with the background of our experimental design (i.e. over-hydration and reduced S-Na and S-Osm).

The use of desmopressin to treat nocturia in elderly patients is becoming more common (Shindel et al 2002). These patient often have concomitant medical conditions (e.g. arthritis) treated with an NSAID. The results of our pharmacodynamic study indicate that the simultaneous use of an NSAID can interfere with the antidiuretic effect of desmopressin, and that the effect can be unpredictable. Whether this has any clinical relevance can only be established in a study design where both drugs are used in long-term treatment, and with the more common administration routes of desmopressin (i.e. intranasally and perorally). Further, it has been reported that predicted steady-state concentrations of piroxicam are higher in elderly women than in either elderly males or young people of either sex (Richardson et al 1985). This implies that an optimal design of a future study should include elderly people as well.

S-Na and S-Osm

Our study showed that moderately over-hydrated healthy subjects drinking tap water under controlled conditions seem to be at risk of hyponatraemia (> 135 mmol L^{-1}) at

some stages after desmopressin administration. The decrease in S-Osm and S-Na probably reflects continuous water intake during maximal antidiuresis (Sjöstrand & Wickström 1990). The extent of the decrease was unexpected, since the excess water load in the body throughout the whole experiment was no more than approximately +1.1 L for each subject (15 mL kg^{-1} for 70 kg body weight).

Hyponatraemia is a rare but well-known complication in adult and paediatric patients taking desmopressin for bleeding disorders and psychogenic polydipsia. Desmopressin-induced hyponatraemia has also been reported in paediatric and adult patients with enuresis. This adverse event can be prevented by fluid restriction and monitoring of serum electrolytes (Shindel et al 2002). Our results of subnormal serum sodium levels in only moderately over-hydrated healthy individuals emphasize the need for fluid restriction and monitoring of serum sodium, especially when using high (i.e. haemostatic) dosages of desmopressin, which cause a maximal antidiuretic effect of long duration.

Validation of IDR model

In our previous study we demonstrated that an IDR model could describe the dynamics of desmopressin satisfactorily. These findings were confirmed in this study with a 50% lower dose (i.e. 0.2 μg). However, the estimate (except k_{out}) of this study differed statistically when compared with the estimates in our previous study. The explanation is probably the difference in the pharmacokinetic parameters (which serves as input in the IDR model) between these two studies. Thus, the pharmacokinetic parameters in the previous study might not be representative due to analytical limitations after a low dose (0.4 μg), and that probably explains why the curve simulation failed. However, the values of the estimates of this study, IC50, γ, k_{in}, etc., should be interpreted cautiously, especially since there is an absence of a sex difference in the parameters. This reflects that simulating data from a selected model has somewhat limited ability to reveal interfering factors of importance, compared with the use of traditionally descriptive statistics.

The urine osmolality, after desmopressin administration, is increased by increasing doses until a certain plateau. The plateau reflects the maximal ability of the drug to concentrate the urine. Further increase of dosage, however, results in prolongation of duration (Rado et al 1976; Lam et al 1996). This pattern is seen in our simulated curve (Figure 4B), where the plateau effect seems to be reached at dose levels > 1.2 μg, and thereafter only the duration and T_{max} are prolonged. After doses of 4 μg intravenously, the mean maximal urine osmolality has been reported to be 909 ± 46 mOsm kg^{-1} at 4 h, and still elevated (> 700 mOsm kg^{-1}) after 7 h (Callréus & Höglund 1998). At dosage of 2 μg subcutaneously or 2 μg intravenously, the urine osmolality has been reported to be around 900–950 mOsm kg^{-1} (Tryding et al 1987; Fjellestad-Paulsen et al 1993), indicating that the maximal amplitude of the antidiuretic response is seen already after this dose. In a study where healthy subjects received desmopressin at much higher (haemostatic) dosage (21 μg, i.v.) the maximal urine osmolality was approx 1000 mOsm kg^{-1}, with a duration of 24 h (Lethagen et al 1998). Although the simulated plateau level in our study (approx. 1150 mOsm L^{-1}) is somewhat higher than in these reports, and the duration differs slightly, the simulated curves have provided a realistic fit for the effect of desmopressin at different doses. This further validates the IDR model as an appropriate model.

Conclusion

The pharmacokinetics of desmopressin after a fixed bolus injection was neither influenced by chronic pre-treatment with piroxicam nor by sex. The pharmacodynamics of desmopressin on the other hand showed a sex difference where females exhibited a more pronounced antidiuretic effect than males. The diminished sex differences after pre-treatment with piroxicam indicates that the mechanism for the difference was prostaglandin mediated.

Our previously described indirect response model for desmopressin was confirmed with the lower dose used in this study, although the modelling could not distinguish a sex difference, indicating a limitation compared with traditional descriptive statistics.

References

Agnoli, G. C., Borgatti, R., Cacciari, M., Lenzi, P., Marinelli M., Stipo, L. (2002) Low-dose desmopressin infusion: renal action in healthy women in moderate salt retention and depletion, and interactions with prostanoids. *Prostaglandins Leukot. Essent. Fatty Acids* **67**: 263–273

Beierle, I., Meibohm, B., Derendorf, H. (1999) Gender differences in pharmacokinetics and pharmacodynamics. *Int. J. Clin. Pharmacol. Ther.* **37**: 529–547

Breyer, M. D., Breyer, R. M. (2000) Prostaglandin E receptors and the kidney. *Am. J. Physiol. Renal Physiol.* **279**: F12–F23

Callréus, T., Höglund, P. (1998) Pharmacokinetics and antidiuretic effect of intravenous administration of desmopressin in orally overhydrated male volunteers. *Pharmacol. Toxicol.* **83**: 259–262

Callréus, T., Odeberg, J., Lundin S., Höglund, P. (1999) Indirect-response modeling of desmopressin at different levels of hydration. *J. Pharmacokinet. Biopharm.* **27**: 513–529

Chang, W. C. (1988) Stimulation of prostaglandin E2 biosynthesis by estradiol in rat kidneys. *Prostaglandins Leukot. Essent. Fatty Acids* **33**: 159–163

Dayneka, N. L., Garg, V., Jusko, W. J. (1993) Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokinet. Biopharm.* **21**: 457–478

Dollery, C. (1999) Desmopressin (acetate). In: Bobbis, A., Rawlins, M., Thomas, S., Wilkins, M. (eds) *Therapeutic drugs*. Churchill Livingstone, Edinburgh, pp D39–D42

Dusing, R., Herrmann, R., Glanzer, K., Vetter, H., Overlack, A., Kramer, H. J. (1981) Renal prostaglandins and water balance: studies in normal volunteer subjects and in patients with central diabetes insipidus. *Clin. Sci. (Lond.)* **61**: 61–67

Fjellestad-Paulsen, A., Höglund, P., Lundin, S., Paulsen, O. (1993) Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. *Clin. Endocrinol. (Oxf.)* **38**: 177–182

Geelen, G., Greenleaf, J. E., Keil, L. C. (1996) Drinking-induced plasma vasopressin and norepinephrine changes in dehydrated humans. *J. Clin. Endocrinol. Metab.* **81**: 2131–2135

Gilbaldi, M., Perrier, D. (1982) *Pharmacokinetics*. Marc Dekker Inc, New York

Harris, R. Z., Benet, L. Z., Schwartz, J. B. (1995) Gender effects in pharmacokinetics and pharmacodynamics. *Drugs* **50**: 222–239

Jackson, E. K. (2001) Vasopressin and other agents affecting the renal conservation of water. In: Hardman, J. G., Limbird, L. E. (eds) *Goodman & Gilman's: The pharmacological basis of therapeutics*. McGraw-Hill, USA, pp 789–808

Jackson Roberts, L. R., Morrow, J. D. (2001) Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In: Hardman, J. G., Limbird, L. E. (eds) *Goodman & Gilman's: The pharmacological basis of therapeutics*. McGraw-Hill, USA. pp 687–731

Juhlin, T., Björkman, S., Gunnarsson, B., Fyge, Å., Roth, B., Höglund, P. (2004) Acute administration of diclofenac, but possibly not long term low dose aspirin, causes detrimental renal effects in heart failure patients treated with ACE-inhibitors. *Eur. J. Heart Failure* In press

Lam, K. S., Wat, M. S., Choi, K. L., Ip, T. P., Pang, R. W., Kumana, C. R. (1996) Pharmacokinetics, pharmacodynamics, long-term efficacy and safety of oral 1-deamino-8-D-arginine vasopressin in adult patients with central diabetes insipidus. *Br. J. Clin. Pharmacol.* **42**: 379–385

Lethagen, S., Frick, K., Sterner, G. (1998) Antidiuretic effect of desmopressin given in hemostatic dosages to healthy volunteers. *Am. J. Hematol.* **57**: 153–159

Lundin, S., Melin, P., Vilhardt, H. (1985) Plasma concentrations of 1-deamino-8-D-arginine vasopressin after intragastric administration in the rat. *Acta Endocrinol. (Copenh.)* **108**: 179–183

Moses, A. M., Moses, L. K., Notman, D. D., Springer, J. (1981) Antidiuretic responses to injected desmopressin, alone and with indomethacin. *J. Clin. Endocrinol. Metab.* **52**: 910–913

Olkkola, K. T., Brunetto, A. V., Mattila, M. J. (1994) Pharmacokinetics of oxicam nonsteroidal anti-inflammatory agents. *Clin. Pharmacokinet.* **26**: 107–120

Pedersen, R. S., Bentzen, H., Bech, J. N., Pedersen, E. B. (2001) Effect of an acute oral ibuprofen intake on urinary aquaporin-2 excretion in healthy humans. *Scand. J. Clin. Lab. Invest.* **61**: 631–640

Rado, J. P., Marosi, J., Szende, L., Borbely, L., Tako, J., Fischer, J. (1976) The antidiuretic action of 1-deamino-8-D-arginine vasopressin (DDAVP) in man. *Int. J. Clin. Pharmacol. Biopharm.* **13**: 199–209

Richardson, C. J., Blocka, K. L., Ross, S. G., Verbeeck, R. K. (1985) Effects of age and sex on piroxicam disposition. *Clin. Pharmacol. Ther.* **37**: 13–18

Robson, W. L., Norgaard, J. P., Leung, A. K. (1996) Hyponatremia in patients with nocturnal enuresis treated with DDAVP. *Eur. J. Pediatr.* **155**: 959–962

Shindel, A., Tobin, G., Klutke, C. (2002) Hyponatremia associated with desmopressin for the treatment of nocturnal polyuria. *Urology* **60**: 344

Sjöstrand, U., Wickström, I. (1990) Dehydrering and hyperhydrering. In: Mossberg, T. (ed.) *Praktisk vätsketerapi*. Nordstedts Förlag AB, Almqvist & Wiksell, Stockholm, pp 22–29

Tryding, N., Sterner, G., Berg, B., Harris, A., Lundin, S. (1987) Subcutaneous and intranasal administration of 1-deamino-8-D-arginine vasopressin in the assessment of renal concentration capacity. *Nephron* **45**: 27–30

Vilhardt, H., Lundin, S. (1986) Biological effect and plasma concentrations of DDAVP after intranasal and peroral administration to humans. *Gen. Pharmacol.* **17**: 481–483

Wang, Y. X., Crofton, J. T., Share, L. (1997) Sex differences in the cardiovascular and renal actions of vasopressin in conscious rats. *Am. J. Physiol.* **272**: R370–R376