The influence of diflunisal on the pharmacokinetics of oxazepam

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1 Single dose pharmacokinetics of oxazepam, 30 mg, have been studied in six healthy male volunteers in the absence of diflunisal and during continuous treatment with diflunisal 500 mg twice daily.

2 During diflunisal treatment, peak plasma concentration of oxazepam significantly decreased from $387 \pm 18 \text{ ng ml}^{-1}$ (mean $\pm$ s.e. mean) to $241 \pm 10 \text{ ng ml}^{-1}$ and total area under the plasma concentration-time curve (AUC) significantly decreased from $5536 \pm 819 \text{ ng ml}^{-1} \text{ h}$ to $4643 \pm 562 \text{ ng ml}^{-1} \text{ h}$. The AUC of oxazepam glucuronide significantly increased from $4771 \pm 227 \text{ ng ml}^{-1} \text{ h}$ to $8116 \pm 644 \text{ ng ml}^{-1} \text{ h}$ and its elimination half-life increased from $10.0 \pm 0.6 \text{ h}$ to $13.0 \pm 1.0 \text{ h}$. Renal clearance for oxazepam glucuronide was significantly reduced from $74 \pm 2 \text{ ml min}^{-1}$ to $46 \pm 3 \text{ ml min}^{-1}$.

3 *In vitro*, diflunisal, at concentrations of 125 to 1000 $\mu\text{g ml}^{-1}$, significantly displaced oxazepam from its plasma protein binding, the free fraction of oxazepam increasing by 28 to 56 %. The free fraction of oxazepam glucuronide, *ex vivo*, increased by $49 \pm 5 \%$ ($n = 3$) during concomitant diflunisal treatment.

4 These data suggest that the observed interaction between oxazepam and diflunisal results from a presystemic displacement of oxazepam from its plasma protein binding sites by diflunisal and from an inhibition of the tubular secretion of oxazepam glucuronide by the glucuronides of diflunisal.

Keywords diflunisal oxazepam pharmacokinetics interaction

Introduction

Oxazepam, a benzodiazepine antianxiety agent and diflunisal, a salicylic acid derived non-steroidal anti-inflammatory agent have some pharmacokinetic characteristics in common. Both are extensively bound to plasma protein: oxazepam 95–98 % and diflunisal 98–99.8 % (Greenblatt, 1981; Tocco *et al.*, 1975; Verbeeck *et al.*, 1979a). The major route of elimination of both oxazepam and diflunisal is by biotransformation to glucuronide conjugates that are

excreted by the kidney: oxazepam forms an ether glucuronide and diflunisal forms both an ester and ether glucuronide (Greenblatt, 1981; Tocco *et al.*, 1975).

As co-administration of these drugs is possible and since interactions between drugs that mainly undergo glucuronidation have been observed both *in vitro* (Pacifi & Rane, 1981) and *in vivo* (Tjandramaga *et al.*, 1983), a study to evaluate the effect of therapy with diflunisal on the phar-

macokinetics of oxazepam in man was undertaken.

Methods

Study subjects

Six healthy male volunteers, ranging in age from 20–22 years and in weight from 68–75 kg participated in the study. Written informed consent was obtained according to institutional policy and the Declaration of Helsinki.

All subjects had a normal history, physical examination and laboratory tests and were ambulatory. They refrained from other drug treatment starting 2 weeks before and lasting throughout the whole study period. Two were mild and one was a heavy smoker.

Trial design

Each subject received, after an overnight fast, a single dose of 30 mg oxazepam (2×15 mg Seresta®, Wyeth) with 200 ml of water. A standard breakfast was allowed 2 h after drug intake. Blood samples were drawn into heparinized tubes immediately before and at 15, 30, 45, 60 min and 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 30, 36, 48 and 72 h after oxazepam intake. Urine was collected immediately before and during the following periods after oxazepam intake: 0–2 h, 2–4 h, 4–6 h, 6–8 h, 8–12 h, 12–24 h, 24–30 h, 30–36 h, 36–48 h and 48–72 h. Plasma and urine samples were stored at -20°C pending analysis.

Following a drug-free interval of 2 days, 500 mg diflunisal (2×250 mg Diflusal®, Merck Sharp & Dohme) was given at 09.00 h and 21.00 h for 9 days. On the morning of the seventh day of treatment with diflunisal, 20 min after the 13th dose of diflunisal, 30 mg oxazepam was administered as above and plasma and urine samples were collected as described above.

Assay procedures

a. Determination of oxazepam Unchanged oxazepam and its glucuronide were estimated in plasma, urine and plasma ultrafiltrate by h.p.l.c. using slight modifications of the procedure described by Vree *et al.*, (1979). Extraction of unchanged oxazepam and the internal standard (desmethylflunitrazepam) was carried out with ether after alkalization with 1 M ammonium acetate buffer pH 11. The organic phase was evaporated and the residue was dissolved in

0.2 ml of the mobile phase (methanol/water, 55:45, v/v). A suitable aliquot of this solution (20–50 μl) was injected onto the stainless steel column (150 $\times$ 4.6 mm) packed with Spherisorb C₆ 5 μm (Alltech, Europe).

For the determination of oxazepam glucuronide, 0.5 ml of the biological material was first adjusted to pH 5 with 0.5 M acetate buffer pH 5, before enzymatic hydrolysis with 2500 Fishman Units of β -glucuronidase (type B-10, Sigma® Chemical Co.). After incubation at 37°C for 24 h, plasma and urine samples were extracted as described above for unchanged oxazepam. Oxazepam glucuronide concentration was obtained by subtracting the concentration of unchanged oxazepam from that of total oxazepam.

A Spectra-Physics liquid chromatograph SP 8700 was used equipped with a Shoefel UV detector SF 770 set at 230 nm. The flow rate was 1 ml min⁻¹. In control plasma, a linear response was found for concentrations ranging from 25 to 1000 ng ml⁻¹. Sensitivity of the method was approximately 5 ng ml⁻¹ in plasma and 10 ng ml⁻¹ in urine. Assay reproducibility ($n = 6$) at plasma oxazepam concentrations of 25, 100 and 500 ng ml⁻¹ yielded coefficients of variation of respectively 2.7, 2.1 and 3.2% for within day assays and 4.7, 2.9 and 1.6% for between day assays. For urine oxazepam concentrations of 0.1, 1.0 and 10 $\mu\text{g ml}^{-1}$, coefficients of variation were respectively 9.0, 2.7 and 2.2% for within day and 3.5, 3.2 and 3.4% for between day assays.

b. Determination of diflunisal Diflunisal concentrations were measured in plasma and urine by h.p.l.c., following a single precipitation step with acetonitrile after addition of the internal standard (fentiazac, Wyeth). Diflunisal glucuronides were determined after acid hydrolysis with 9 N HCl for 30 min in a boiling waterbath.

A Spectra-Physics chromatograph SP 8700 was used. The separation was performed on a stainless steel column (125 $\times$ 4 mm) packed with Li Chrosorb RP 18 5 μm (Merk, Darmstadt, W. Germany). The mobile phase consisted of a mixture of methanol and 0.03 M phosphate buffer pH 7.0 (65 : 35 v/v). The flow rate was 1 ml min⁻¹. A Shoefel fluorescence detector FS 970 was used with excitation and emission wavelength cut-off filter set at 300 nm and 370 nm respectively. In control plasma a linear response was found in the range between 2 and 200 $\mu\text{g ml}^{-1}$.

Coefficients of variation for diflunisal concentrations of 5, 25 and 100 $\mu\text{g ml}^{-1}$ in plasma were respectively 4.7, 2.3, and 2.7% and in urine 3.2, 2.6 and 1.9% respectively. Sensitivity of the

method was approximately $1 \mu\text{g ml}^{-1}$ in plasma and urine.

Plasma protein binding

Protein binding of oxazepam and oxazepam glucuronide was determined in the absence and presence of diflunisal, by ultrafiltration using the Amicon Centrifree TM Micropartition system (Amicon Corp. MA 01923, USA) (MacMahon *et al.*, 1983).

Oxazepam binding to fresh human plasma was measured after incubation at 37°C for 1 h using 1 ml samples spiked with 500 ng ml^{-1} and under the same conditions in plasma samples from the same subjects after prior incubation during 1 h with various concentrations of diflunisal. All ultrafiltrations were performed in triplicate and $300 \mu\text{l}$ ultrafiltrate was used for the determination of free oxazepam by h.p.l.c. as described above.

Oxazepam glucuronide protein binding was measured in plasma samples from three volunteers, who participated in the interaction study. In order to obtain sufficient material for analysis, five consecutive samples of each subject, collected following the intake of oxazepam alone and following the intake of oxazepam plus diflunisal, were pooled. In each of these six pools, the concentration of oxazepam and of oxazepam glucuronide was determined and the remaining plasma was subjected to ultrafiltration. In the ultrafiltration unbound oxazepam and oxazepam glucuronides were determined in duplicate. The coefficient of variation ($n = 6$) of oxazepam binding at oxazepam concentrations of 250, 500 and 1000 ng ml^{-1} was respectively 0.3, 0.3 and 0.1%. There was no free ligand adsorption to the filtration membrane (mean recovery $100.2 \pm 4\%$, $n = 9$) and more than 99.9% of protein was retained by the membrane using the microprotein assay of Peterson (1977).

Pharmacokinetic and statistical analysis

Pharmacokinetic symbols used in this paper are those suggested by Rowland & Tucker (1982). Maximum plasma concentration (C_{max}) and the time of its appearance (t_{max}) were obtained from plasma data. Elimination half-life ($t_{1/2}$) was calculated from the equation:

$$t_{1/2} = \frac{0.693}{\lambda_z}$$

where λ_z is the first order elimination rate constant obtained from the slope of the terminal

exponential phase which was determined by linear least squares regression analysis. No weighting was used.

The area under the plasma concentration-time curve ($\text{AUC}_{0-\infty}$) was determined by the trapezoidal rule and extrapolated to infinity (AUC) by adding the final plasma concentration divided by λ_z . For unchanged oxazepam, the ratio of the volume of distribution (V) and of total clearance (CL) over the bioavailability factor (F) were estimated by:

$$\frac{V}{F} = \frac{D}{\text{AUC} \cdot \lambda_z}$$

$$\frac{\text{CL}}{F} = \frac{D}{\text{AUC}} \text{ where } D \text{ is the dose given.}$$

Renal clearance of oxazepam glucuronide was determined by the total amount of oxazepam glucuronide recovered in the urine [$A_{\text{e}(\infty)}$] divided by the total AUC of oxazepam glucuronide in plasma. The amount of oxazepam glucuronide excreted after the last urine collection was determined as the quotient of the excretion rate at 60 h and the urinary excretion rate constant ($\lambda_{\text{z ur}}$) derived from the slope of the least-squares regression line for the log transformed excretion rates as a function of time.

Statistical analysis was performed using Student's paired *t*-test and the nonparametric Wilcoxon sign rank test. Corresponding *P* values are given as $P_{t\text{-test}}$ and P_{Wilcoxon} . *P* values equal to or less than 0.05 were considered to indicate statistically significant differences. Results in the text and tables are given as mean $\pm$ s.e. mean.

Results

Plasma

The mean plasma concentration-time profiles in the absence and presence of diflunisal are shown in Figure 1 for unchanged oxazepam and in Figure 2 for oxazepam glucuronide. When oxazepam was administered during diflunisal, plasma concentrations of diflunisal and its glucuronides had reached steady-state values of respectively 90 ± 9 and $24 \pm 5 \mu\text{g ml}^{-1}$.

Individual and mean pharmacokinetic parameters in the absence and presence of diflunisal for unchanged oxazepam are given in Table 1 and for oxazepam glucuronide in Table 2. The mean values in these Tables and Figures do not include the data of subject 5 who represents a particular case that will be discussed separately.

Figure 1 Mean ($\pm$ s.e. mean) plasma concentrations of unchanged oxazepam after a single oral dose of 30 mg oxazepam in the absence (●) and presence (○) of diflunisal ($n = 5$).

The data show that diflunisal causes alterations in the pharmacokinetics of oxazepam. There was no significant difference in mean time to peak, but the maximum concentration of

oxazepam decreased significantly by $38 \pm 2\%$. The mean terminal half-life of oxazepam slightly increased during concomitant treatment with diflunisal but the increase was statistically not

Figure 2 Mean ($\pm$ s.e. mean) plasma concentrations of oxazepam glucuronide after a single oral dose of 30 mg oxazepam in the absence (▲) and presence (△) of diflunisal ($n = 5$).

Table 1 Pharmacokinetic parameters of unchanged oxazepam after administration of 30 mg oxazepam p.o. in the absence and in the presence of diflunisal treatment.

Subject	Oxazepam				Oxazepam + diflunisal							
	C_{max} (ng ml ⁻¹)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (ml min ⁻¹)	V/F (l)	AUC (ng ml ⁻¹ h)	C_{max} (ng ml ⁻¹)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (ml min ⁻¹)	V/F (l)	AUC (ng ml ⁻¹ h)
1	314	2.0	9.9	177	152	2815	208	4.0	8.3	190	136	2614
2	413	3.0	9.4	74	60	6716	227	3.0	11.6	93	93	5405
3	392	3.0	12.1	69	73	7248	258	1.5	14.2	86	105	5810
4	407	3.0	7.0	110	67	4533	247	2.0	10.8	115	108	4334
5*	283	1.0	1.6	370	52	1351	240	1.5	6.3	259	141	1930
6	407	3.0	10.9	79	74	6368	265	3.0	11.2	99	96	5054
Mean	387	2.8	9.9	102	85	5536	241	2.7	11.2	117	108	4643
s.e. mean	18	0.2	0.8	20	17	819	10	0.4	0.9	19	8	562
P t-test							< 0.01	NS	NS	< 0.01	NS	< 0.05
P Wilcoxon							0.1	NS	NS	0.1	NS	0.1

*Data of subject 5 are not included in the mean.

Table 2 Pharmacokinetic parameters of oxazepam glucuronide after administration of 30 mg oxazepam p.o. in the absence and in the presence of diflunisal treatment.

Subject	Oxazepam				Oxazepam + diflunisal							
	C_{max} (ng ml ⁻¹)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng ml ⁻¹ h)	$A_{q(5)}$ (% dose)	CL _R (ml min ⁻¹)	C_{max} (ng ml ⁻¹)	t_{max} (h)	$t_{1/2}$ (h)	AUC (ng ml ⁻¹ h)	$A_{q(5)}$ (% dose)	CL _R (ml min ⁻¹)
1	438	3.0	10.9	5295	74.4	70.3	533	5.0	10.5	9832	80.3	40.8
2	287	4.0	8.5	4882	69.1	70.8	244	6.0	13.6	6805	70.9	52.1
3	275	3.0	11.2	5197	80.0	77.0	347	5.0	16.6	9462	69.2	36.6
4	282	5.0	8.4	4276	66.2	77.4	323	4.0	11.6	7620	71.8	47.1
5*	342	2.0	5.4	4136	60.2	72.8	457	4.0	7.5	7068	78.7	55.6
6	264	5.0	11.0	4207	63.5	75.5	325	4.0	12.6	6862	73.0	53.2
Mean	309	4.0	10.0	4771	70.6	74.2	354	4.8	13.0	8116	73.0	46.0
s.e. mean	32	0.4	0.6	227	3.0	1.5	48	0.4	1.0	644	1.9	3.2
P t-test							NS	NS	NS	< 0.01	NS	< 0.01
P Wilcoxon							NS	NS	NS	0.1	NS	0.1

*Data of subject 5 are not included in the mean.

significant. The AUC decreased significantly during treatment with diflunisal by $14 \pm 4\%$. The parameter CL/F increased significantly by $17 \pm 5\%$ in the presence of diflunisal and V/F increased by $36 \pm 13\%$ although the latter increase did not reach statistical significance.

Oxazepam glucuronide appeared rapidly in plasma to reach peak values which were slightly but not significantly higher in the presence of diflunisal. The slight difference in $t_{\max}$ was also not significantly different. The elimination half-life of the glucuronide, however, significantly increased from 10.0 to 13.0 h and the AUC significantly increased by $70 \pm 9\%$.

The oxazepam plasma data of subject 5, shown in Figure 3, differed from those of the other subjects, both in the absence and presence of diflunisal. Prominent was the much faster elimination of oxazepam, evidenced by a much shorter $t_{1/2}$, a much higher clearance and a much smaller AUC compared with the other subjects (Table 1). The relative increase in oxazepam half-life during diflunisal treatment was much more pronounced and there was a rather pronounced decrease in oxazepam clearance while in all other subjects the clearance showed an increase in the presence of diflunisal. The increase in V/F was also much greater and the AUC increased while it decreased in the other subjects. The elimination of oxazepam glucuronide was also faster in this subject although the difference was not as marked as for the

elimination of unchanged oxazepam (Figure 4, Table 2).

Protein binding

The *in vitro* binding of oxazepam, 500 ng ml^{-1} , to fresh human plasma in the absence and in the presence of increasing concentrations of diflunisal is given in Table 3. The free fraction of oxazepam was found to be between 2.5 and 3.6% which is well within the reported values in the literature (Greenblatt, 1981).

Diflunisal displaced oxazepam the free fraction of which increased by 28 to 56%. There seemed to be no further increase in displacement of oxazepam between 250 and $1000 \text{ } \mu\text{g ml}^{-1}$ of diflunisal.

The binding of oxazepam glucuronide in plasma from the three volunteers who participated in the interaction study is given in Table 4. The mean free fraction of oxazepam glucuronide in the absence of diflunisal was found to be $20 \pm 2\%$. The free fraction increased by approximately 50% during steady-state concentrations of diflunisal.

Urine

Oxazepam in urine was almost entirely present as the glucuronide. Less than 0.1% of the administered dose was excreted as unchanged oxazepam during the first 24 h after drug admini-

Figure 3 Plasma concentration-time profile of unchanged oxazepam after a single oral dose of 30 mg oxazepam in the absence (●) and presence (○) of diflunisal in subject 5.

Figure 4 Plasma concentration-time profile of oxazepam glucuronide after a single oral dose of 30 mg oxazepam in the absence (▲) and presence (Δ) of diflunisal in subject 5.

stration. The cumulative 72 h urinary recovery of oxazepam glucuronide, shown in Figure 5, was not different in the absence or presence of diflunisal treatment. However, in the presence of diflunisal, the elimination of oxazepam glucuronide was slightly delayed during the first 12 to 24 h.

In all subjects (including subject 5) the renal clearance of oxazepam glucuronide decreased under the influence of diflunisal, resulting in a statistically significant decrease from 74.2 ± 1.5 to $46.0 \pm 3.2 \text{ ml min}^{-1}$ (Table 2).

Renal clearance of diflunisal glucuronides during combined treatment was $19.4 \pm 1.0 \text{ ml min}^{-1}$.

On the basis of these plasma and urine data and assuming a glomerular filtration rate of 120 ml min^{-1} in our healthy subjects, the relative

contribution of glomerular and tubular components to the renal clearance of oxazepam glucuronide in the absence and presence of diflunisal can be calculated. Similarly, using a value of 10% for the free fraction of diflunisal glucuronides in plasma, as reported earlier from our laboratory (Verbeeck *et al.*, 1979b) the glomerular and tubular contribution to the renal clearance of diflunisal glucuronides can also be calculated. The results of these calculations are summarized in Table 5.

Discussion and conclusions

Oxazepam belongs to the class of poorly cleared drugs which means that its clearance is sensitive to changes both in protein binding within the

Table 3 Free fraction (%) of oxazepam (500 ng ml^{-1}) in the absence (control) and presence of diflunisal *in vitro* ($n = 5$).

	Control	125	+ Diflunisal ($\mu\text{g ml}^{-1}$)		
			250	500	1000
Mean	3.1	4.1	4.7	3.9	4.0
s.e. mean	0.2	0.1	0.1	0.2	0.2
Range	2.5–3.6	3.8–4.4	4.5–5.2	3.3–4.5	3.5–4.5
% Change		+ 28	+ 56	+ 29	+ 32
<i>P</i> <i>t</i> -test		< 0.05	< 0.01	< 0.05	< 0.05

Table 4 Free fraction (%) of oxazepam glucuronide in the absence (control) and presence of diflunisal *ex vivo* ($n = 3$).

Subject	Control	+ Diflunisal	% Change
1	17	24	+41
2	24	36	+50
3	18	28	+56
Mean	20	29	+49
s.e. mean	2.3	3.5	4.6

blood and in intrinsic hepatic clearance (Wilkinson & Shand, 1975). When administered during diflunisal maintenance treatment a number of changes occur in the kinetic parameters of oxazepam and of its glucuronide.

The observed decrease in $C_{\max}$ and AUC for unchanged oxazepam could theoretically be due to a smaller extent of intestinal absorption but this is not the case, as the 72 h cumulative urinary recovery is not altered in the presence of diflunisal. It is probably also not due to a slower absorption since $t_{\max}$ remains unchanged. A further, and in this case more likely explanation for the observed decreased in $C_{\max}$ is an increase in the presystemic elimination of the drug by the liver. Since both drugs are highly bound to plasma protein it is conceivable that a number of oxazepam binding sites are occupied by diflunisal which is present in much higher concentrations in portal blood. This would lead to less extensive

binding of oxazepam in portal blood whereby more free oxazepam would be available for first-pass extraction by the liver. This explanation is supported by the observation that in the presence of diflunisal the decrease in $C_{\max}$ of unchanged oxazepam is associated with an increase in the $C_{\max}$ of oxazepam glucuronide and by a significant increase in the ratio of the AUC of oxazepam glucuronide over oxazepam during the first 4 h after dosage as shown in Figure 6.

The observed increase in V/F and CL/F can be explained by displacement of oxazepam from protein binding sites in the systemic circulation and/or by the decreased availability (F) of oxazepam due to presystemic elimination. The relative contribution of these two mechanisms can, however, not be inferred from our data as it would require i.v. administration of oxazepam in the absence and presence of diflunisal. The hypothesis of displacement of oxazepam by

Figure 5 Cumulative urinary recovery ($\pm$ s.e. mean) of total oxazepam after single doses of 30 mg oxazepam, given alone (■) and in the presence (□) of diflunisal

Table 5 Components of renal excretion of diflunisal- and oxazepam-glucuronides.

	Free fraction (%)	CL_R ($ml\ min^{-1}$)	Glomerular filtration ($ml\ min^{-1}$)	Tubular secretion ($ml\ min^{-1}$)	% of CL_R
Diflunisal glucuronides	10	20	12	8	40
Oxazepam glucuronides					
Control	20	74	24	50	68
+ diflunisal	29	46	35	11	24

diflunisal is strengthened by *in vitro* experiments where it is shown that in the presence of diflunisal at concentrations of 125–1000 $\mu g\ ml^{-1}$, the free fraction of oxazepam clearly increases, indicating displacement of oxazepam from protein binding sites by diflunisal (Table 3).

As far as oxazepam glucuronide is concerned, the observed increase in mean C_{max} during diflunisal treatment is probably due to a greater presystemic formation of the glucuronide metabolite during the absorption phase. It could also partly be due to the observed slower elimination of the conjugate. The slower elimination, evidenced by an increase in half-life and an increase in AUC of the glucuronide during the elimination phase, could be ascribed to a competition for tubular secretion between the glucuronides of diflunisal and oxazepam. The glucuronides of both drug indeed undergo substantial tubular secretion and the tubular secretion of diflunisal glucuronides largely exceeds

that of oxazepam glucuronide due to the much higher plasma concentrations of diflunisal glucuronides. That competition for tubular secretion occurs is further shown by the decreased renal clearance of oxazepam glucuronide in spite of its increased glomerular clearance resulting from its displacement from plasma protein binding sites in the presence of diflunisal (Table 5). This displacement accounts for an increase in glomerular clearance from 24 to 35 $ml\ min^{-1}$. On a total renal clearance of the oxazepam glucuronide, in the presence of diflunisal, of 46 $ml\ min^{-1}$ this would leave a net tubular clearance of only 11 $ml\ min^{-1}$ as compared to 50 $ml\ min^{-1}$ in the absence of diflunisal.

Subject 5 deserves special comment. The much faster elimination pattern could, theoretically, result from a higher free fraction in the systemic circulation and/or from a higher intrinsic liver clearance. The first possibility is unlikely in view of a plasma protein binding similar to that

Figure 6 Oxazepam (O) and oxazepam glucuronide (O-G) concentration ($\pm$ s.e. mean) during the first 4 h following administration of 30 mg oxazepam in the absence (left panel) and presence (right panel) of diflunisal.

in the other subjects. A higher intrinsic clearance is therefore the most likely explanation. It is noteworthy that this subject is a cigarette smoker (25/day) and it is known that tobacco smoking can induce liver enzymes including oxazepam glucuronyl-transferase (Ochs *et al.*, 1981). The unexpected decrease in clearance in this subject during diflunisal can be explained by assuming a competition between diflunisal and oxazepam for glucuronidation. The latter phenomenon is not observed in the other subjects where the hepatic clearance of oxazepam is much smaller and where competition between oxazepam and diflunisal for glucuronidation, if it exists at all, would certainly be smaller and could be compensated by an increase in hepatic clearance resulting from the increase in the concentration of unbound oxazepam.

In conclusion, this study demonstrates the existence of a pharmacokinetic interaction

between oxazepam and diflunisal involving two main mechanisms, firstly a displacement of oxazepam from its plasma protein binding in the blood leading to a slightly increased presystemic hepatic extraction and a slightly decreased systemic availability and secondly a competition between glucuronides of oxazepam and diflunisal for tubular secretion.

As to the clinical significance of this interaction one could, from this single dose experiment, expect that chronic treatment with oxazepam and diflunisal would lead to a somewhat lower steady-state concentration of oxazepam. The clinical significance of this would probably be small.

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