A pharmacokinetic comparison of three pharmaceutical formulations of nimesulide in healthy volunteers

Dušan Jovanović*, Vesna Kilibarda*, Veljko Todorović†, Olivera Potrebic†

Military Medical Academy, National Poison Control Centre, *Institute of Toxicology and Pharmacology, †Clinic of Emergency and Clinical Toxicology and Pharmacology, Belgrade

Background/Aim. Switching the patient from one pharmaceutical formulation of the same drug to another, may lead to therapeutic inadequacy in some cases. To minimize the risk, careful pharmacokinetic studies are desired in the pre-registration period and afterwards.

Methods. A randomized, crossover design with one-week wash-out period between each dose was applied. Serum samples, obtained before dosing and at various appropriate time points up to 15 hours, were analyzed for nimesulide content by a high-performance liquid chromatographic method with ultraviolet (UV) detection. The pharmacokinetics and relative bioavailability of three different pharmaceutical formulations containing nimesulide, manufactured by the same pharmaceutical factory, were studied prospectively in 12 healthy subjects of both sexes. A single 100-mg oral dose of nimesulide was given to the volunteers in the form of conventional tablets, mouth dissolving tablets or as a suspension. Analysis of variance, power analysis, 90% confidence intervals, and two one-sided tests were used for the statistical analysis of pharmacokinetic parameters.

Results. The tolerability of all preparations was excellent. The respective confidence intervals of the ratios of geometric means of C_max and AUC_0-∞ of nimesulide were out of acceptable limits either for conventional tablets in comparison with suspension or for mouth dissolving tablets when compared with conventional tablets. A comparison of mouth dissolving tablets with suspension showed a statistically significant difference between C_max values (suprabioavailability of mouth dissolving tablets), while the point estimate of the ratio of geometric means of AUC_0-∞ was 0.945 with the corresponding 90% confidence interval of 0.902−0.991. At the 5% level of significance, there were no differences between the formulations under the study in times elapsed to peak serum concentrations, as revealed by the non-parametric Wilcoxon signed ranks test. Conclusion. Only a 90% confidence interval for the relative differences of log-transformed AUC_0-∞ values of nimesulide absorbed from mouth dissolving tablets vs. suspension was included in the 80% to 125% interval proposed by the Food and Drug Administration (FDA). On that basis, mouth dissolving tablets (Nimulid-MD™) were considered bioequivalent to Nimulid™ suspension according to the extent of drug absorption. Concerning the comparable amounts of nimesulide available in the systemic circulation after application of these formulations the one might not expect therapeutic failure after switching the patient from one to another.

Key words: anti-inflammatory agents, non-steroidal; chemistry, pharmaceutical; dose-response relationship, drug; pharmacokinetics; biological availability; therapeutic equivalency.

Introduction

Nimesulide is a synthetic sulfonanilide derivative. It belongs to a class of non-steroidal anti-inflammatory drugs (NSAID) which selectively inhibit cyclooxygenase-2 but, also, interfere with the production/action of mediators other than prostaglandins such as enzymes, toxic oxygen derivatives, cytokines, platelet-activating factor and histamine (1,
2). A combination of a variety of mechanisms makes nimesulide a NSAID with marked anti-inflammatory, analgesic and antipyretic properties (2, 3). Several recent experimental studies have also demonstrated a significant neuroprotective effect of nimesulide on chronic cerebral hypoperfusion (4), global cerebral ischemia (5,6), diffuse traumatic brain injury (7, 8) or cerebral infarction induced by permanent middle cerebral artery occlusion (9).

The pharmacokinetic profile of orally administered nimesulide is characterized by rapid and complete absorption, the rate and the extent of which do not differ significantly whether the drug was administered in a tablet or in a suspension form. The time to reach maximal concentration was reported to vary from 1.9h in children to more than 2.5h in healthy volunteers (3).

Nimesulide is 99% bound to human plasma proteins, mainly albumins and it is principally distributed in the extracellular fluid compartment. The drug undergoes extensive metabolism in the liver. Metabolic biotransformation can occur at both the phenoxy ring moiety and the aromatic nitro group. The principal metabolite is 4-hydroxynimesulide, which contributes to the anti-inflammatory activity of the compound. Another degradation product, 2-(4'-hidroxyphe- 

only 1% to 3% of the dose is excreted unchanged in the urine (70%) or the feces (20%) in both free and conjugated form. The terminal elimination half-life of nimesulide is shorter in children than in adults (approx. 2.4h vs. up to 5h) while the elimination half-times of metabolites are nearly 2-times higher than that of the parent compound (3).

The purpose of this study was to evaluate the tolerability, pharmacokinetic property and comparative bioavailability of the three different pharmaceutical formulations of nimesulide, all manufactured by the drug company Panacea Biotec Ltd. from India, that are available at the Serbian market. Particular attention has been made to the pharmacokinetic profile of flavoured dispersible tablets (MD tablets) with fast mouth dissolving characteristics thereby providing immediate relief of the drug (10). Although this had not been a classic bioequivalence study, it was of therapeutic interest to compare their bioavailability with that of the liquid formulation (suspension) which is usually thought to be a 100% bioavailable.

Methods

A single-dose, open-label, randomized, six-sequence, three-period crossover study design was used to evaluate the bioavailability of nimesulide, prepared either as a 100-

mg conventional tablets (Nimulid™ tablets), 100-mg mouth dissolving tablets (Nimulid-MD™ tablets) or a 50 mg/5 ml suspension (Nimulid™ suspension). Twelve subjects of both sexes in good physical condition, as determined by the complete medical and laboratory examinations before the study, were enrolled and provided written informed consent prior to any study related procedure. The study was approved by the Drug Commission and the Ethics Committee of the Military Medical Academy, on September 20, 2001.

The enrolled subjects were randomly assigned to one of the six sequence groups such that upon the completion of the study each subject received all three regimens. Dosing in each of the three consecutive periods was separated by 7-day washout period. A single dose of 100 mg nimesulide (one conventional tablet, one MD tablet or a 10-ml volume of suspension) was given with 200 ml of non-carbonated mineral water following an overnight fast of at least 10 hours.

Venous blood samples (approx. 8 ml) were collected prior to dosing (hour 0) and afterwards at time-points 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 12 and 15 hours. The samples were centrifuged within one hour of collection and the serum was separated and frozen at -20°C until assayed.

Assay method

The High Performance Liquid Chromatography (HPLC) set was equipped with a pump (Model 2150, LKB, Bromma, Sweden), an automatic sample system (Model AS-100, Bio-Rad Laboratories, Inc., Hercules, CA, USA), a variable wavelength UV detector (Model 1801, Bio-Rad Laboratories, Inc., Hercules, CA, USA) and an integrator (Model 2221, LKB, Bromma, Sweden). For the acquisition and integration of analytical data, a Bio-Rad Value Chrom software, operated by Pentium microprocessor, was used. Separations were performed on a reverse phase column (BioSil C18 HL, 4.6 x 250 mm; Bio-Rad Laboratories, Inc., Hercules, CA, USA) at the ambient temperature.

The HPLC assay for determination of unchanged nimesulide was fully developed and completely validated in our laboratory. Its development, however, had been based on the data published by Gupta (11) but due to differences in the equipment and materials being on disposal, it had to be generally modified.

The HPLC analysis was performed by using a mobile phase consisted of 0.01M ammonium-dihydrogen-orthophosphate and acetoniitrile (1:1), previously filtrated and degassed by a membrane degasser. The flow rate was 1.0 ml/min, an injector loop volume was 20 μl, and an UV detector wavelength set at 300 nm throughout the assay. Under these conditions the retention time of nimesulide was about 6.47 min. All the chemicals were of HPLC, and p.a. purity, and had been purchased commercially.

After being refrigerated, the samples were allowed to melt spontaneously at room temperature. Aliquots of 0.5 ml of serum were dispensed into glass tubes and were deproteinized using 0.5 ml of acetonitrile. The mixture was strongly vortexed for 60 seconds to ensure adequate mixing and afterwards centrifuged at 8000 g for 2 minutes. The obtained supernatant amounting 20-μl was injected into the HPLC system and further analyzed using the validated HPLC-UV method. Standard solutions (range of concentrations 0.05–10.0 μg/ml) and
spiked serum samples (range of concentrations 0.1–5.0 µg/ml) were also determined under the same assay conditions.

**Pharmacokinetic analysis**

The pharmacokinetic parameters of nimesulide were estimated using non-compartmental techniques. The peak plasma concentration ($C_{\text{max}}$) and the time elapsed to peak concentration ($t_{\text{max}}$) were obtained directly from the data. The elimination rate constant ($k_e$) was obtained from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. Half-life ($t_{1/2}$) was calculated as $\ln(2)/k_e$ ($\ln(2) = 0.693$). The area under the nimesulide serum concentration-time curve ($AUC^{0-\infty}$) was calculated using the linear trapezoidal rule while the area under the concentration-time curve from time 0 to the infinite time ($AUC^{0-\infty}$) was calculated as the sum of $AUC^{0-15}$ and $C_t/k_e$, where $t$ was the time of the last measurable concentration ($C_t$) and $k_e$ was the elimination rate constant.

Parameters $C_{\text{max}}$, $t_{\text{max}}$, and $AUC^{0-\infty}$ were accepted as the main variables, while the values of $AUC^{0-15}$, residual areas [$AUC^{0-15}/AUC^{0-\infty} \times 100$], $k_e$ and $t_{1/2}$ served as the secondary pharmacokinetic objectives.

**Statistical analysis**

The following main pharmacokinetic parameters, which completely describe the rate and the extent of absorption of nimesulide, derived from the individual serum concentration-time profiles were subjected to statistical analysis: $C_{\text{max}}$, $AUC^{0-\infty}$ and $t_{\text{max}}$. The comparison of secondary kinetic variables was only descriptive.

Following logarithmic transformation of $AUC^{0-\infty}$ and $C_{\text{max}}$ the values were subjected to analysis of variance (ANOVA) including terms for subjects, treatment (sequence) and period. For evaluation of bioequivalence the point estimates and 90% confidence intervals for the differences between test and reference formulations were constructed using the residual mean square error, obtained from the multifactorial ANOVA. The point estimates and the 90% confidence intervals were then back transformed to give estimates of the ratio of the geometric means and the corresponding 90% confidence intervals for the ratios of the two formulations in the comparison. A non-parametric test (Wilcoxon Signed Rank’s Test) was performed for $t_{\text{max}}$.

Bioequivalence between the formulations was accepted if the back transformed 90% confidence intervals for the geometric mean ratios of $AUC^{0-\infty}$ and $C_{\text{max}}$ had fallen within 0.80–1.25 range (12–14) and if the differences in $t_{\text{max}}$ between the two formulations had been not statistically different ($p > 0.05$).

**Results**

**HPLC method**

Under the described assay conditions, linearity was observed in serum standard curves of nimesulide over the range of 0.05 – 10.0 µg/ml with a correlation coefficient greater than 0.999. The lower limit of quantification (LOQ) of unchanged nimesulide was 0.1 µg/ml while the limit of detection was 0.05 µg/ml of serum. Concentrations below LOQ were reported as 0.0 µg/ml. The mean recovery of extraction of nimesulide from serum samples ranged from 93% to 109%. The same working standard solutions had been stable during a 5-day working period when kept in the refrigerator.

**Study in healthy volunteers**

A total of 12 subjects, whose demographic characteristics are summarized in Table 1, were selected to participate in the study. There were no differences in their age, weight or height that might compromise the validity of the planned pharmacokinetic trial.

![Table 1](image)

**Table 1**

<table>
<thead>
<tr>
<th>Demographic data of subjects (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Mean*</td>
</tr>
<tr>
<td>SE†</td>
</tr>
<tr>
<td>Min.</td>
</tr>
<tr>
<td>Max.</td>
</tr>
<tr>
<td>CV‡ (%)</td>
</tr>
</tbody>
</table>

Males, $n = 9$ Females, $n = 3$

*Mean – arithmetic mean, †SE – standard error of the mean, ‡ CV – coefficient of variation

The mean serum concentration-time profile of nimesulide absorbed from the 3 different pharmaceutical preparations is shown in Figure 1. Marked differences were observed between the amounts of drug that had reached the systemic circulation during the period of 15 hours. However, the tolerability of all three preparations containing nimesulide was reported as excellent.

The relevant pharmacokinetic parameters of nimesulide for each preparation are listed in Table 2. As it can be seen, the residual area (relation between $AUC^{0-15}$ and $AUC^{0-\infty}$) of nimesulide (7.3% suspension, 5.5% conventional tablets, 5.0% MD tablets) accounted for less than 20% of the area from time 0 to the time of the last measurable concentration. Therefore, the stated criterion ($AUC^{0-15}/AUC^{0-\infty} \times 100 > 80\%$) was fulfilled and the residual area had no sizeable impact on the calculation of $AUC^{0-\infty}$ and, thus, on bioavailability.
The rate of nimesulide absorption from the tested pharmaceutical formulations, as well as their half-lives of the terminal phase, agreed very well after the application of the three nimesulide-containing preparations. Marked differences in the maximal concentrations were noted between MD tablets and the other two formulations following the same dosages of the drug.

The statistical evaluation of the main pharmacokinetic variables, which describe the rate and the amount of absorption of nimesulide, is presented in Table 3. The respective point estimates of the ratios of geometric means of log-transformed $C_{\text{max}}$ and $AUC_{0-\infty}$ of nimesulide were 0.655 and 0.498 (conventional tablets vs. suspension), 1.244 and 0.945 (MD tablets vs. suspension), and 1.827 and 2.103 (MD tablets vs. conventional tablets). The corresponding 90% confidence intervals were 0.260–0.607, 0.335–0.603, 1.004–1.542, 0.902–0.991, 1.358–2.457 and 1.575–2.810, respectively. For the median $t_{\text{max}}$ values, at a 5% level of significance, there were no significant differences between the formulations in the study, as revealed by the non-parametric Wilcoxon signed ranks test.

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suspension</th>
<th>Conventional tablets</th>
<th>MD tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg.ml$^{-1}$)</td>
<td>4.53 0.67</td>
<td>4.11 1.03</td>
<td>5.88 0.98</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>3.0 0.4</td>
<td>2.8 0.5</td>
<td>2.8 0.3</td>
</tr>
<tr>
<td>$k_e$ (h$^{-1}$)</td>
<td>0.2214 0.0201</td>
<td>0.2764 0.0327</td>
<td>0.2645 0.0318</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>3.5 0.4</td>
<td>2.8 0.2</td>
<td>3.0 0.3</td>
</tr>
<tr>
<td>$AUC_{0-15}$ (µg.h.ml$^{-1}$)</td>
<td>34.75 6.55</td>
<td>24.70 8.09</td>
<td>33.86 6.17</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µg.h.ml$^{-1}$)</td>
<td>38.44 7.55</td>
<td>26.56 8.95</td>
<td>36.01 6.80</td>
</tr>
<tr>
<td>Residual area (%)</td>
<td>7.3 1.7</td>
<td>5.5 1.5</td>
<td>5.0 1.3</td>
</tr>
</tbody>
</table>

* Mean – arithmetic mean, †SE – standard error of the mean

**Table 3**

### Statistical evaluation of bioequivalence of nimesulide from the Nimulid™ 100-mg conventional tablets, Nimulid-MD™ 100-mg mouth dissolving tablets or Nimulid™ suspension (50-mg/5 ml)

<table>
<thead>
<tr>
<th>Statistical test</th>
<th>Pharmacokinetic parameter</th>
<th>Nimulid™ tablets vs. Nimulid™ suspension</th>
<th>Nimulid-MD™ tablets vs. Nimulid™ suspension</th>
<th>Nimulid-MD™ tablets vs. Nimulid™ tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point estimate of the ratio of geometric means</td>
<td>$C_{\text{max}}$</td>
<td>0.655</td>
<td>0.498</td>
<td>–</td>
</tr>
<tr>
<td>90% confidence interval of the ratio of geometric means*</td>
<td>–</td>
<td>0.260–0.607</td>
<td>0.335–0.603</td>
<td>–</td>
</tr>
<tr>
<td>Wilcoxon signed ranks test†</td>
<td>–</td>
<td>–</td>
<td>p = 0.5465</td>
<td>–</td>
</tr>
<tr>
<td>Point estimate of the ratio of geometric means</td>
<td>$AUC_{0-\infty}$</td>
<td>1.244</td>
<td>0.945</td>
<td>–</td>
</tr>
<tr>
<td>90% confidence interval of the ratio of geometric means*</td>
<td>–</td>
<td>1.004–1.542</td>
<td>0.902–0.991</td>
<td>–</td>
</tr>
<tr>
<td>Wilcoxon signed ranks test†</td>
<td>–</td>
<td>–</td>
<td>p = 0.5050</td>
<td>–</td>
</tr>
<tr>
<td>Point estimate of the ratio of geometric means</td>
<td>$t_{\text{max}}$</td>
<td>1.827</td>
<td>2.103</td>
<td>–</td>
</tr>
<tr>
<td>90% confidence interval of the ratio of geometric means*</td>
<td>–</td>
<td>1.358–2.457</td>
<td>1.575–2.810</td>
<td>–</td>
</tr>
<tr>
<td>Wilcoxon signed ranks test†</td>
<td>–</td>
<td>–</td>
<td>p = 0.7518</td>
<td>–</td>
</tr>
</tbody>
</table>

* - Acceptance range (bioequivalence): 0.80 – 1.25
† - Bioequivalence: p > 0.05
Discussion

The analysis of the assay data indicated that the chosen HPLC-UV method was simple, precise and enough accurate for performing a valid bioequivalence study. According to the literature, its sensitivity was comparable to the results of the HPLC methodologies that had been applied so far to determine nimesulide concentration by other investigators (15–20). As expected, however, the HPLC method we used was less sensitive than liquid chromatography coupled to tandem mass spectrometry (21).

Twelve subjects were arbitrarily chosen to participate into the study. That number corresponded to the smallest sample that is accepted as sufficient to assess bioequivalence (22, 23) and might represent a reliable number of participants for the bioequivalence decision. The post-study calculations based on the log–ANOVA error data [AUC0−∞ CV-intra = 6.68%; suspension vs. MD tablets] revealed a sample size of 7 subjects to be quite enough to show the difference of 20% between the AUC0−∞ values of the test and the reference articles. The type I and type II errors would not exceed 5% and 20%, respectively.

The overall pharmacokinetic profile of nimesulide in the present study, independently from the pharmaceutical formulation being used, was close and in agreement with the data previously published for oral nimesulide preparations in the relevant publications (3, 15–18, 24–29). However, on the basis of maximum serum concentrations and AUC0−∞ values of nimesulide it was concluded that they had been significantly different after the administration of suspension and conventional tablets. Oppositely, the total amount of nimesulide (AUC0−∞) absorbed from the suspension and MD tablets did not differ significantly with the power (derived from ANOVA) greater than 0.999.

Only 90% confidence intervals for AUC0−∞ geometric mean ratios of MD tablets, when compared to suspension, were included in the 80% to 125% interval proposed by the U.S. Food and Drug Administration (12, 13) and the difference between tmax values was statistically insignificant. On that basis, tablets Nimulid-MD™ were considered bioequivalent to suspension Nimulid™ according to the extent of absorption of nimesulide. The differences in Cmax values, indicating suprabioavailability of MD tablets according to the rate of absorption, were judged not to be therapeutically important since, nimesulide is not a medicine which pharmacodynamic action is closely related to the level of maximal concentrations in the blood.

Conclusion

From the pharmacokinetic point of view, concerning the total amount of drug that is available at the site of action, suspension and MD tablets could be marked as interchangeable by a physician and might not be expected to produce therapeutic failure after switching the patient from one to another. An increased maximal concentration in the serum after MD tablets would definitely not increase the toxicity level of nimesulide due to its wide therapeutic window in patients.

Acknowledgment

The authors wish to thank Ms. Vera Mladenović, Mrs. Snežana Stevanović and Mr. Dragoslav Savić for their perfect and helpful assistance during the clinical and analytical phases of the study.

REFERENCES


The paper was received on June 20, 2005.

**Abstract**


FARMAKOKINETIČKO POREDENJE TRI FARMACEUTSKA OBLIKA NIMESULIDA KOD ZDRAVIH DOBROVOLJACA

**Uvod/Cilj.** Prevođenje bolesnika sa jednog na drugi farmaceutski oblik istog leka, može u pojedinim slučajevima, rezultirati promenjenim terapijskim efektom. U cilju smanjenja ovakvih rizika, u preregistracionom periodu, ali i nakon puštanja u promet leka, nužno je sprovesti ozbiljna farmakokinetička ispitivanja. **Metode.** Izvedeno je randomizirano i unakrsno ispitivanje sa „wash-out“ periodom od sedam dana. Uzorci seruma uzimani su pre davanja lekova, a nakon toga, u definisanim vremenskim tačkama, tokom 15 sati. Prospektivna studija obuhvatila je 12 zdravih osoba oba pola u cilju ispitivanja farmakokinetike i relativne biološke raspoloživosti nimesulida primenjenog u tri različita farmaceutska oblika, koje proizvodi isti proizvođač. Dobrovoljcima je davana jednokratna doza nimesulida od 100 mg, u obliku standardnih tableta, tableta koje se tope u ustima ili suspenzije. Određivanje nime-
nimesulida vršeno je tečnom hromatografijom visoke rezolucije sa UV detekcijom. Za statističku analizu izračunatih farmakokinetičkih parametara korišćene su: analiza varijanse, „power“ analiza, 90% interвали poverenja i dvostruki, jednostrani t-test.

Rezultati. Podnošljivost svih ispitivanih farmaceutskih oblika nimesulida bila je odlična. Kod standardnih tableta, u poređenju sa suspenzijom, ili tableta koje se tope u ustima, u poređenju sa standardnim tabletama, izračunati interвали poverenja za relativne razlike logaritamski transformisanih vrednosti maksimalnih koncentracija i površina ispod krive bili su van definisanih, i stručno prihvaćeni granica. Poređenje bitnih farmakokinetičkih parametara za tablete koje se tope u ustima i suspenziju ukazalo je na statistički značajne razlike među vrednostima maksimalnih koncentracija („supra” biološka raskoloživost tableta koje se tope u ustima), dok je kritična vrednost odnosa geometrijskih sredina niza logaritamski transformisanih vrednosti površina ispod krive iznosila 0,945, a odgovarajući 90% interval poverenja kretao se u rasponu od 0,902 do 0,991. Pri nivou značajnosti od 5%, primenom neparimetrijskog Wilcoxonovog testa suma rangova, među ispitivanim farmaceutskim oblicima nimesulida nisu ustanovljene razlike u vremenima potrebnim da se dostigne maksimalna koncentracija leka u crvi. Zatim, analiza dobijenih rezultata pokazala je da je jedino 90% interval poverenja za relativne razlike logaritamski transformisanih vrednosti površina ispod krive nimesulida apsorbovanog iz tableta koje se tope u ustima, u poređenju sa suspenzijom, bio uključen u raspon 80–125%, koji prihvata Agencija za hranu i lekove SAD. Stoga je donet zaključak da su samo tablete koje se tope u ustima (Nimulid-MD™), ceneći ukupnu količinu leka koja je dospela u sistemsku cirkulaciju, biološki ekvivalentne formulacije koja, po definiciji, ima apsolutnu biološku iskoristljivost (Nimulid™ suspenzija). Pod navedenim uslovima ne mogu se očekivati promene u terapijskoj efikasnosti tokom prevođenja bolesnika sa jednog na drugi farmaceutski oblik nimesulida.

Ključne reči: antiinflamatorici, nesteroidni; lekovi, oblici; lekovi, odnos doza – reakcija; farmakokinetika; bioraspoloživost; terapijska ekvivalentnost.