

Research Article**Comparative bioavailability studies of binary and ternary solid dispersions of Nabumetone in Wistar albino rats****Jim Joseph^{*}, Jyoti Harindran***Department of Pharmaceutical Sciences, Centre for Professional and Advanced Studies, Cheruvandoor Campus, Ettumanoor-686631, Kottayam, Kerala, India.*

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Abstract

Objective: The study investigated the comparative bioavailability of Nabumetone (NBT) from binary (BSD) and ternary (TSD) solid dispersions prepared by melt method with that of the commercially available marketed tablet formulation in wistar albino rats. **Materials and methods:** Female Wistar albino rats (three groups with four animals in each) were selected for bioavailability (BA) study. Same sex was desired to maintain similar metabolic pattern throughout the study. Test (T1 and T2) and standard (S1) samples were suitably diluted and administered to the animals after acclimatization. Plasma samples were collected periodically from the tail vein and tested using HPLC for quantification of the active metabolite 6-methoxy-2-naphthyl acetic acid (6-MNA) upto a period of 48h. **Results:** C_{max}, and AUC of 6-MNA were significantly increased by administration of BSD (T1), while the distribution and clearance were decreased when compared to that of marketed sample (S1). T1 was able to increase the 6-MNA bioavailability in 1.79-fold compared to 6-MNA from TSD (T2). T1 increased the 6-MNA bioavailability by 1.26-fold while comparing with S1. **Conclusion:** Administration of BSD (T1) increased the systemic exposure level of 6-MNA *in vivo* with elevated AUC, whereas, TSD (T2) decreased both. The T_{max}, V_d and Cl of 6-MNA was found to be higher for T2 compared to T1 and S1, suggesting a possible distribution of 6-MNA to the high affinity synovial fluid which is the site of action in case of OA or RA. However, this has to be confirmed with further studies.

Key words: Binary and ternary solid dispersions, Nabumetone, 6-MNA, bioavailability

Introduction

Nabumetone (NBT) is a non-acidic prodrug that undergoes hepatic first pass metabolism by cytochrome enzymes to produce the active antiarthritic metabolite 6-MNA. It was first synthesized in laboratory at research division of Beecham pharmaceuticals. The compound when tested in Wistar albino female rats has shown significant anti-inflammatory activity (Goudie et al., 1978). The parent compound was not detected in urine or plasma of experimental animals like rats, mice, rabbits, rhesus monkeys or even healthy human subjects and the major circulatory metabolite was identified as 6-MNA with known anti-inflammatory activity. Metabolic pathways involving O-

demethylation, reduction of the ketone group and oxidation of the butanone side chain to acetic acid occurred in all species but with different ratios of metabolic end-products. In rat about half of the administered dose was oxidized to 6-MNA (Haddock et al., 1984). Unchanged NBT is usually not detectable in plasma after oral administration. 6-MNA is extensively bound to plasma proteins, greater than 99% and it diffuses readily into synovial fluid, achieving peak concentrations in 4 to 12h. The terminal phase half-life of 6-MNA is about 24h. Plasma concentrations of the active metabolite are increased 1.5-to 2-fold in elderly arthritic patients compared with healthy young subjects after oral administration of NBT 1000mg (Friedel and Todd, 1988). Co-administration with food enhances the bioavailability of NBT, in contrast to other NSAIDs and the reason may be attributed to the increased splanchnic blood flow by food results in faster first-pass metabolism of NBT. 6-MNA was reported to penetrate inflammatory exudates as it is having acidic characteristics and excellent protein binding capability (Haddock et al., 1983).

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HPLC was proved to be a better technology to detect plasma concentration of 6-MNA even in low levels (Al-Momani, 1997). A number of experiments were performed using HPLC to quantify the metabolite in plasma as well as urine (Mikami et al., 2000; Wanwimolruk, 1990; Ray and Day, 1984). Modified HPLC methods were employed for determination of 6-MNA in human plasma (Jang et al., 1995; Kobylińska et al., 2003). Radiotracer and auto-radiographic methods were also used to study the bio-distribution pattern of 6-MNA and its pharmacokinetic profile (Haddock et al., 1984, 1983). Simultaneous determination of 6-MNA and herbal components were also reported using modified HPLC method (Balap et al., 2016). Binary and ternary solid dispersions of NBT prepared by melt-method that exhibited better product characteristics upon evaluation were chosen for in-vivo studies to find out the bioavailability. The results were compared with that of commercially available tablet dosage form. Female Wistar albino rats (three groups with four animals in each) were selected for BA study. Same sex was desired to maintain similar metabolic pattern throughout the study. Plasma samples were collected periodically from the tail vein and tested using HPLC for quantification of 6-MNA. Studies were designed in such a way that the animals will receive same amount of the active substance, irrespective of the dosage form through dose calculation.

Materials and methods

Nabumetone (Divis Laboratories Ltd., Hyderabad), Polyethylene glycol 4000 (Merck speciality chemicals, Mumbai) and Glucosamine (Sonia Organics, Bangalore) were used to prepare BSD and TSD. BSD and TSD along with commercial tablets procured from the market (Nabumetone tablets USP 500mg; Fannin UK Ltd.) were used in the study. 6-MNA standard was procured from Cayman Chemical, USA (Item No: 70620; Batch: 192292). All other chemicals used were of HPLC grade.

Animals

Female wistar albino rats weighing 200-250g were selected for study and obtained from CARE Keralam Ltd., Koratty, Thrissur. The animals were 12-14 weeks old and were identified using cage cards and picric acid body marking. Animals were housed under standard laboratory conditions with individual ventilated cage system (Air changes 15 per hour), room temperature 22±3°C, relative humidity 50-60%, with 12h light and 12h dark cycle. Single animals were housed in a standard poly-sulphonate cage (Size: L 300 x B 170 x H 140 mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. Sterilized paddy husk was provided as bedding material. The animals were acclimatized for a minimum period of five

days to laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was recorded on the day of receipt and on 5th day of acclimatization. The animals were fed *ad libitum* throughout the acclimatization and study period. Laboratory rodent feed (Manufactured by Feed mill of School of Animal Nutrition and Feed Technology, Kerala Veterinary & Animal Sciences University, Mannuthi, Thrissur) was provided. Water was provided *ad libitum* in polycarbonate water bottles with stainless steel sipper tubes.

Animal Protocol

A total of twelve female wistar albino rats (200-250g) were utilized in this study. Animals were of same sex to maintain uniformity in metabolic changes. Animals were maintained under standard laboratory conditions (22±3°C room temperature and 50-60% humidity) with alternating light and dark cycles of 12hrs and provided with food and water (*ad libitum*). The rats were fed with pellet diet and were acclimatized to laboratory conditions for 5 days prior to the experiment. Animals were divided into three groups of four animals each (n = 4) and were treated as mentioned in table 1.

Table 1. Animal grouping for *in-vivo* studies.

Groups	No. of animals	Treatment
I	4	Binary Solid dispersion NBT: PEG 4000 (1:4) (T1) 7.5 mg/kg body wt. per oral
II	4	Ternary Solid Dispersion NBT: GLS: PEG 4000 (1:1:1) (T2) 7.5 mg/kg body wt. per oral
III	4	Marketed sample of Nabumetone tablet (S1) 7.5 mg/kg body wt. per oral

T1, T2 and S1 were administered by oral gavage at a single dose of 7.5 mg/kg. 500µL of blood samples were withdrawn from the tail vein. The animals were anaesthetized animals using inhalant anaesthetic. Samples were collected into heparinised micro tubes at the following times: 0, 0.5, 1, 2, 4, 8, 12, 24 and 48h after dosing. The blood samples were centrifuged at 7200rpm for 10min. The supernatant were collected, transferred to tightly sealed plastic tubes and were stored at -20°C until analysis by HPLC. After each sampling the same volume were replaced with saline solution.

Sample preparation and administration

The weighed test items were suspended in distilled water to get desired concentration as per the dose (mg/kg body weight). Formulation of the test item was prepared shortly before dosing. The homogeneity of the test formulation was maintained by continuous stirring with a glass rod during dosing.

The test item was administered orally by gavage to each rat as a single dose, using gavaging needle (animal feeding needle). The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The dose volume was 1mL/100 g body weight for all animals.

HPLC Analysis

Instruments

HPLC: Agilent Technologies 1200 Infinity Series

Balance: Shimadzu

Preparation of Plasma Samples:

The frozen plasma samples were thawed out and centrifuged (2000 rpm*10min). To 0.5 ml of plasma in 15 ml glass tube, 25 μ L of 36% aqueous hydrochloric acid were added. Plasma was shortly vigorously shaken. Diethyl ether (3 ml) was added and vortexed-mixed for 1 minute. After centrifugation (3000rpm*20 minutes) the tubes were stored in a deep freezer (-34°C for 1h) until the water layer froze to ice. The organic layer containing the analyte was decanted in to another clean tube and the solvent was evaporated. The dry extract in the glass tube was reconstituted in 600 μ L of the mobile phase and transferred in to the vial of the auto sampler.

Standard Preparation:

Accurately weighed and dissolved 1.73mg 6-MNA standard in 3.5mL methanol (500ppm). 5mL of the solution was diluted to 25mL using methanol. From this 10 ppm standard was prepared.

Chromatographic conditions

Mobile phase: 0.01N acetic acid: Acetonitrile (55:45)

0.01N acetic acid: 0.28 ml acetic acid in 500 ml water

Column: C18 4.6x250mmx5 μ m

Flow rate: 1mL/Minute

Injection volume: 10 μ L

Wavelength: 225nm

Run time: 10minutes

Column temp.: 28°C

Pharmacokinetic parameters

AUC (area under the curve which is also known as plasma drug concentration versus time curve): It is an indicator of extent of absorption of an orally administered drug formulation.

C_{max}: peak plasma concentration after a single oral dose of drug.

T_{max}: Time at which the maximum concentration (C_{max}) is observed.

Ke: the first-order elimination rate constant for a one-compartment drug.

t_{1/2}: first-order elimination half-life. The time it takes for 50% of

drug present to be eliminated; it is constant for a given patient receiving a particular drug.

V_d: apparent volume of distribution. The volume of plasma required to dilute a given dose of drug, resulting in its observed plasma drug concentration.

Cl: systemic clearance of a drug is a pharmacokinetic parameter that indicates the volume of plasma from which all drug is removed (cleared) per unit time.

Pharmacokinetic parameters were estimated using the model-independent method. The terminal elimination rate constant (Ke) was estimated by a linear regression analysis of the terminal portion of the log linear blood concentration–time profile of test sample.

The terminal elimination half-life (t_{1/2}) was calculated from Ke using the formula $t_{1/2} = 0.693/Ke$. The maximum observed plasma concentration (C_{max}) and the time taken to reach it (T_{max}) were obtained from the curve plotting plasma concentration vs. time.

The area under each drug concentration time curve (AUC, h μ g/mL) to the last data point was calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of C_{Last}/Ke, where C_{Last} is the concentration of the last measured plasma sample.

The apparent body clearance (Cl) was calculated using the equation $Cl = \text{dose}/AUC$.

The apparent volume of distribution (V_d) was calculated by the equation $V_d = \text{dose}/Ke AUC$.

Statistical Analysis

The data generated from the present study were subjected to computer statistical analysis using GraphPad Prism® software, Version 5.00, USA. 2007. The values are expressed in Mean \pm SD. One way ANOVA with Dunnett's post-test was done for T1 and T2 treated groups comparing with S1 treated group. All analysis and comparisons was evaluated at 5% significance level.

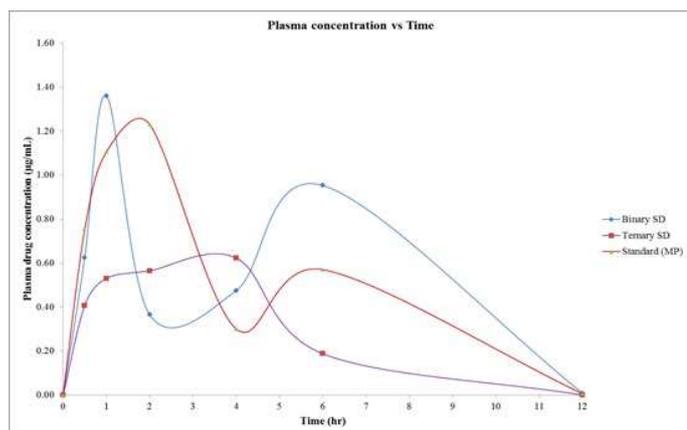
Results and discussion

The mean 6-MNA plasma concentration–time profiles after oral administrations of 7.5mg/kg of NBT in different formulations (T1 and T2), and commercial tablet (S1), were expressed in table 2.

From the above Table 2, it is evident that the metabolite 6-MNA was not traceable at 24h and above time points. Upto 12h, presence of 6-MNA was seen with some animals in the treatment group. Maximum concentration was seen from 0.5h to 6h in all groups administered with NBT. The plot corresponding to plasma concentration of various samples has been given below in figure 1.

Table 2. Plasma Concentration of 6-MNA in various samples at different time intervals

Groups	Animal No	Plasma concentration of 6-MNA ($\mu\text{g/mL}$)									
		Time intervals of blood collection \rightarrow	0h	0.5h	1h	2h	4h	6h	12h	24h	48h
Group I (T1)	1		0	0.47	1.74	ND	0.33	0.9	0.02	0	0
	2		0	0.95	0.95	0.35	0.48	0.76	0.01	0	0
	3		0	0.28	1.73	ND	0.56	1.33	0	0	0
	4		0	0.8	1.02	0.38	0.53	0.82	0	0	0
Group II (T2)	5		0	0.38	0.63	0.67	1.16	0.24	0	0	0
	6		0	0.48	0.46	0.49	0.36	0.11	0	0	0
	7		0	0.36	0.58	0.77	0.68	0.27	0	0	0
	8		0	ND	0.45	0.33	0.29	0.13	0	0	0
Group III (S1)	9		0	0.47	1.16	0.95	0.34	0.53	0	0	0
	10		0	1.19	0.99	1.51	0.22	0.7	0.02	0	0
	11		0	0.47	1.02	ND	0.26	0.33	0	0	0
	12		0	0.88	1.25	ND	0.38	0.72	0	0	0

**Figure 1.** Pharmacokinetic parameters following single oral administration of different NBT samples.

The raw data obtained were subjected to statistical evaluation and calculated various pharmacokinetic parameters and described in table 3.

Table 3 summarizes the relevant pharmacokinetic parameters. After the oral administration of S1, the drug was absorbed

quickly, and a maximum plasma concentration (C_{max}) of 6-MNA approximately $1.235 \pm 0.206 \mu\text{g/mL}$ was reached in 1h (T_{max}). Thereafter, the 6-MNA plasma concentration decreased, as the drug was distributed and metabolized, resulting in a $t_{1/2}$, approximately 1.6h. 6-MNA was detected up to 12h after administration. In case of groups administered with T1 and T2, 6-MNA was absorbed into the circulatory system and reached its peak concentration approximately 1 and 2h respectively, after administered individually. The C_{max} of 6-MNA was increased after administration of T1 compared to group administered with S1 and 6-MNA was detected up to 12 hr after administration similar to S1. Administration of T1 also increased the C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of 6-MNA whereas decreased the $t_{1/2}$, V_d and Cl of 6-MNA when compared to group treated with S1, which were not significant statistically. Administration of T2 decreased the C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and $t_{1/2}$ of 6-MNA whereas increased T_{max} , V_d and Cl of 6-MNA in comparison with group treated with S1, but these changes were not found to be statistically significant. Compared to S1 group, the C_{max} of T1 group

Table 3. Pharmacokinetic parameters following single oral administration of different NBT samples to Wistar albino rats

Groups	AUC_{0-t} (h $\mu\text{g/mL}$)	$AUC_{0-\infty}$ (h $\mu\text{g/mL}$)	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	K_e (1/h)	$t_{1/2}$ (h)	V_d (L/kg)	Cl (L/h/kg)
Group I (T1)	4.776 ± 1.190	5.563 ± 0.513	1.360 ± 0.434	1.00	0.546 ± 0.295	1.481 ± 0.525	3.385 ± 1.266	1.648 ± 0.414
Group II (T2)	2.830 ± 1.211	3.101 ± 1.310	0.7175 ± 0.328	2.00	0.561 ± 0.171	1.319 ± 0.372	6.258 ± 4.193	3.083 ± 1.392
Group III (S1)	3.838 ± 2.184	4.415 ± 1.912	1.235 ± 0.206	1.00	0.424 ± 0.026	1.641 ± 0.100	5.731 ± 2.699	2.443 ± 1.246

was increased 1.1 fold. The increase in C_{max} indicates that the PEG formulation was effective in increasing drug absorption, and the delayed T_{max} observed in T2 group demonstrates an obvious sustained release of 6-MNA. The clearance of 6-MNA from the T1 was 1.5-fold lower, whereas that of T2 group was 1.26 fold higher than that of S1. The T1 dispersion decreased the 6-MNA volume of distribution by 6.9-fold compared to S1. But, the $t_{1/2}$ of 6-MNA from the T1 and T2 decreased to 1.48 h and 1.32h respectively, while for S1 the $t_{1/2}$ was 1.64 h. Between the two formulations, the 6-MNA from T1 presented a relative bioavailability 1.79-fold superior to that of the 6-MNA from T2. Compared to the S1, the T1 increased the 6-MNA bioavailability 1.26-fold while T2 decreased the bioavailability of 6-MNA by 1.42-fold. Hence from the observations made in the present study, it could be suggested that administration of binary SD (T1) increased the systemic exposure level of 6-MNA *in-vivo* with elevated AUC, whereas, ternary SD (T2) decreased the systemic exposure level of 6-MNA *in-vivo* with decreasing AUC. The data indicates that some constituents of T2 might have decreased the plasma concentration of 6-MNA in rats.

In the present study, pharmacokinetic interaction of Glucosamine with NAB was observed by a marked decrease in C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of 6-MNA by administration of T2. Increase in elimination of 6-MNA was observed with reduced $t_{1/2}$ when administered with T2. Increase in Cl and Vd, also, of T2 treated group, indicated the increase in elimination. Hence, it could be said that administration of T2 decreased the systemic exposure level of 6-MNA *in vivo*.

Conclusion

In conclusion, the present study revealed that C_{max} and AUC of 6-MNA were significantly increased by administration of binary solid dispersion of NBT: PEG 4000 prepared by melt-method, while the distribution and clearance were decreased compared to that of commercially available sample of Nabumetone tablets USP 500mg (S1). T1 was able to increase the 6-MNA bioavailability in 1.79-fold compared to 6-MNA from ternary SD (T2). Compared to S1, T1 increased the 6-MNA bioavailability by 1.26-fold. Hence, it could be suggested that administration of binary solid dispersion (T1) increased the systemic exposure level of 6-MNA *in vivo* with elevated AUC, whereas, ternary solid dispersion (T2) decreased the systemic exposure level of 6-MNA *in-vivo* with decreasing AUC. The T_{max} , Vd and Cl of 6-MNA was found to be higher for ternary SD (T2) compared to T1 and S1, suggesting a possible distribution of 6-MNA to the high affinity synovial fluid which is the site of action in case of OA or RA. However, this has to be confirmed with further studies.

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Conflicts of interest: None

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