OnLine Journal of Biological Sciences 2 (11): 769-770, 2002
ISSN 1608-4127

Analysis of Blood for Secnidazole in Female Volunteers by HPLC Method

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Abstract: The biokinetics parameters were investigated following oral dose of 1g secnidazole in healthy female volunteers. The plasma samples were deproteinized by $ZnSO_4$ and NaOH analyzed by HPLC. The mean maximum concentration in plasma was found to be 23.08 ± 0.8 after 2.5 h of drug administration. The area under curve (AUC) was 349.05 ± 20.9 h mg L^{-1}. The clearance and volume of distribution were 0.44 ± 0.37 L h^{-1} and 11.09 ± 7.81 L respectively. The elimination half life was 13.86 ± 0.92 h. The values for absorption rate constant and absorption half life were 2.97 ± 0.46 L h^{-1} and 0.27 ± 0.03 h. The maximum concentration 16.24 ± 0.48 mg L^{-1} was achieved in 1.77 ± 0.16 h. These parameters showed that protein deproteinization method and analysis techniques are valid for the protein analysis of secnidazole.

Key words: Secnidazole, plasma, female volunteers, HPLC

Introduction

Protozoal infections occurs throughout the world, the most common amoebic infection of man is amoebiasis caused by *Entamoeba histolytica*, *Giardia lamblia* exists worldwide and in children it can cause serious symptoms, mainly in gastrointestinal tract leading to acute diarrhoea, then dehydration and malabsorption. Secnidazole, a synthetic derivative of nitroimidizole series 1-[(2-hydroxypropyl)-2-methyl-(5-nitroimidazole)] is a new therapeutic resource that is effective and well tolerated for the safe treatment of amoebiasis and giardiasis and can be administered in a single dose (Thami and Kanwar, 1998).

Metronidazole, the first nitroimidazole was introduced as a treatment of trichomonasis asexually transmitted disease caused by *Trichomonas vaginalis*. But secnidazole is reported to be still more effective and have a high cure rate in the treatment of human trichomoniasis. Secnidazole single dose treatment has eliminated the possibility of relapse and also facilitates the treatment for both partners in urethritis and vaginitis due to *Trichomonas vaginalis* (Saracoglu *et al.*, 1998).

Toxicity of secnidazole for animal is low (LD for mice by mouth, 2.4 g kg^{-1} body weight). When given orally to man, active concentrations persist in blood (Benazet and Guillaume, 1976).

Recommended dose of 2 g of secnidazole single administration produced mean peak concentration equal to 40.5 ± 9.4 μg mL^{-1} at 2 h in blood (Tenenbaum *et al.*, 1993).

Secnidazole is rapidly and completely absorbed after oral administration having terminal elimination half life approximately 17-29 h (Gillis and Wiseman, 1996).

Several studies have shown that biodisposition kinetics of indigenous investigation of drugs differ foreign data (Nawaz, 1994). For the analysis HPLC method was preferred over other because it was routinely used in the pharmaceutical labs for quality assurance and detection of drug (Jessa, 1996).

Materials and Methods

Volunteers: A total of 10 healthy female human volunteers having mean age 23 years and mean body weight 60 kg participated in this study.

Drug used: Secnidazole obtained as film coated tablets by the name of secnidal (secnidazole 1 g) from Rhone Poulenc Rorer Pakistan (Pvt.) Limited was used for oral administration to volunteers.

Sample collection and handling: After an overnight fasting, a control blood sample was drawn from each volunteer. Each volunteer received single oral dose of 1 g secnidazole and took standard breakfast two hours following drug administration. The venous blood was drawn in heparinized centrifuged tubes at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 h and one sample after 24 h, following oral administration of drug. The blood samples were centrifuged at 4000 rpm for 10 min and plasma was stored at -20°C till further analysis.

Preparation of working standards and plasma samples: Stock solution of secnidazole equivalent to 1 mg mL^{-1} was prepared in HPLC grade methanol. Different working standard solutions containing 0, 1, 5, 10, 20, 40 μg mL^{-1} of secnidazole were prepared from stock solution in drug free control plasma. In a 1.5 mL Eppendrof vessel, 900 μL 50% $ZnSO_4$ and 150 μL 56% NaOH added to 0.5 mL of plasma blank and plasma standard. The mixture was agitated and centrifuged (Microcentaure EYELE) for 12 minutes at 10,000 rpm. Supernatant was filtered with microsyringe using 0.2 μ pore size filter of 13 mm diameter.

Chromatographic systems and conditions: Chromatography was performed with reversed phase HPLC with U.V. detection. The pump was Jasco-PU-980 (Intelligent HPLC Pump) and UV Spectrophotometer Jasco-UV-975 (intelligent U.V/visible detector) set at 276 nm. The output of the detector was monitored with an integrator Jasco-807-IT. The column (250 x 4.6 mm^2 I.D.) packed with 5 μm ODS was used to separate the peaks. The mobile phase consisted of 10% (v/v) acetonitrile in 0.02 M sodium acetate buffer pH 4.0 (adjusted with acetic acid) (Sekhar *et al.*, 1997). The samples (20 μL) were injected into the column manually. The column temperature was maintained at 40°C using as isocratic mode. The flow rate was 1.0 mL min^{-1} the retention time was 9.366 min in plasma.

Pharmacokinetics parameters: The data on plasma concentration at different time intervals was analyzed by single compartment model (Baggot, 1977) using computer Programme MW, PHARM Version 3.02 (Rombout, 1987).

Results and Discussion

The average $\pm$SE values for plasma concentrations of secnidazole at various time intervals have been plotted in Fig. 1.

The plasma concentration at 0.5 h was 8.76 ± 1.01 μg mL^{-1} which increases with the passage of time due to absorption. The mean maximum plasma concentration 23.08 ± 0.8 μg mL^{-1} was achieved at 2.5 h. When elimination began, the plasma concentration began to decrease and after 24 h there was mean value of 6.26 ± 0.47 μg mL^{-1}. The mean plasma concentration of secnidazole after 24h was 7.18 ± 0.44 μg mL^{-1} in male volunteers. But the mean maximum plasma concentration 22.76 ± 1.31 μg mL^{-1} was achieved after 4 h (Shahid, 2001). Biokinetics data was analyzed according to one compartment model. Mean $\pm$SE values of biokinetics parameters are given in Table 1.

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Table 1: Mean ± SE values of biokinetics parameters of secnidazole following oral administration of 1g secnidazole

Parameters	Mean ± SE	Minimum	Maximum
Area under the curve (AUC) [h mg L^{-1}]	349.05 ± 20.90	328.15	369.95
AUC polyexponential (t = 24)	241.17 ± 7.31	233.86	248.48
AUC trapezoidal rule (t = 24)	5.14 ± 228.50	238.50	237.78
Clearance (CL)	0.44 ± 0.31	0.13	0.75
Volume of distribution (Vd) [L]	11.09 ± 7.81	3.28	18.90
Elimination Half-life ($t_{1/2}$ ß) [h]	13.86 ± 0.92	12.94	14.78
Rate constant (K10) [L h^{-1}]	0.05 ± 0.002	0.048	0.052
Mean residence time (MRT) [h]	20.61 ± 1.36	19.25	21.97
Absorption rate constant (Ka) [L h^{-1}]	2.97 ± 0.46	2.51	3.43
Absorption half life ($t_{1/2}$ α) [h]	0.27 ± 0.03	0.24	0.30
Lag-time (T_0) [h]	0.22 ± 0.05	0.17	0.27
Time to peak (T_{max}) [h]	1.77 ± 0.16	1.61	1.93
Peak concentration (C_{max}) [mg L^{-1}]	16.24 ± 0.48	15.76	16.72

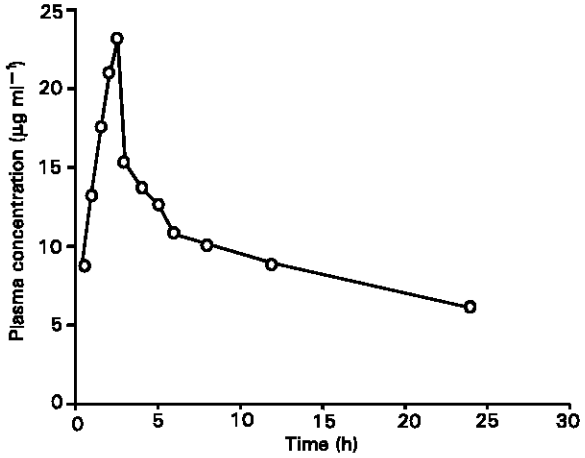

Fig. 1: Average representation plot of plasma concentration versus time after oral administration of 1 g secnidazole tablet

The area under curve (AUC) was 3.49.05 ± 20.9 h mg L^{-1}. Whereas the area under curve in male colunteers was 674.49 ± 71.74 h mg L^{-1} (Shahid, 2001). The difference may be due to difference in absorption, the dosage of medicine, biochemical and physiological variations. The clearance (Cl) of secnidazole in female volunteers was 0.44 ± 0.31 L h^{-1} while in male it was 1.605 ± 0.19 L h^{-1} (Shahid, 2001). The volume of distribution (V_d) female volunteers 11.09 ± 7.81 L. Shahid (2001) calculated the volume of distribution of secnidazole in male volunteers and the value was 60.26 ± 5.39L. Elimination half life ($t_{½}$ß) in present study was 13.86 ± 0.92 h.

Tenenbaum *et al.* (1993) reported that apparent elimination half life was 28.8 h in blood after taking 2 g tablet of secnidazole.

Gills and Wiseman (1996) reported that secnidazole has a longer terminal elimination half life approximately 17-29 h. Absorption half life ($t_{½}$α) was 0.27 ± 0.03 h in female volunteers whereas in male it was 1.087 ± 0.19 h (Shahid, 2001).

Absorption rate constant (Ka) in present study was 2.97 ± 0.46 L h^{-1}. Shahid (2001) calculated the absorption rate constant of the same drug and its value was 0.812 ± 0.169 L h^{-1} in male. The present result differs from previous study.

Time to peak concentration (T_{max}) of secnidazole in female was 1.77 ± 0.16 h. Shahid (2001) reported 5.028 ± 0.65 h for 1 g; Tenenbaum *et al.* (1993) 2-3 h (2g tablet) and Sekhar *et al.* (1997) reported 2.67 ± 1.11 h (1g).

Peak plasma concentration (C_{max}) in present study of secnidazole was 16.24 ± 0.48 mg L^{-1} C_{max} in male volunteers was 14.95 ± 1.012 mg L^{-1} (Shahid, 2001). Sekhar *et al.* (1997) calculated C_{max} in six male volunteers was achieved having value 25.68 ± 3.44 mg L^{-1}.

Tenebaum *et al.* (1993) calculated the C_{max} 40.5 ± 9.4 µg mL^{-1} of 2 g of secnidazole orally.

The results of this study indicated that biokinetics parameters in young healthy female volunteers have variation range with the similar studies conducted elsewhere because of geodetical influence (Nawaz *et al.*, 1988).

So the study supports the need for comprehensive evaluation of drug under our own environmental conditions to obtain biodisposition kinetic parameters on which rational dose regimens of drug could be based.

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