A COMPARISON OF METRONIDAZOLE DISTRIBUTION FOLLOWING INTRAVENOUS METRONIDAZOLE AND METRONIDAZOLE PHOSPHATE DISODIUM IN MICE

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SUMMARY

The distribution of metronidazole following intravenous metronidazole (MTZ) and metronidazole phosphate disodium (MNP) in mice was investigated by HPLC. The results showed no significant differences in the metronidazole concentrations of liver, kidney, heart, spleen, fat, brain, lung, stomach, jejunum, skeletal muscle, ovary, testis and epididymis ($P > 0.05$). It can be concluded that using MNP as a water-soluble substitute for MTZ should not influence the distribution of metronidazole in mice.

KEY WORDS

metronidazole, metronidazole phosphate disodium, distribution, mice

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INTRODUCTION

Metronidazole (MTZ) has diverse uses including treatment or prevention of anaerobic bacterial infections, *Trichomonas* vaginitis, amebiasis, giardiasis, balantidiasis, dracunculiasis, cutaneous leishmaniasis /1, 2/ and as a radiosensitizer of hypoxic tumor cells /3/. In some situations a high concentration injectable solution is necessary, especially as a radiosensitizer, because in radiotherapy a large dose of metronidazole, 6 g/m^2 or more, is required and oral administration of this large dose frequently causes severe nausea and vomiting which sometimes is difficult to control with antiemetics. Due to its low solubility in water a high concentration injectable solution is not available. To improve its water-solubility a series of water-miscible cosolvents, solubilizing agents and water-soluble pro-drugs have been suggested /4, 5, 6/. Among these the phosphate ester disodium salt (metronidazole phosphate disodium, MNP) is one of the best candidates because it is freely soluble in water, stable *in vitro* at physiological pH and easily hydrolyzed to the parent compound *in vivo*.

It has been demonstrated in our laboratory that the half-life of hydrolysis of MNP to therapeutic concentrations of MTZ was 4 min in rabbits (to be published). For developing a high concentration injectable solution an injectable powder of metronidazole phosphate disodium salt was prepared in our laboratory. In this paper we report a comparison of metronidazole distribution following intravenous metronidazole and metronidazole phosphate disodium in mice.

MATERIALS AND METHODS

Chemicals

Metronidazole was obtained from Wuhan Pharmaceutical Factory with a purity of 99.6% and which met the standards of C.P. A 0.5% solution of metronidazole for injection was prepared in our laboratory.

Metronidazole phosphate disodium salt was synthesized by our research group and its chemical structure was ascertained by elemental analysis, UV, IR and NMR. The solution for injection which contained 0.915 g of MNP (equal to 0.5 g of MTZ) per 3 ml was freshly

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prepared in our laboratory by an aseptic technique for administration on the same day.

All other chemicals used in this study were of reagent grade.

Mice experiment

Twelve Kun-Ming mice (weight 23±3.7 g), equal numbers of male and female, were supplied by the Experimental Animal Center of West China University of Medical Sciences and randomly separated into two groups. The mice of one group were injected with MTZ solution at the dose of 0.1 mg/g via the tail vein, the other group was injected with MNP at the same parent dose (0.183 mg of MNP/g). Three hours after administration the mice were sacrificed. Blood was collected, heparinized and centrifuged, and the plasma was stored at -20°C until analysis. The liver, kidney, heart, spleen, fat, brain, lung, stomach, jejunum, skeletal muscle, ovary or testis and epididymis were removed, cleaned and frozen at -20°C for future analyses.

Measurement of metronidazole

Apparatus and HPLC conditions

A Shimadzu LC-6A high-performance liquid chromatograph equipped with a SPD-6A variable wavelength UV detector, a Model 7125 injection valve with a 20 μl loop and a C-R4A chromatopac for data analysis was used. The column (150 mm by 4.5 mm) and pre-column were packed with octadecylsilane (C18).

The mobile phase contained 0.1 M phosphate buffer solution and methanol (8:2), used at a flow rate of 1 ml/min. The absorbance detector was set at 319 nm and attenuation was set at 0.02 absorbance units full scale; the column oven temperature was 40°C. Under these conditions the retention time of metronidazole was 6.5 min without interference from blank tissues or metabolites.

Procedure

The isolated tissues and organs were cut into small pieces, of which an ascertainable amount (about 100 mg) was weighed accurately, homogenized and transferred to a centrifuge tube to which 900 μl 0.1 M zinc sulfate solution was added. The extractions with 1 ml of acetic

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ether were repeated twice on a vortex mixer. The combined extracts were evaporated to dryness on a water-bath at 37°C under a stream of dry nitrogen. The solid residue was redissolved in 100 μl methanol and the solution was centrifuged for 5 min at 10,000 rpm. 8 μl of supernatant was injected onto the HPLC.

RESULTS

Reliability of measuring method

Chromatograms of metronidazole in mouse tissues following intravenous MTZ or MNP are shown in Figure 1.

In these assays an external standard method was used. A standard curve was prepared on every experimental day by adding standard solutions of drug to the blank tissues and assaying the standards in the same manner as mentioned above. The standard curve was constructed by plotting the peak area against added standard amount. In the concentration range of 0.4-20.74 μg/ml the correlation coefficients of different tissues and days were over 0.992.

The absolute recoveries were estimated by determining the added standards in blank tissues and in final solvents. The results obtained are shown in Table 1.

Precision of the measurements was evaluated by repeated determination of the same samples. The results from Table 1 show that the coefficients of variation (CV) within the day were 1.8-7.7% (n = 4) for the high concentration (20.74 μg/ml) and 3.3-11.1% (n = 4) for the low concentration (1.04 μg/ml), respectively. The coefficients of variation among days were 7.2% (n = 5) for the intestinal samples at the concentration of 20.74 μg/ml and 8.8% (n = 4) for the liver samples at the concentration of 1.04 μg/ml.

Comparison of tissue levels of metronidazole

The comparison of tissue levels of metronidazole following intravenous MTZ or MNP are shown in Table 2 and Figure 2.

Student's t-test was used to decide whether the mean differences were significant, and no significant differences were found in the tissues following intravenous MTZ or MNP. The probability values are shown in Table 2 (> 0.05).

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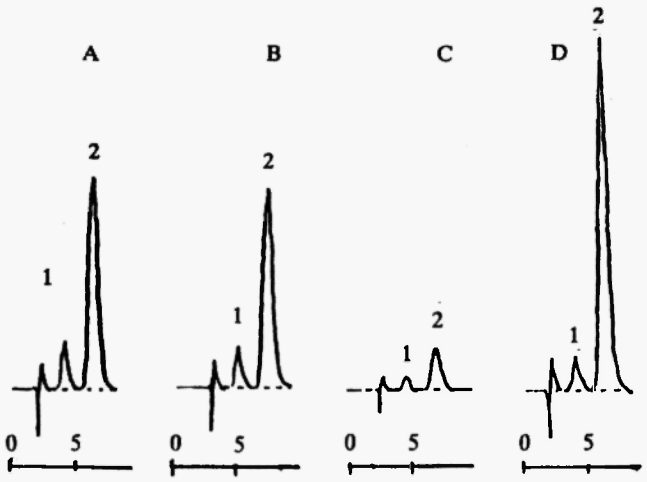

Fig. 1: Chromatograms of metronidazole in mouse tissues following i.v. MTZ or MNP. A: Blood, B: Kidney, C: Liver, D: Stomach. 1: Metabolite, 2: Metronidazole.

TABLE 1
Absolute recovery of metronidazole following i.v. MTZ or MNP in mice (n = 4)

	Level (20.74 μg/ml)		Level (1.04 μg/ml)	
Tissues	**mean±SD**	**CV%**	**mean±SD**	**CV%**
Testis	61.4±4.4	7.2	56.6±1.8	3.3
Epididymis	68.5±7.0	10.2*	62.9±3.3	5.1*
Blood	58.4±1.4	2.3	62.2±6.9	11.1
Plasma	57.4±2.0	3.5	59.4±4.2	7.0
Liver	57.1±1.5	2.6	56.7±5.0	8.8
Kidney	60.9±2.4	3.9	57.2±3.5	6.1
Heart	62.9±4.5	7.2	64.5±6.8	10.5
Spleen	66.6±1.2	1.8	63.2±6.2	9.7
Fat	60.2±4.4	7.3	59.5±4.2	7.1
Brain	59.4±1.7	2.8	55.8±5.5	9.8
Lung	65.3±2.1	3.2	63.4±4.3	6.8
Stomach	61.2±2.6	4.2	59.4±4.0	6.7
Jejunum	62.4±4.5	7.2	59.2±5.9	9.9
Muscle	61.3±4.7	7.7	59.5±4.6	7.8
Ovary	67.6±2.4	3.6*	64.0±3.7	5.8*
mean	62.1±3.6	5.7	60.2±2.9	4.9

*n = 2

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TABLE 2
Comparison of tissue levels of metronidazole following i.v. MTZ or MNP in mice (n=6)

Tissues	MNP mean±SD	MTZ mean±SD	P
Blood	12.04±3.07	13.44±6.51	0.71
Plasma	11.70±3.86	14.00±6.64	0.57
Heart	8.73±3.56	7.98±4.92	0.81
Liver	4.58±2.49	3.38±0.78	0.39
Kidney	10.13±3.48	9.02±2.88	0.64
Spleen	9.02±2.19	8.76±2.38	0.88
Lung	7.07±2.90	7.93±2.09	0.65
Stomach	11.32±1.65	11.31±3.96	1.00
Jejunum	3.34±1.14	5.07±1.66	0.06
Brain	9.05±2.35	9.13±3.08	0.96
Muscle	9.05±3.20	8.95±3.08	0.96
Fat	3.83±2.16	2.05±2.16	0.18
Ovary	8.96±1.50*	7.91±6.26	>0.05
Testis	12.43±3.38*	10.97±4.78	0.69
Epididymis	9.59±3.72*	6.61±1.71	0.28

Unit: μg/100 mg or μg/100 ml

*n=3

DISCUSSION

In previous experiments in our laboratory it has been demonstrated that the half-life of hydrolysis of MNP to MTZ was 5 min in rabbits, and no MNP could be detected one hour after i.v. MNP. In the present distribution study it was difficult to measure quantitatively the amounts of MNP in tissues, although a method of measuring both components in the same sample was developed in our laboratory.

Although the distribution of metronidazole following a single i.v. injection of MNP has not been reported up to now, Placidi and his colleagues published the distribution of ^{14}C-metronidazole in mice following a single i.v. injection of MTZ /7/. Their results are similar to ours, except that lower levels of MTZ were found in the liver in our experiments. It is possible that the radiochemical method did not

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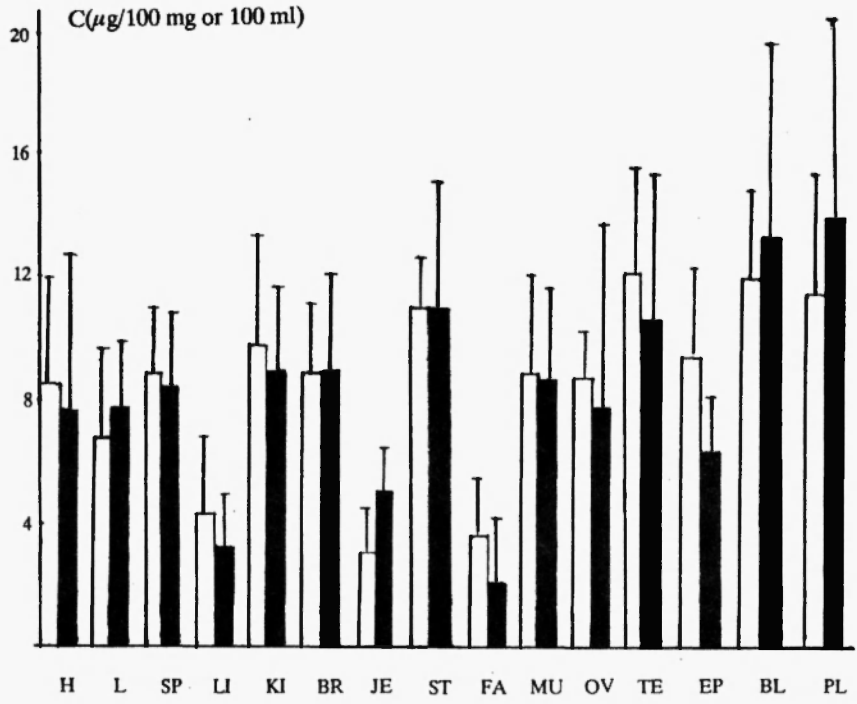

Fig. 2: Comparison of tissue levels of metronidazole following i.v. MTZ (■) or MNP (□) in mice. Vertical bar represents standard deviation. H: heart, L: lung, SP: spleen, LI: liver, KI: kidney, BR: brain, JE: jejunum, ST: stomach, FA: fat, MU: muscle, OV: ovary, TE: testis, EP: epididymis, BL: Blood, PL: plasma.

separate metronidazole from its metabolites, and the liver is the main site of drug metabolism.

Because there were no significant differences of metronidazole distribution following i.v. MTZ or MNP, it can be concluded that using MNP as a water soluble MTZ substitute should not influence the distribution of metronidazole in mice. More experiments need to be carried out to extend this work to other species including man.

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