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Long-Term Effects of Mesocaval Shunt on the Pharmacokinetic Parameters of Metronidazole in Lewis Rats

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ABSTRACT
Portacaval shunts are commonly used to prevent gastroesophageal variceal bleeding secondary to portal hypertension. Ammonia encephalopathy is a common complication and it's management includes antibiotics such as metronidazole, neomycin and recently, rifaximin. Metronidazole is often used in this context but its pharmacokinetics may be altered in these patients. Compare the pharmacokinetic parameters of two groups of rats, one of them after one year of having undergone mesocaval shunt. A randomized, experimental, prospective study in twenty adult male Lewis rats divided into two groups. The first group underwent mesocaval shunt and the other was the control group. Analyses of the following parameters were performed in both groups: (1) Pharmacokinetics of metronidazole by high-performance liquid chromatography, (2) Transaminase levels and (3) Body and liver weight. Differences between the MCS group and the control group were found for the pharmacokinetic parameters: Cmax ($p = 0.004$), Ka ($p = 0.009$), Ka t1/2 ($p = 0.007$), Vd ($p = 0.01$), AUC ($p = 0.05$) and tmax ($p = 0.01$) and in ALT levels ($p = 0.03$) and liver weight ($p = 0.00$). The results obtained in this experimental model during one year of mesocaval shunt led us to suggest that metronidazole dosage should be decreased, without altering the administration schedule, because the drug levels reached in the mesocaval shunt group caused the animals to behave as if they had been given higher doses.

Key words: Lewis rat, long-term effect, mesocaval-shunt, metronidazole, pharmacokinetics parameters

INTRODUCTION

Acute Hepatic Encephalopathy (HE) is associated with portosystemic shunts, in which portal venous blood does not flow normally through the liver. Therefore, products absorbed in the gastrointestinal tract and not properly eliminated and they pass into the systemic circulation system. Some of these products cause reversible neurological dysfunction (Colombato, 2007; Martin, 1993; Sereda and Adin, 2005). A rat model with portacaval shunts and chronic liver failure is considered the best animal model (Butterworth *et al*., 2009).

In the therapeutic management of these ammonia encephalopathies used antibiotic such as metronidazole (Martin, 1993; Dbouk and McGuire, 2006; Shah and Kamath, 2006).

Metronidazole is effective in eradicating anaerobic Gram-negative and Gram-positive bacilli (Muller, 1983; Phillips and Stanley, 2007; Lofmark *et al*., 2010). This drug is absorbed rapidly, low plasma protein binding and less than 20% is metabolised in the liver and its elimination is renal. The main products, excreted in rat urine are metronidazole, its glucuronide and sulphate conjugates (Farrell and Zaluzny, 1983; Muller, 1983; Martin, 1993; Wibawa *et al*., 2001). Because there are reports in the literature that patients with various degrees of hepatic impairment due to cirrhosis or chronic hepatitis, may suffer intoxication when treated with metronidazole even at standard doses, is necessary to determine if this type of pathologies with anatomical and physiological changes cause alterations in the pharmacokinetics of metronidazole, for this to have with the

pharmacokinetic criteria for an adjustment of dosage of this drug in these patients and avoid problems of intoxication; under the hypothesis the Mesocaval Shunt (MCS) affects the pharmacokinetics of metronidazole in rats after a year of surgery. Therefore the aim of the study was to determine whether differences exist in the pharmacokinetic parameters of metronidazole in two groups of elderly rats mesocaval shunt and laparotomized after a one year of surgery.

MATERIALS AND METHODS

A randomized, experimental, prospective study in twenty male Lewis rats, weighing 250 g, was performed from the biotherium of the "Dr. Joaquín Cravioto" Research Tower at the National Paediatric Institute (INP), Mexico. At 85±5 days of age, two groups of 10 animals each were randomized using a random number table in group A underwent mesocaval shunt and group B underwent only laparotomized (control). The present study was approved by the Institutional Research Committee and Internal Committee for the Care and Use of Laboratory Animals of the INP. This study adhered to the regulations of the NOM-062-ZOO-1999 Official Mexican Standard and the Guide for the care and use of experimental animals.

Anaesthetic procedure: After fasting for 12 h, all animals were anaesthetised with 3 mg kg^{-1} xylazine (Rompun®, Bayer, Germany), 35 mg kg^{-1} ketamine (Anesket®, PiSA, Mexico) and 0.05 mg kg^{-1} atropine (Atropisa®, PiSA, Mexico) intramuscularly to perform the MCS and control procedures and to place a central catheter for blood sampling.

Surgical procedure: To achieve a controlled reduction of hepatic portal blood flow through a portosystemic shunt in the experimental group, the technique described by Yang *et al.* (1994) and previously standardised in our laboratory was used, in which the liver is not fully deprived of splanchnic blood flow. This condition is similar to the "Spontaneous" portosystemic shunt observed in patients with cavernous transformation of the portal vein.

Once the animals recovered from the residual effects of the anaesthetic, they were placed in individual plastiglass boxes in an environment with a controlled temperature and 12 h light/dark cycles. Later, they were allowed to consume food and water *ad libitum* (Laboratory Rodent Diet®, USA 5001 with 23% protein). They remained under observation and their weight was recorded every third day for a one year.

At the end of this period, both groups were subjected to a pharmacokinetic study and liver function blood tests. For these tests, venesection from the right external jugular vein was performed in each rat and a Silastic® catheter 20 was introduced, the end of which was exteriorised at the nape. Upon the completion of the study, all animals were euthanised by an overdose of 120 mg kg^{-1} sodium pentobarbital (Pisabental® PiSA, Mexico) and the livers were extracted and weighed.

Pharmacokinetic study and assay of transaminases: The technique described by Guille *et al.* (2005) was used, administering a dose of 60 mg kg^{-1} metronidazole (Flagyl® Kendrick, Mexico) through an orogastric tube. Through the catheter placed in the jugular vein, 0.5 mL of blood was drawn for the determination of Alanine Transaminase Enzyme (ALT) and aspartate aminotransferase enzyme (AST) as markers of liver function. Subsequently, 12 samples of 0.3 mL each were drawn at 0, 0.25, 0.50, 1, 2, 3, 4, 8, 12, 15, 18 and 24 h after antibiotic administration.

Analytical procedure: Metronidazole plasma concentrations were determined by HPLC with the method proposed by (Hackett and Dusci, 1979) which was previously validated, with the following results: Linear range of 1-50 mcg mL^{-1}, recovery of 100.33±3.5%, accuracy of 99.7±1.28% and limit of detection of 0.5 mcg mL^{-1} (Guille *et al.*, 2005).

To determine ALT and AST concentrations, semi-automatic Microlab 200 Merck equipment was used.

Pharmacokinetic analysis: The pharmacokinetic profiles of metronidazole in plasma were prepared from the data for concentration versus time which were adjusted by the nonlinear regression method using the weighted least-squares procedure (I/C) with WINNONLIN software version 1.1. The fit of the experimental data in the pharmacokinetic model was assessed by the following criteria: sum of squares of the residuals, the F test, Akaike's information criterion (Yamaoka *et al.*, 1978) and lack of systematic deviations from the fitted curves. All kinetic studies were fitted to a one-compartment open model (Gibaldi and Perrier, 1982).

Liver weight: Each extracted liver was immediately weighed on an analytical Sartorius scale with accuracy to 0.01 g.

Statistical analysis: Descriptive statistics was performed. Student's t-test for non-paired samples was used for the following variables: Body weight, liver weight, tmax, Vd, Cl, t1/2 ka and k 10 and values are represented in the tables as X±SD; the Mann-Whitney U test was used for data with large dispersion (ALT, AST, t1/2 ke, k 01, AUC and Cmax). The Statistical Package for the Social Sciences (SPSS) version 19 Inc, Chicago IL USA was used.

RESULTS

All animals survived to complete the study. Table 1 shows the somatometric characteristics and serum transaminase levels of the rats.

There were no significant differences in body weight; however, liver weight was lower in group A than in group B (10.93±2.22 vs. 18.77±2.54 g).

Serum ALT levels were higher in group A compared to group B (70.5 vs. 36 U L^{-1}), respectively. No significant differences were found in serum AST levels; however, the values were greatly scattered in both groups.

Table 1: Somatometric characteristics and serum transaminase levels of the studied rats

Variables	Group A (MCS)	Group B (control)	p-value
Age (days)	381.00±28.86	385.25±13.45	
Weight (g)	492.00±81.01	545.00±78.57	0.07*
Hepatic weight (g)	10.93±2.22	18.77±2.54	0.000*
Variables	Median (v-v+)	Median (v-v+)	p-value
ALT (U L^{-1})	70.5 (23-107)	36 (22-56)	0.03***
AST (U L^{-1})	222 (115-277)	153 (110-360)	0.06***

Somatometric data was normally distributed and therefore, they are presented as the median and standard deviation (X±SD). Serum transaminase levels were not normally distributed and are represented as the m: Mean, v-: Minimum and v+: Maximum values. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, U/L: Units/litre, *Student's t-test, ***Mann-Whitney U test

Table 2: Pharmacokinetic parameters of the studied rats

Pharmacokinetic parameters	Group A (MCS) X±SD	Group B (control) X±SD	p-value
t_{max} (h)	4.73±1.33	6.38±1.32	0.01*
Vd (mL g^{-1})	1.08±0.322	1.41±0.221	0.01*
Clt (mL g^{-1} h^{-1})	0.16±0.065	0.23±0.082	0.06*
$t_{1/2}k_a$ (h)	2.77±1.36	4.43±0.917	0.007*
k_{10} (h)	0.156±0.058	0.16±036	0.47*
Variables	Median (v-v+)	Median (v-v+)	p-value
$t_{1/2}k_e$ (h)	4.29 (2.79-13.1)	4.64 (3.24-5.59)	0.45***
k_{01} (h)	0.161 (0.052-0.248)	0.148 (0.122-0.211)	0.009***
AUC (µg mL^{-1} h^{-1})	375.97 (225.98-774.54)	299.81 (164.97-439.26)	0.05***
C_{max} (µg mL^{-1})	27.64 (14.82-46.36)	15.62 (12.42-21.07)	0.004***

t_{max}: Maximum time, V_d: Volume of distribution, Cl_t: Clearance, $t_{1/2}k_a$: Absorption half-life and k_{10} were normally distributed and are presented as the mean and standard deviation. The parameters of $t_{1/2}K_e$: Elimination half-life, constant k_{01}, AUC: Area Under the Curve and C_{max}: Maximum concentration were not normally distributed and are represented as the median, v-: Minimum and v+: Maximum values. *Student's t-test, ***Mann-Whitney U test

Table 2 shows the pharmacokinetic parameters of both groups and there were significant differences between the groups in most parameters. The volume of distribution was lower in group A versus group B ($p = 0.01$) and the half-life of absorption was faster in the MCS group compared to the control group ($p = 0.007$). Conversely the rats with MCS reached a higher C_{max} and in a less time ($p = 0.004$) and t_{max} ($p = 0.01$), the AUC is bigger in group A versus the group B ($p = 0.05$) and the group of the rats with MCS has a clearance more accelerated than the control group ($p = 0.06$).

DISCUSSION

The treatment of HE is complex and depends on the conditions of each individual. A common strategy is to modify the intestinal bacterial flora with antibiotics. Neomycin, metronidazole, vancomycin and rifaximin have been shown to be effective in the acute and chronic treatment of HE (Poh and Chang, 2012).

The choice of antibiotics is still controversial and there are limited studies on adverse events and bacterial resistance (Patidar and Bajaj, 2013). Loft *et al.* (1987) described an increased risk of peripheral neurotoxicity due to prolonged metronidazole elimination in patients with HE; nevertheless, it is still in use.

In a previous study comparing healthy rats to rats subjected to portacaval shunts, it was shown that the pharmacokinetics of metronidazole changed. Increases in the maximum concentration (Cmax) and elimination half-life (t1/2 kel), as well as fast clearance (Clt) (Guille *et al.*, 2005), were observed in the rats subjected to portocaval shunts. This model behaves in a similar way to patients with HE (Butterworth *et al.*, 2009) which confirms the result reported by Loft *et al.* (1987).

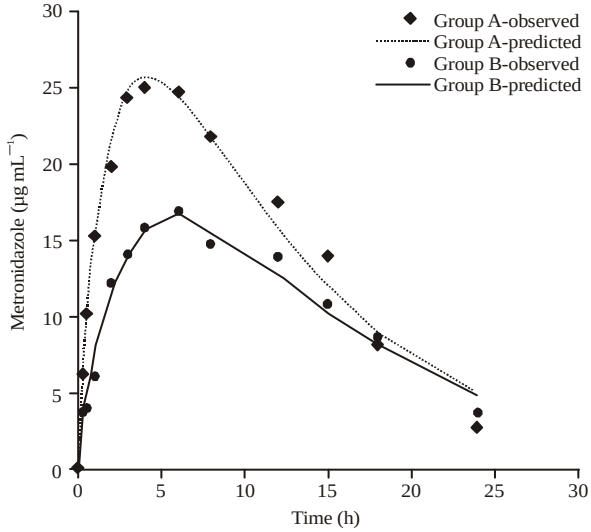

Fig. 1: Pharmacokinetic profiles of both groups

In the present study, rats with MCS showed marked changes in most pharmacokinetic parameters such as absorption and volume of distribution; the most obvious changes were found in plasma drug concentrations (Fig. 1) which were almost double the levels achieved by the control group. These levels were also achieved faster. There were no changes in the elimination of the drug.

The high metronidazole concentration found in the rats with portacaval shunts is consistent with the results of Aller *et al.* (2008) in rats after one month of portacaval shunt which showed that the REDOX system (oxidoreduction) activity decreased; therefore, the hepatic metabolism of this drug is altered. The first-pass effect is decreased due to the

mesocaval shunt. Therefore, once the drug is absorbed through the digestive tract, most of the antibiotic reaches the circulation system unchanged and is removed in the same state (Aller *et al*., 2008). A similar phenomenon occurs in patients in whom portal venous blood does not normally flow through the liver because of partial prehepatic obstruction or portosystemic shunts. Therefore, the products absorbed from the gastrointestinal tract such as amino acids, free fatty acids, mercaptans and ammonia produced by the intestinal bacterial flora are not properly metabolised or purified and their levels rise in the circulation system (Tams, 1985; Martin, 1993; Blei and Cordoba, 2001; Sereda and Adin, 2005; Colombato, 2007).

The present research shows that the duration of the mesocaval shunt can further modify the pharmacokinetics of metronidazole and possibly increase the risk of adverse events with which it is associated. Conversely, decreased liver weight and increased liver function enzymes were found in rats with MCS which were attributed to liver damage resulting from nutrient deprivation, low liver perfusion, decreased hepatic oxidative metabolism and a 25% loss in deoxyribonucleic acid (Takemura *et al*., 2006; Aller *et al*., 2012). The aim of this study was only to determine whether differences exist in the pharmacokinetic parameters of this drug in two groups of elderly rats (MCS) and laparatomized after a one year of surgery.

CONCLUSION

The results obtained in this study suggest that metronidazole dose adjustment should be considered in individuals undergoing mesocaval shunt for an extended period of time due to the risk of adverse events associated with high concentrations of this drug.

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