BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **19**: 479–482 (1998)

SHORT COMMUNICATION

In Vitro Dissolution and Urinary Excretion Study of Metoclopramide Tablets

Pablo A.M. Quiroga, Patricia M. Bustillo and María G. Volonté*
Cátedra de Ensayo y Valoración de Medicamentos, Division Farmacia, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115, 1900 La Plata, Argentina

Key words: metoclopramide; dissolution; excretion

Introduction

Metoclopramide was the first of the benzamides with motor effects on the gut. It also has actions within the central nervous system, characteristic of the dopaminergic receptor blockage. It is widely used in the treatment of nausea and vomiting [1,2].

It is rapidly and completely absorbed following oral dosing, but the first-pass metabolism reduces its bioavailability by approximately 75%. The drug is rapidly distributed in most of the tissues, and easily permeates the blood–brain barrier. Up to 30% of the drug is excreted unchanged in urine, and the rest is eliminated in urine and bile, after its conjugation with glucuronic acid or sulphate [3].

In the present study, bioavailability of two lots of tablets containing metoclopramide hydrochloride is analysed, using urine as body fluid, and the *in vivo* results were correlated with those obtained *in vitro*.

Materials and Methods

Tablets

Metoclopramide hydrochloride (10 mg), produced in the Medicament Production Unit (MPU) of the School of Exact Sciences, La Plata National University (UNLP) and identified as Formulation A. Metoclopramide hydrochloride (10 mg) commercially available in the Argentine pharmaceutical market and identified as Formulation B.

Chemicals

Metoclopramide hydrochloride, drug powder, commercial use, USP 23 degree. Methanol and acetonitrile, HPLC grade (Merck, Darmstadt, Germany); sterile water for injection (Roux Ocefa, B.A., Argentina); triethylamine, reagent grade (Sintorgan, B.A., Argentina) and acetic acid, RSE (Erba, Milan, Italy).

Equipment

The liquid chromatograph used was a Konik KNK 500G with a double piston pump (Konik, Barcelona, Spain) and equipped with a variable wavelength detector, model 204 (Linear, NA, USA), a Rheodyne model 7125, 20 μL loop injector (Rheodyne, Cotati, CA, USA), a KNK 029-375 program microprocessor and an integrator model Datajet SP 4600 (Spectra Physics, San Jose, CA, USA). A Lichrocart RP-18 column (125 × 4 mm i.d. and 5 μm particle size) (Merck, Darmstadt, Germany), with a guard column (50 × 4.6 mm i.d.), packed with 40 μm Pelliguard LC-18 (Supelco, Bellefonte, PA, USA) was used. Other equipment used was as follows: centrifuge, CR 150, Rolco (Rolco, B.A., Argentina); Metler Toledo AG 204 (Metier, Greifensee, Switzerland) balance; UV/VIS spectrophotometer, Beckmann model 25 (Beckman, CA, USA) and a Sotax AT7 (Sotax AG, Basel, Switzerland) dissolution equipment.

In Vitro Dissolution Study

This was carried out according to the USP 23 [4] using the basket method, employing water and HCl 0.1 M as dissolution medium, both at 37°C and 50 rpm. The samples were centrifuged at 3500 rpm for 15 min and analysed by UV at 272 nm. The sampling times were 5, 10, 20, 30, 40, 50 and 60 min, respectively.

In Vivo Study

Eight healthy volunteers, four females and four males, between 24 and 30 years, with a body weight of 52–75 kg, participated in this study. The following admission criteria were established: clinical histories, non-smokers, and non-consumers of medicaments or alcoholic beverages in a period not less than 1 week before the experiment.

* Correspondence to: Cátedra Ensayo y Valoración de Medicamentos, Departamento Ciencias Biológicas, Facultad Ciencias Exactas, UNLP, 47 y 115, (1900) La Plata, Argentina. e-mail: kv@biol.unlp.edu.ar

CCC 0142–2782/98/070479–04$17.50

Received 25 June 1997
Accepted 13 May 1998

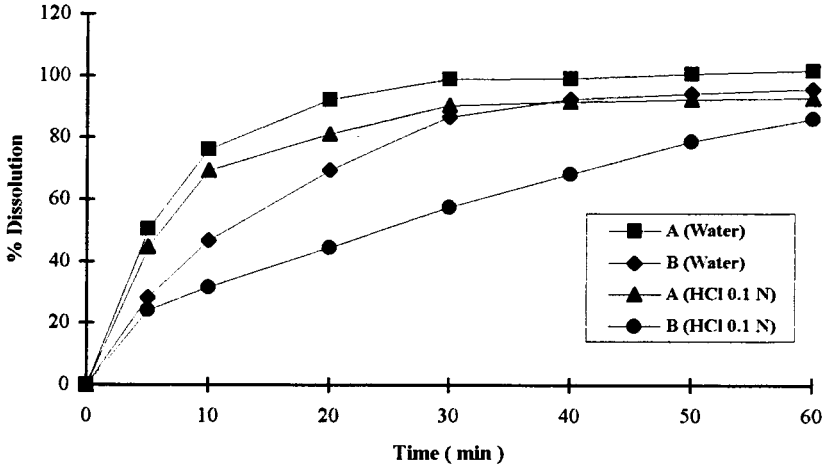

Figure 1. *In vitro* dissolution profiles of metoclopramide formulations A and B

A single dose modality was used, administering two tablets of metoclopramide hydrochloride (20 mg) with approximately 200 mL of water, after 12 h fasting, for each one of the formulations assayed, assuring the quantifiable levels of the drug in the biological fluid of the study. This administered dose does not represent any risk for the subjects undergoing the study. A randomized, crossover, and compensated design was used, with a washout period of not less than 1 week, between two consecutive administrations.

During the day of treatment, the intake of food was standardized, recording the hours of each meal. Volunteers could not take beverages containing xanthines or alcohol during this day.

Urine samples were collected for monitoring the drug at the following times: 0.5, 1.0, 1.5, 2.5, 3.5, 4.5, 5.5, 8.5, 11.5, 14.5, and 24.5 h. In preliminary studies, where the sampling was carried out up to 36.5 h after intake of the tablets, it was determined that at 24.5 h the elimination was total. Therefore, it was taken as the last time for sampling. After measuring the urine volume, a small portion of the urine was kept frozen until the HPLC analysis.

Drug analysis

Urine samples were analysed by HPLC, with the following chromatographic conditions: mobile phase was water with 1.3% triethylamine, adjusted to pH 6.8 with acetic acid solution (1:10):methanol:acetonitrile (77:16:8), and the flow-rate was set at 1.2 mL min^{-1}; the injection volume was 20 μL, temperature 30°C and detection at 309 nm.

Prior to injection into the chromatograph, samples were processed as follows: 2 ml of centrifuged urine (15 min at 3500 rpm) were diluted to 5 mL with mobile phase and filtered by 0.45 μm membrane.

Method Validation

The proposed analytical method was validated by obtaining a calibration curve using standard metoclopramide hydrochloride in a matrix of a pool of blank urine from the volunteers participating in the study, with a range of concentrations between (0.3–6 μg mL^{-1}). The data were analysed by linear regression analysis. The linearity, precision and recovery were evaluated.

Bioavailability Parameters

The following parameters were obtained from the experimental concentration profiles of metoclopramide hydrochloride in urine, as a function of time: $E_{24.5}$, maximum amount of metoclopramide excreted after 24.5 h of administration; $(dE/dt)_{max}$, maximum rate of excretion and t_{max}, time of maximum rate.

Statistical Analysis

The mean values of the above mentioned parameters were calculated with the respective standard error of the mean. The values of volunteer no. 1 were not taken into account due to anomalous behavior in comparison with the rest; after applying a statistical test for outlier identification, Dixon's test [5,6], as it is indicated in the *in vivo* bioequivalence guidances from USP-NF [7].

An analysis of variance (ANOVA) [8] was applied in order to evaluate the dissolved percentages and the data of $E_{24.5}$ and $(dE/dt)_{max}$.

Results and Discussion

Linearity of the calibration curve was found in the range of concentrations between 0.3 and 6 μg mL^{-1}. The intercept (a) and the slope (b), with the respective confidence intervals of 95% were: $a =$

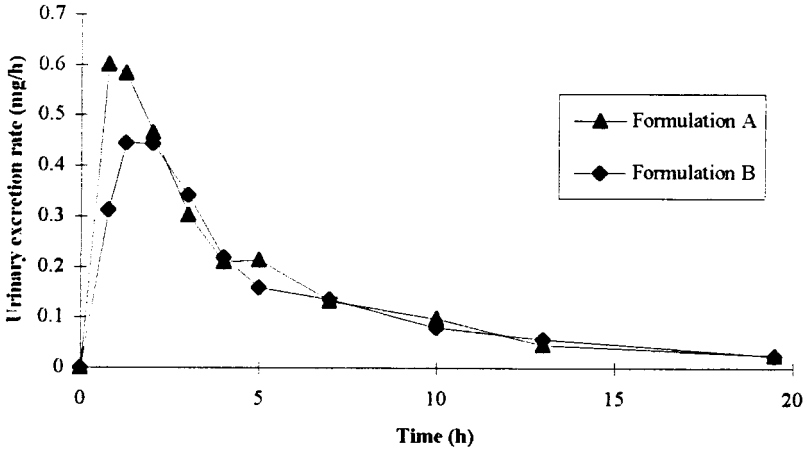

Figure 2. Average urinary elimination rate, as a function of time

-0.328 ± 0.26; $b = 5.944 \pm 0.09$, with a coefficient of correlation $r = 0.999$. In order to confirm the regression analysis, we analysed the response factor: area/concentration of metoclopramide, as a function of concentration. This factor, as it was expected, was constant along the range of concentrations of the calibration curve. Residual (Ri) analysis was carried out (Ri versus concentration) obtaining an aleatory pattern of distribution and the summatory of them was 4.35×10^{-14}. These analyses confirm that the model used for our analytical method is lineal [9].

The precision of the system, calculated as the coefficient of variation (CV) of six injections of the same solution of the standard was 0.9%; the precision of the method, evaluated according to dispersion of five samples was 3.8% and the inter-day precision, calculated as the CV of four injections of the same sample in four different days was 3.5%. In order to establish the method accuracy, a recovery assay was performed using nine samples prepared with the metoclopramide standard added to blank urine, in concentrations between the range of the calibration curve. The result expressed as recovery% $\pm$ SEM was 97.43 ± 0.52.

Table 1. Bioavailability parameters for formulations A and B, from urine levels

Subjects	$E_{24.5}$ (mg)		$(dE/dt)_{max}$ (mg h^{-1})		t_{max} (h)	
	A	B	A	B	A	B
1*	6.77	9.99	1.67	1.33	2.00	2.00
2	2.51	2.66	0.74	0.46	0.75	2.00
3	2.99	3.29	0.75	0.69	2.00	0.75
4	2.59	2.59	0.58	0.46	0.75	2.00
5	3.52	2.35	0.46	0.42	1.50	1.25
6	2.63	3.53	0.59	0.56	2.00	3.00
7	3.85	3.36	1.31	0.61	1.25	1.25
8	2.29	2.01	0.58	0.37	0.75	2.00
$\bar{X}$	2.91	2.83	0.72	0.51	1.29	1.75
SEM	0.22	0.21	0.11	0.04	0.21	0.38

* Outlier.

The dissolution profiles of both formulations are shown in Figure 1. The mean percentages of dissolution with its typical mean deviation at 60 min for the formulation A were 101.73 ± 0.65 and 92.86 ± 0.41, in water and HCl 0.1 M, and for the formulation B 95.82 ± 1.4 and 86.28 ± 0.69, respectively. The ANOVA performed with the dissolved percentages at different sample times, in water as well as in hydrochloric acid, indicates that there are no significant differences ($p > 0.05$).

The average urinary elimination rate as a function of time, of metoclopramide for both formulations can be observed in Figure 2. The $E_{24.5}$, $(dE/dt)_{max}$ and t_{max} values appear in Table 1 for each subject, and for each formulation, with their mean values and standard error of the mean.

The ANOVA of the urinary excretion values and the data of maximum rate of excretion show no significant differences ($p > 0.05$) between both formulations, indicating a similar statistical behavior in terms of absorption and elimination of the drug. At the same time they also correlate with those found *in vitro*.

Acknowledgements

This project received financial support from: Universidad Nacional de La Plata, Colegio de Farmacéuticos de la Provincia de Buenos Aires and Laboratorios Bagó. P.M. Bustillo is a fellow of Laboratorios Bagó.

References

1. L. Teng, R.B. Bruce and L.K. Dunning, Metoclopramide metabolism and determination by high-pressure liquid chromatography. *J. Pharm. Sci.*, **66**, 1615–1618 (1977).
2. C. Dollery, *Therapeutics Drugs*, Livingstone, Madrid, 1991, pp. 148–152.
3. A. Goodman-Gilman, L.S. Goodman, T.W. Rall and F. Murad, *Las Bases Farmacológicas de la Terapéutica*, Médica Panamericana, Buenos Aires, 1990, pp. 901–903.

4. *U.S. Pharmacopeia*, 23rd, U.S. Pharmacopeial Convention, Rockville, MD, 1995, p. 1012.
5. R.B. Dean and W.J. Dixon, Simplified statistics for small numbers of observations. *Anal. Chem.*, **23**, 636–638 (1951).
6. K.A. Connors, *Curso de Análisis Farmacéutico*, Reverté, Barcelona, 1980, pp. 631–632.
7. *U.S. Pharmacopeia*, 23rd, U.S. Pharmacopeial Convention, Rockville, MD, 1995, p. 1932.
8. R.R. Sokal and F.J. Rohlf, *Introducción a la bioestadística*, Reverté, Barcelona, 1984, pp. 157–180.
9. R.H. Myers, *Classical and Modern Regression with Applications*, Duxbury Press, Boston, 1986.