



# Pharmacokinetics of Celgosivir (MX-3253), a Novel $\alpha$ -Glucosidase-I Inhibitor, in Loperamide-treated and Diarrhoea-induced Rats

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## Abstract

Celgosivir (MX-3253) is a novel antiviral agent currently in clinical development for the treatment of chronic hepatitis C virus (HCV) infection. Celgosivir (6 O-butanoyl castanospermine) and its primary metabolite, castanospermine, are potent inhibitors of  $\alpha$ -glucosidase-I, an enzyme that alters processing of viral glycoproteins. Orally administered celgosivir is well tolerated in humans producing mainly side effects of flatulence and mild to moderate diarrhoea. The purpose of the present study was to determine the effect of the anti-diarrhoeal drug loperamide hydrochloride on the pharmacokinetics (PK) of orally administered celgosivir. The effect of diarrhoea was also investigated. Male CD rats were orally administered loperamide at 0.35 mg/kg followed by oral dosing of celgosivir at 35 mg/kg. The dosages of loperamide and celgosivir were based on human doses adjusted to total body surface area. Another group of rats was fasted overnight and then administered castor oil (5 mL/kg) to induce diarrhoea. These animals were immediately given access to food followed by dosing with celgosivir one hour later. Blood samples were collected over 24 hours. Celgosivir PK was investigated by following the plasma concentration of its primary metabolite, castanospermine, using LC/MS. Oral administration of a 35 mg/kg dose of celgosivir to normal rats yielded mean  $C_{max}$ ,  $T_{max}$  and AUC values of 8.8  $\mu$ g/mL, 0.44 hr and 10.5  $\mu$ g.hr/mL, respectively. Comparable results were obtained for celgosivir in loperamide-treated animals, indicating that this anti-diarrhoeal agent had no significant effect on the PK of celgosivir. The  $C_{max}$  and AUC values in diarrhoea-induced rats were reduced by 54% and 44%, respectively, compared with normal rats. The difference in the  $C_{max}$  was determined to be statistically significant. In conclusion, the concomitant administration of loperamide had no effect on the PK of celgosivir in normal rats and could be considered a viable treatment option for reducing gastrointestinal effects that may be associated with celgosivir treatment. Since diarrhoea-induced rats showed a reduction in castanospermine  $C_{max}$  and AUC, treatment with loperamide might prevent lowered systemic drug exposure in patients experiencing diarrhoea.

## Introduction

Infection with the Hepatitis C virus (HCV) is a global health problem. According to a World Health Organization report, the number of deaths attributable to HCV increased to 54,000 in 2003 [1]. The importance of finding new effective treatments for HCV infection is highlighted by the spread of the virus, which now affects between 170 and 300 million patients worldwide, 85% of whom become chronic carriers, 30% evolving to liver cirrhosis and 5% evolving to liver cancer [2, 3, 4].

Celgosivir (MX-3253) is a novel antiviral agent currently in clinical development for the treatment of chronic HCV infection. Celgosivir (6 O-butanoyl castanospermine) and its primary metabolite, castanospermine, inhibit the mammalian enzyme  $\alpha$ -glucosidase-I. This enzyme plays a critical role in the processing of viral glycoproteins. It is believed that inhibition of  $\alpha$ -glucosidase-I will result in misfolded HCV glycoproteins, which in turn will inhibit virus maturation and infectivity.

Orally administered celgosivir is well tolerated in humans producing side effects mainly targeted to the gastrointestinal tract including flatulence and mild to moderate diarrhoea. Loperamide is the active ingredient found in over-the-counter medications used for symptomatic relief of acute and chronic diarrhoea.

The purpose of this study was to investigate the effect of loperamide hydrochloride on the pharmacokinetics (PK) of orally administered celgosivir. In addition, the effect of diarrhoea on celgosivir PK was also investigated. Celgosivir pharmacokinetics were assessed by following the plasma profile of its primary metabolite, castanospermine.

## Methods

### Treatment Groups

Male Sprague-Dawley rats (CrI:CD) were obtained from Charles River Laboratories (Montreal, Canada). Animals weighed approximately 200-400 g at the time of the study and dose levels were adjusted according to the weight of each animal. Dose levels of celgosivir and loperamide were based on human doses adjusted to total body surface area.

**Treatment Group 1 (Normal Controls):** Six rats were administered a single oral dose of celgosivir at 35 mg/kg.

**Treatment Group 2 (Loperamide-treated):** Six rats were administered a single oral dose of loperamide at 0.35 mg/kg. Ten minutes later, each animal was given a single oral dose of celgosivir at 35 mg/kg.

**Treatment Group 3 (Diarrhoea-induced):** A standard castor oil model was used to induce diarrhoea in a total of six rats. Animals were fasted for approximately 18 hours but had free access to water. Castor oil was then administered as a single oral dose of 5 mL/kg and the animals immediately given free access to food. One hour after castor oil administration, each animal was administered a single oral dose of celgosivir at 35 mg/kg.

**Treatment Group 4 (Fasted Controls):** A total of 10 animals were fasted for approximately 18 hours with free access to water. After fasting, all animals were allowed free access to food for approximately 30 minutes prior to administration of a single oral dose of celgosivir at 35 mg/kg.

### Blood Sampling and Bio-analysis

At various time-points after celgosivir administration, blood samples were withdrawn from rotating groups of 2-3 animals via the tail vein. Plasma samples were generated and stored frozen until analyzed.

Plasma samples were analyzed for castanospermine, the primary metabolite of celgosivir, using LC/MS. Samples were extracted using solid-phase extraction followed by separation using reversed-phase HPLC with MS detection in electrospray positive mode. The range of the bio-analytical assay was 0.1 to 50  $\mu$ g/mL.

### PK Analysis

Celgosivir pharmacokinetics were assessed by following the plasma concentration of its primary metabolite, castanospermine. Pharmacokinetic parameters were calculated according to a two-compartment model with bi-exponential decay using the method of residuals.

$C_{max}$  values between treatment groups were compared using a one-way analysis of variance (ANOVA).

## Results

Figure 1. Overlaid Castanospermine Plasma Concentration Versus Time Plots For Normal Control (Treatment Group 1) And Loperamide-treated (Treatment Group 2) Rats

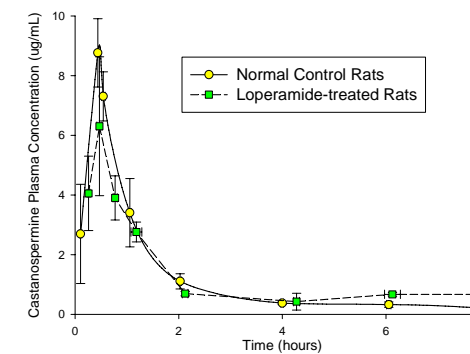


Figure 2. Overlaid Castanospermine Plasma Concentration Versus Time Plots For Fasted Control (Treatment Group 4) And Diarrhoea-induced (Treatment Group 3) Rats

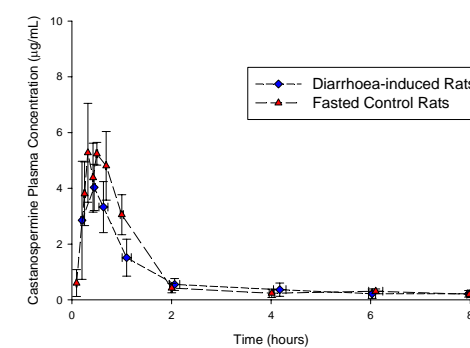
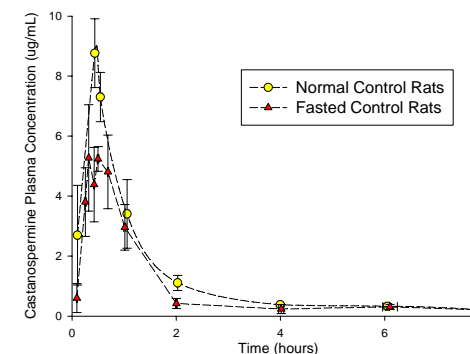


Figure 3. Overlaid Castanospermine Plasma Concentration Versus Time Plots For Normal Control (Treatment Group 1) And Fasted Control (Treatment Group 4) Rats



## Results (Continued)

Table 1. Summary of Pharmacokinetic Parameters

Treatment Group	Description	$C_{max}$ ( $\mu$ g/mL)	$T_{max}$ (hours)	AUC ( $\mu$ g-hour/mL)
1	Normal Controls	8.76 $\pm$ 1.15	0.44 $\pm$ 0.01	10.5
2	Loperamide-treated	6.30 $\pm$ 2.33	0.47 $\pm$ 0.05	9.5
3	Diarrhoea-induced	4.03 $\pm$ 0.83 *	0.44 $\pm$ 0.01	5.9
4	Fasted Controls	5.28 $\pm$ 1.77 *	0.32	7.2

\*  $C_{max}$  values significantly different from Normal Control rats (Treatment Group 1) using ANOVA (P=0.017)

## Discussion

The primary objective of this study was to investigate the effect of the anti-diarrhoeal drug, loperamide, on the pharmacokinetics of celgosivir in rats. Upon oral administration, celgosivir is rapidly converted to its major metabolite, castanospermine [5]. Oral administration of a 35 mg/kg dose of celgosivir to normal control rats (Treatment Group 1) resulted in castanospermine  $C_{max}$ ,  $T_{max}$  and AUC values of 8.8  $\mu$ g/mL, 0.44 hour and 10.5  $\mu$ g-hour/mL, respectively. Comparable results were obtained in animals that had been pre-administered a 0.35 mg/kg dose of loperamide (Treatment Group 2) (Figure 1 and Table 1).

The effect of diarrhoea on the pharmacokinetics of celgosivir was also investigated as part of this study. When comparing normal control animals (Treatment Group 1) with diarrhoea-induced animals (Treatment Group 3), the castanospermine  $C_{max}$  values were reduced by 54% (Table 1). This difference was determined to be statistically significant ( $p < 0.05$ ). Induction of diarrhoea with castor oil required overnight fasting (approximately 18 hours) followed by administration of castor oil with immediate access to food. In order to determine the effect of overnight fasting on celgosivir pharmacokinetics, a fasted control group (Treatment Group 4) was added to the study. Castanospermine  $C_{max}$  and AUC values in these animals were in between those obtained for normal control (Treatment Group 1) and diarrhoea-induced animals (Treatment Group 3) (Figures 2 and 3 and Table 1). These results suggest that both fasting and diarrhoea may reduce systemic exposure upon oral administration of celgosivir to rats.

## Conclusions

Concomitant administration of loperamide had no significant effect on the PK of celgosivir in normal rats and could be considered as a viable option for reducing gastrointestinal effects that may be associated with celgosivir treatment.

Diarrhoea-induced rats showed a reduction in castanospermine  $C_{max}$  and AUC. Treatment with loperamide might prevent lowered systemic drug exposure in patients experiencing diarrhoea.

## References

- World Health Organization, *The World Health Report, in changing history*, 2004
- Poynard T. *et al.* (2003) *Lancet* 362 (9401): 2095-2100
- Manns M.P *et al.* (2001) *Lancet* 358 (9283): 958-965
- Maddrey, W. *Conquering Hepatitis*, (2000) Hamilton: BC Decker Inc.: 1-108
- Stoltz M.L. *et al.* (1996) 11<sup>th</sup> International AIDS Conference, Vancouver British Columbia - July 7-12, 1996