Pharmacokinetics of Celecoxib in the Presence and Absence of Interferon-Induced Acute Inflammation in the Rat: Application of a Novel HPLC Assay

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Abstract Purpose. Celecoxib (CEL) is a relatively new cyclooxygenase-2 specific inhibitor nonsteroidal anti-inflammatory drug with low incidents of the toxic side effects. We developed and validated an HPLC assay for CEL and delineated pharmacokinetics of the drug in the rat in the presence and absence of inflammation. **Methods.** Rat plasma (0.1 mL plasma) was spiked with CEL and ibuprofen as internal standard. The solution was acidified and constituents were extracted with isooctane-isopropanol (95:5). The organic solvent was separated, evaporated and the residue was dissolved in the HPLC mobile phase [acetonitrile-water-acetic acid-triethylamine (47:53:0.1:0.03)]. The HPLC system consisted of an auto-injector, an isocratic pump, a 10 cm C_{18} analytical column packed with 5-μm of reversed-phase particles, a UV detector set at 254 nm, and an integrator. Control adult male Sprague-Dawley rats were dosed with CEL [5 mg/kg *i.v.* (n=8), *p.o.* (n=6) or *i.p.* (n=3)]. Acute inflammation was brought about by two (12 and 1 h pre-CEL) *s.c.* injection of 50,000IU/200μL interferonα2a. Inflamed rats (n=6) received 5 mg oral CEL. Serial blood samples were collected via a inserted catheter at the right jugular vein, and plasma samples were analyzed for CEL. **Results**. The assay yielded linear response within the examined ranges of 20-1000 ng/mL and 1-100 μg/mL (r^2>0.99) with an extraction efficiency of >70%, intra- and inter-day variability of <10% and accuracy of >90%. In control rats, CEL had an oral bioavailability of 0.59 due mainly to presystemic hepatic metabolism. A multi-compartmental disposition kinetics with an average terminal $t_{1/2}$ of 2.8 ± 0.7 h, and volume of distribution of 2.3 ± 0.6 L/kg were found. Acute inflammation had no significant effect on the pharmacokinetics of CEL, although a trend towards increased plasma concentration was noticed. **Conclusions.** The validated assay has sufficient accuracy and precision for pharmacokinetic studies of CEL in the rat. The lack of change in CEL pharmacokinetics after acute inflammation maybe due to 1) insensitivity of its metabolic system to the acknowledged inhibitory effect of inflammation, and/or 2) the relatively low pre-systemic metabolism of the drug.

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INTRODUCTION

Celecoxib (CEL), a member of the new generation of non-steroidal anti-inflammatory drugs (NSAIDs) (Figure 1), specifically acts on cyclooxygenase-2 (COX-2), the main cyclooxygenase isomer expressed during inflammation (1-4).

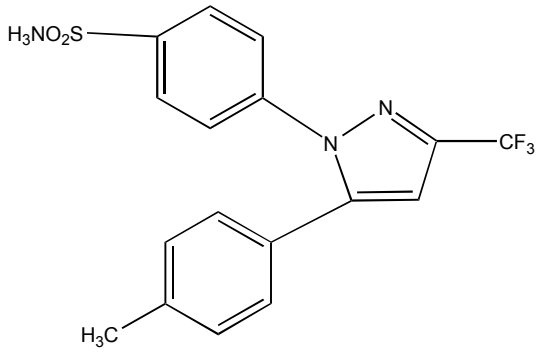

Figure 1: The Chemical Structure of CEL.

Previous generations of NSAIDs inhibit both cyclooxygenase –1 (COX-1) and COX-2. COX-1, is constitutively expressed and is associated with maintenance of the cell (e.g., mucosal lining of gut integrity) hence, its inhibition results in clinically significant toxicity(5, 6). This toxicity may be prevented with the introduction of NSAIDs with high selectivity in COX-2 inhibition(4-6). At present, CEL is primarily used in the treatment of pain and inflammation associated with rheumatoid arthritis and osteoarthritis. Inflammatory conditions such as rheumatoid arthritis and administration of interferon have been reported to significantly inhibit hepatic metabolism of many drugs (7-9). Therefore, we developed an assay suitable for delinea-

tion of pharmacokinetics of CEL in the rat and studied the effect of acute inflammation caused by administration of interferonα2a. The reported high performance liquid chromatography (HPLC) assays (10) use solid-liquid extraction procedures. Herein, we report an HPLC assay, which involves a convenient and cost-effective liquid-liquid extraction method.

METHODS AND MATERIALS

Chemicals

Celecoxib was extracted from Celebrex 100 mg Capsules (Searle MI, USA). Racemate ibuprofen was obtained as a gift from Upjohn Canada (Don Mills, Canada). Isooctane and isopropanol (Assurance grade) were purchased from BDH Chemicals Canada (Edmonton, Canada). HPLC grade acetonitrile and methanol as well as analytical grade triethylamine, acetic acid and sulfuric acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Interfronα2a (Referon-A) was from Hoffmann-La Roche Ltd (Mississauga, Ontario, Canada).

ASSAY PREPARATION

The HPLC system (Shimadzu, Japan) consisted of a Sil-9A model autoinjector, a SPD-6A model variable UV spectrophotometer detector set at 254 nm and a CR601 model Chromatopac integrator, and a Waters 6000 A model HPLC pump (Waters, Mississauga, Canada). The mobile phase, which was filtered through a 0.45 μm nylon filter before use, consisted of acetonitrile-water-acetic acid-triethylamine (47:53:0.1:0.03) and was pumped at a flow rate of 1 ml/min. A 10 cm x 4.6 mm I.D. C_{18} analytical column packed with 5 μm reversed-phase particles (Phenomenex, Torrance, CA, USA) and a Novo-Pak C8 guard column (Waters, Millipore Corp., Milford, MA, USA) were used for separation.

Celecoxib powder were prepared from the contents of 20 capsules, which were suspended in 50 mL of HPLC grade methanol, filtered, allowed to evaporate, and dissolved in and recrystallized from acetonitrile. The identity and purity of the CEL was determined utilizing melting point, differential scanning calorimetry, and nuclear magnetic resonance (data not shown). Standard solutions were prepared by adding 100 μL of ibuprofen stock solution (100 μg/mL) and specific volumes of CEL stocks solutions (1 μg and 100 μg/mL) to 100 μl of rat blank plasma to make the final concentrations of 20 ng/mL to 100 μg/mL. Two calibration curves with different concentration ranges were prepared. The low range curve included solutions containing 20, 50, 100, 250, 500, 1000 ng/mL and the high range curve consisted covered solutions of 1.0, 2.5, 5.0, 25, and 100 μg/mL of CEL. To each spiked standard solution was added 0.2 ml of 0.6 M H_2SO_4 and 5 ml of isooctane-isopropanol (95:5). The tubes were vortex-mixed for 30 s and centrifuged at 2500 g for three min. The organic layer was transferred to clean tubes and evaporated (Savant Speed Vac concentrator-evaporator, Emerson Instruments, Scarborough, Canada). The residue was reconstituted in 200 μL of mobile phase and aliquots of 150 μL were injected to the chromatographic system.

The method's extraction efficiency was determined for concentrations of 50, 100 and 250 ng/mL. The peak areas of the extracted plasma samples were compared with the peak areas obtained after direct injection of 150 μL of solutions containing 50, 100 and 250 ng/mL CEL.

Accuracy was determined by examining calibration curves prepared in triplicate on three consecutive days.

PHARMACOKINETIC EXPERIMENTS

Male Sprague-Dawley rats (250-300 g) were anesthetized using pentobarbital (65 mg/kg *i.p.*) and a silastic catheter was inserted into the right jugular vein. Animals were allowed to recover for 24 h before dosing. On the day of the experiment animals were placed in metabolic cages and dosed with 5 mg/kg *i.v.* (n=8), *p.o.* (n=6) or *i.p.* (n=3) of CEL. Serial blood samples (200 μL) were collected. The catheter was flushed with 200 μL of saline after each sample collection. Samples were stored at -20° until analyzed. The effect of inflammation was assessed by dosing animals (n=6) with interferonα2a (INF, 200 μL *s.c.*; is saline-diluted to a concentration of 5x 10^4iu/200 μL immediately before use) 12 and 1 h before administration of CEL . Control rats were dosed with 200 μL *s.c.* normal saline.

DATA ANALYSIS

Accuracy was expressed as the mean % error, [(mean measured concentration)/(expected concentration)]x

100. Precision was expressed as percent coefficient of variation [(standard deviation/mean)×100].

Areas under the plasma concentration versus time curves (AUC) were calculated using the linear trapezoidal method. Elimination rate constants (β) were calculated for each individual rat from the slope of the regression line of the log-linear terminal elimination phase of the concentration-time curves using at least 3 data points. The oral clearance (CL_{oral}) was calculated from Dose/$AUC_{(0-\infty)}$. Volume of distribution was calculated from CL/β. Bioavailability (F) was calculated from AUC_{oral} or $AUC_{i.p}$ /$AUC_{i.n.}$. The significance of the difference between any two groups was tested using paired two-tailed Students t-test. Determination of significance of differences between multiple groups was done using ANOVA followed by the Duncan New Multiple Range test. The level of significance was set at 0.05. Data are expressed as mean ± standard deviation.

RESULTS

Assay Validation

Ibuprofen and CEL were eluted 8 and 11 min following injection into HPLC, respectively. The peaks were resolved with no interfering peaks (Figure 2). Extraction efficiency was found to be 69.8 % (Table 1).

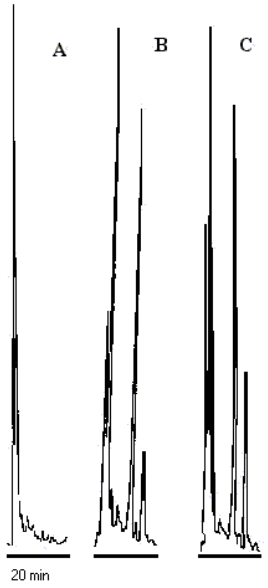

Figure 2: Representative chromatographs of: A) blank rat plasma, B) plasma spiked with 100 ng/mL ibuprofen (peak 1) and 100 ng/mL CEL (peak 2,) and, C) plasma sample from a rat 4 h after administration of 5 mg/kg, the concentration of 2 is equivalent to 151 ng/mL.

Table 1: Extraction efficacy of CEL from rat plasma at various concentrations, mean ± standard deviation (n=3)

Concentration (μg/L)	Extraction Efficacy
50	68.4 ± 4.6
100	69.9 ± 2.3
250	71.2 ± 5.0
Average Extraction Efficacy	69.8 ± 1.4

The relationship between the area ratio of CEL to internal standard *versus* concentration was linear (r^2<0.99) for both low and high range calibration curves. Intra- and inter-day variability did not exceed 10% with less that 10% error throughout the examined range (Table 2 and 3). The limit of quantification of the assay was, therefore, set at 20 ng/mL of CEL based on 0.1 mL rat plasma sample.

Table 2: Within-day precision and accuracy for the CEL assay in rat plasma.

Standard Concentration (μg/L)			
Actual	Observed	Precision[a](%)	Accuracy[b](%)
20	20.3	4.6	101.6
50	47.5	3.8	95.0
100	89.9	7.2	93.5
250	227.8	5.3	91.1
500	496.5	1.3	99.3
1000	997.6	0.9	99.8

[a] *Expressed as relative standard deviation.*

[b] *Expressed as [(mean observed concentration / actual concentration)x100]*

Table 3: Intra-day variability of the CEL assay over three consecutive days in rat plasma.

Standard Concentration (μg/L)			
Actual	Observed	Precision[a](%)	Accuracy[b](%)
20	21.3	9.2	106.7
50	47.2	6.1	94.3
100	92.2	6.9	92.2
250	226.1	5.3	90.4
500	511.7	5.6	102.3
1000	991.7	0.8	99.2

[a] *Expressed as relative standard dev.*

[b] *Expressed as [(mean observed concentration / actual concentration)x100].*

Table 4: **Table 4. Mean (± standard deviation) pharmacokinetic indices of CEL in control rats after various routes of administration and after p.o. administration in interferon-treated rats of 5 mg/kg.**

Administration Route and Dose	T_{max} (h)	C_{max} (μg/mL)	$t_{½}$ (h)	AUC (μg*h/mL)	Vd (L/kg)	CL (mL/min/kg)
i.v. (n= 8)	nd	nd	2.8 ± 0.7	9.3 ± 1.8	2.3 ± 0.6	10 ± 1.7
i.p. (n= 3)	0.28 ± 0.22	1.2 ± 0.19	5.2 ± 1.8	6.6 ± 1.7*	5.5 ± 0.5	13 ± 3.3
p.o. (n=6)	1.6 ± 1.2	0.94 ± 0.35	3.1 ± 0.8	4.6 ± 2.2*	5.2 ± 3.0	23 ± 3.3
INF-treated *p.o.*(n=6)	3.1 ± 2.0	0.92 ± 0.23	2.5 ± 0.3	5.4 ± 0.78*, NS	3.3 ± 0.9	15 ± 0.33

ND not determined
** Significantly different from i.v. (Duncan New Multiple Range Test p <0.05).*
NS not significantly different from p.o. control

Celecoxib pharmacokinetics

Following *i.v.* administration of CEL, the concentration-time curve of CEL exhibited a multi-compartment pattern with a steep initial declines followed by a log-linear terminal phase with a $t_{1/2}$ of 3 ± 1 h (Figure 4).

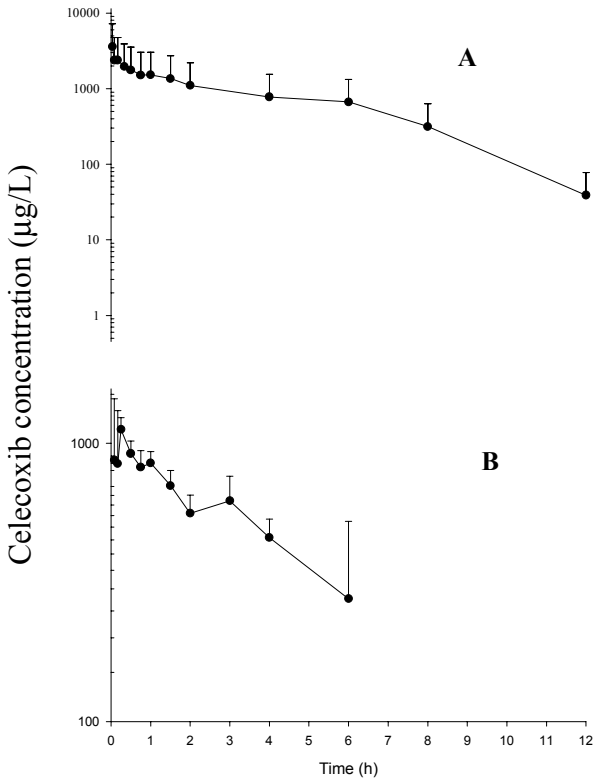

Figure 3: **Concentration-time curve following (A) 5mg/kg CEL administered *i.v.* (n=8) and (B) *i.p.* (n=3). Data presented as mean ± standard deviation**

CEL has a relatively large volume of distribution and a medium to low hepatic extraction (Table 4). Numerical differences were found in AUC values when CEL was administered through different routes with the greatest and smallest values after *i.v.* and *p.o.*, respectively (Table 4). These differences, however, were significant only between the *i.v.* and other routs.

Interferon administration was associated with significant increases in nitrite, a stable metabolite of nitric oxide, and segmented neutrophil indicating inflammation (11). Interferon therapy did not significantly alter pharmacokinetics of CEL although a trend toward increased AUC was observed (Table 4 and Figure 4).

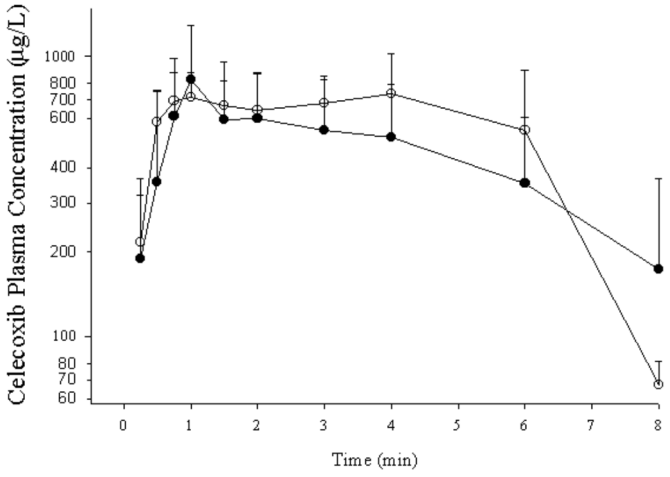

Figure 4: **Concentration-time curve for CEL administrated orally (5mg/kg) to control (●) and interferon-treated (○) rats. Data presented as mean ± standard deviation (n=6/group).**

DISCUSSION

The present assay is sensitive, reproducible and convenient. The liquid-liquid extraction method used is more convenient and cost-effective than the solid-phase

extraction procedure utilized by others.(10, 12, 13). This method provides a sensitivity of at least 20 ng/mL based on 100 μL sample volume, which is suitable for pharmacokinetic studies of CEL and is comparable with the assays reported by others (10). In a preliminary experiment we confirmed that the assay can also be used to determine CEL in human plasma.

Our basic pharmacokinetic indices consistent with values reported previously (12). In addition, a comparison of the AUC values following administration through different routes indicates that the drug is cleared mainly by the liver with the possibility of some metabolism and/or incomplete absorption taking place in the gut (AUC: *i.v.*>*i.p.*>*p.o.*); i.e., the *i.v.* dose which by passes first pass metabolism has the greatest AUC while the *p.o.* dose which undergoes both gut and liver first-pass clearance yielded the smallest AUC. The involvement of the gut, however, is not certain since the numerical difference between the AUC of *p.o.* and *i.p.* routes did not indicate significance. Pharmacokinetic data following *i.v.* administration (Table 4) suggest a CL_{TB} of 10 mL/min/kg for CEL.

Considering the significant contribution of the hepatic metabolism in clearance of CEL and the rat hepatic blood flow of 65 mL/min/kg, the drug may be classified as one with a low hepatic extraction. The relatively large volume of distribution of approximately 2 L/kg (Table 4) suggests extensive deep tissue binding and/or distribution. These findings in the rat suggest somewhat similar disposition kinetics to those reported in humans (14).

Both acute inflammation induced by interferon (11) and chronic arthritis (15) are reported to reduce clearance of drugs. This effect is mainly observed with drugs that are cleared in the liver. The extent of this inhibition increases with the efficiency of the hepatic metabolism; i.e., drugs with high hepatic extraction such as propranolol (16, 17) and verapamil (11, 15) are more affected than those with intermediate or low extraction ratio such as hyroxychloroquine (18). The diminishing effect of inflammation on clearance of drugs is attributed to increased expression of pro-inflammatory cytokines (7, 8) and/or nitric oxide (7, 8) and subsequent reduction in cytochrome function. Acute inflammation caused by interferon did not significantly altered pharmacokinetics of CEL despite a trend towards increased AUC of the drug in the inflamed as compared with control rats. Al least two explanations may be offered: 1) The enzymes responsible for metabolism of CEL are not affected by acute inflammation, and/or 2) CEL is a drug with a relatively low liver extraction efficiency, hence, its clearance is not sensitive to the inhibitory effect of inflammation.

CONCLUSIONS

The determination of CEL plasma concentrations using a newly validated liquid-liquid extraction HPLC assay has allowed us to characterize CEL PK in the rat, as well as determine CEL bioavailability. The effect of inflammation on CEL has been found to be minimal. Further extermination is required with different inflammatory models before we can conclusively determine the effects of inflammation on CEL PK.

ACKNOWLEDGEMENTS

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