ORIGINAL ARTICLE

Contrasting effects of cord injury on intravenous and oral pharmacokinetics of diclofenac: a drug with intermediate hepatic extraction

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Study design: Laboratory investigation in rats submitted to experimental spinal cord injury (SCI).

Objective: To determine the effect of acute SCI on the pharmacokinetics of diclofenac, a marker drug of intermediate hepatic extraction, administered by the intravenous and the oral routes.

Methods: Female Wistar rats were submitted to complete section of the spinal cord at the T8 level. SCI and sham-injured rats received 3.2 mg kg⁻¹ of diclofenac sodium either intravenously or orally, diclofenac concentration was measured in whole blood samples and pharmacokinetic parameters were estimated. Diclofenac was not selected as test drug because of its therapeutic properties, but because to its biopharmaceutical properties, that is, intermediate hepatic extraction.

Results: Diclofenac bioavailability after intravenous administration was increased in injured rats compared with controls due to a reduced clearance. In contrast, oral diclofenac bioavailability was diminished in SCI animals due to a reduction in drug absorption, which overrides the effect on clearance.

Conclusion: Acute SCI induces significant pharmacokinetic changes for diclofenac, a marker drug with intermediate hepatic extraction. SCI-induced pharmacokinetic changes are not only determined by injury characteristics, but also by the route of administration and the biopharmaceutical properties of the studied drug.

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INTRODUCTION

Spinal cord injury (SCI) is a life-threatening event that not only results in a sensory-motor loss below the site of lesion, but also causes autonomic alterations including gastrointestinal and hemodynamic changes.^{1–4} These changes are known to modify drug availability. Significant changes in volume of distribution and clearance have been reported in patients after cord lesion for various drugs, including aminoglycosides⁵ and methylprednisolone.⁶ Likewise, SCI-induced changes in oral drug absorption have been reported for paracetamol,^{4,7} dantrolene⁸ and theophylline,⁹ and it has been suggested that these changes could have an impact on the efficacy and safety of a variety of drugs in this population.¹⁰

Characterization of the pathophysiological alterations underlying pharmacokinetic changes that occur in patients with SCI has been complicated because of the important variability induced by differences in injury characteristics, as well as in the individual conditions of each patient.¹⁰ Moreover, not all drugs exhibit altered disposition after SCI. For example, it has been reported that bioavailability of riboflavin⁷ and fampridine¹¹ is not significantly different from that observed in able-bodied individuals. Therefore, depending on which physiological mechanisms are involved in the absorption, distribution and elimination of a given drug, SCI will or will not change its pharmacokinetic parameters.¹⁰ There is evidence suggesting that the

magnitude of the pharmacokinetic changes will depend on variables related to the injury itself: site of injury, intensity and the time elapsed after lesion.^{4,7,10} It then appears that it is not possible to generalize on the impact of SCI on drug disposition and thus systematic research is required to fully elucidate this issue. Therefore, animal models, where the lesion can be standardized, are useful to understand these phenomena.^{12–15}

Aside from the characteristics of cord injury, the biopharmaceutical properties of a given drug will have an important role in the potential alterations of drug disposition.^{13–15} It has been documented that SCI reduces liver blood flow.³ It is well known that changes in liver blood flow importantly alter the elimination of drugs submitted to high hepatic extraction, but not of drugs with low hepatic extraction, in which clearance depends primarily on the hepatic enzymatic capacity.¹⁶ Consistently, we have observed that SCI impairs the clearance of phenacetin, but not of naproxen, marker drugs of high and low hepatic extraction, respectively.¹⁴ However, at present there is no information on the effect of SCI on the handling of intermediate extraction drugs, in which clearance depends on both, liver blood flow and hepatic enzymatic capacity.¹⁶

Therefore, to complete the screening of drugs with diverse degrees of hepatic extraction, we determined the pharmacokinetic parameters of diclofenac, a marker drug with intermediate extraction.¹⁷ As it is

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known that SCI can alter both, drug clearance and absorption,¹⁰ we studied diclofenac pharmacokinetics after intravenous and oral administration in rats submitted to SCI and in sham-lesioned controls.

MATERIALS AND METHODS

Animals

Female Wistar rats from our own animal facility were used, weighing between 240 and 260 g. At 24 h before the start of the study, food was withdrawn, although animals continued to have free access to drinking water. At the end of the study rats were euthanized in a CO₂ chamber. The protocol was approved by our local Animal Care Committee.

Study design

Experimental cord injury was performed as described previously.¹⁵ Briefly, animals were anesthetized with an intramuscular injection of a mixture of ketamine (77.5 mg kg⁻¹) and xylazine hydrochloride (12.5 mg kg⁻¹), and laminectomies were performed at T8 level. The spinal cords were completely sectioned using micro-scissors; control rats were only laminectomized. Surgical planes were sutured separately using 4–0 nylon. After the surgical procedure, rats received appropriate postsurgical care.

Twenty-four hours after sectioning the cord at T8, animals were lightly anesthetized with ether and a cannula was introduced into the caudal artery for blood sampling.^{14,15} Then, rats were divided into four groups of six animals each. All groups received the same dose of diclofenac, that is, 3.2 mg kg⁻¹. Diclofenac was administered as the sodium salt (Novartis Farmacéutica S.A., Mexico City, Mexico). Control group 1 and SCI group 3 received diclofenac intravenously; control group 2 and SCI group 4 received the drug orally. Intravenous administration of diclofenac sodium dissolved in isotonic saline was performed by inserting a cannula into the jugular vein, whereas oral diclofenac sodium suspended in 4% carboxymethyl cellulose was given via an intragastric cannula.¹⁵

Blood samples of 100 µl were drawn at the following times: 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 480 min after drug administration. To minimize hemodynamic changes, the total blood volume drawn did not exceed 2 ml and the extracted blood volume drawn by sampling was immediately replaced with an equivalent volume of saline.¹⁴ Diclofenac concentration was determined in whole blood by an high-performance liquid chromatography method described previously.¹⁸

For each animal, a diclofenac blood concentration curve against time was constructed. Pharmacokinetic parameters were estimated by non-compartmental analysis, as describe previously.^{14,15} For intravenous administration, the following parameters were determined: area under the curve extrapolated to infinity (AUC), clearance, volume of distribution (V_d) and half-life. For the oral route, the parameters estimated were AUC, the maximal observed concentration (C_{max}) and the time at which this maximum occurs (T_{max}). Absolute oral bioavailability (F) was estimated as the ratio between mean oral AUC and mean intravenous AUC.¹⁵

Statistics

Comparisons of pharmacokinetic parameters between SCI and control animals were performed using the Student's *t*-test. Differences were considered significant when $P < 0.05$.^{12–15}

Statement of ethics

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research.

RESULTS

All animals exhibited normal locomotor activity before starting the study. After cord transection, rats exhibited flaccid paralysis. Rats subjected only to laminectomy showed normal gait. After intravenous administration, diclofenac blood concentrations were higher in the SCI rats compared with controls (Figure 1). Pharmacokinetic parameters are shown in Table 1. AUC was significantly increased,

clearance significantly diminished and half-life significantly longer in injured rats. There was no significant difference in V_d . Opposite SCI-induced changes in diclofenac bioavailability were observed with the oral route. Notwithstanding an impaired clearance, diclofenac blood levels after oral administration were lower in injured rats (Figure 2). Hence, AUC, C_{max} and absolute bioavailability (F) were significantly reduced after lesion. There was no significant difference in T_{max} (Table 2).

DISCUSSION

Pharmacokinetics of oral and intravenous diclofenac was studied in rats subjected to spinal cord section or laminectomy. Diclofenac was chosen not because of its therapeutic effects, but because of its biopharmaceutical properties. Diclofenac is a drug of intermediate hepatic extraction and extensively (99%) bound to plasma proteins.¹⁷ It was observed that SCI results in changes in bioavailability that occurred in opposite directions, depending on the route of administration. In SCI animals, AUC was increased when diclofenac was given intravenously. AUC, however, was reduced after oral administration.

AUC is an indicator of drug bioavailability and depends on both, drug absorption and elimination.¹⁶ When the drug is administered by the intravenous route, it is considered that the absolute bioavailability is 100%, as the totality of the dose reaches the circulation. Oral bioavailability is lower due to incomplete absorption and/or to first-pass metabolism. Absolute oral bioavailability is expressed as F , which is estimated as the ratio between oral AUC and intravenous AUC.^{15,16} Oral and intravenous administrations, however, must be performed on subjects with comparable physiological conditions, as certain pathological states may alter absorption, elimination or both.¹⁶ The design of this study, in which diclofenac was given intravenously and orally to rats with SCI and to sham-lesioned controls, allows to

Figure 1 Diclofenac blood concentrations observed after the intravenous administration of a single 3.2 mg kg⁻¹ dose to female Wistar rats submitted to SCI at the T8 level (○) and in sham-lesioned controls (●). Data are expressed as the mean ± s.e.m. of six animals.

Table 1 Pharmacokinetic parameters of diclofenac after administration of a single 3.2 mg kg^{-1} dose by the intravenous route to rats submitted to spinal cord injury (SCI) at the T8 level and to sham-lesioned animals

Group	AUC ($\mu\text{g h mL}^{-1}$)	Clearance ($\text{L h}^{-1} \text{ kg}^{-1}$)	Volume of distribution (L kg^{-1})	Half-life (h)
Sham	12.31 ± 2.24	0.32 ± 0.17	1.58 ± 0.77	2.90 ± 0.64
SCI	22.57 ± 2.48^a	0.14 ± 0.04^a	1.14 ± 0.30	4.98 ± 0.67^a

Data are expressed as mean $\pm$ s.e.m.^aDenotes a statistically significant difference ($P < 0.05$), as determined by the Student's *t*-test. **Figure 2** Diclofenac blood concentrations observed after the oral administration of a single 3.2 mg kg^{-1} dose to female Wistar rats submitted to SCI at the T8 level (○) and in sham-lesioned controls (●). Data are expressed as the mean $\pm$ s.e.m. of six animals.

characterize SCI-induced changes in diclofenac pharmacokinetic parameters by both routes, including absolute oral bioavailability.

Intravenous administration allowed detecting an impaired diclofenac clearance in SCI rats leading to increased bioavailability. Owing to diclofenac biopharmaceutical properties,¹⁷ a reduced clearance could result from hemodynamic alterations affecting liver blood flow and/or impairment of hepatic intrinsic metabolic function.¹⁶ The latter possibility seems unlikely, as there is evidence that indicators of liver function are not modified by SCI.¹⁴ On the other hand, we have shown that the spinal shock occurring after cord injury leads to blood flow redistribution, diminishing liver blood flow.³ It has been suggested that this is at the origin of an impaired clearance of phenacetin, a marker drug submitted to high hepatic extraction, in SCI rats.^{14,15} Hence, our data indicate that SCI reduces the clearance of diclofenac, a drug submitted to intermediate hepatic extraction, in a manner analogous to that of phenacetin. Nonetheless, SCI did not produce any significant alteration of diclofenac distribution, despite that a

Table 2 Pharmacokinetic parameters of diclofenac after administration of a single 3.2 mg kg^{-1} dose by the oral route to rats submitted to spinal cord injury (SCI) at the T8 level and to sham-lesioned animals

Group	AUC ($\mu\text{g h mL}^{-1}$)	C_{max} ($\mu\text{g mL}^{-1}$)	T_{max} (h)	<i>F</i>
Sham	7.95 ± 0.65	2.14 ± 0.06	0.19 ± 0.02	0.85 ± 0.30
SCI	5.72 ± 0.20^a	1.21 ± 0.08^a	0.25 ± 0.09	0.5 ± 0.13^a

Data are expressed as mean $\pm$ s.e.m.^aDenotes a statistically significant difference ($P < 0.05$), as determined by the Student's *t*-test.

reduced V_d has been reported for phenacetin.¹⁴ This discrepancy can be explained by drug binding to plasma proteins, which is one of the main determinants of drug distribution.^{16,17} It has been reported that SCI reduces the V_d of drugs with low plasma protein binding, such as gentamicin and phenacetin, regardless of the mechanism of drug clearance, whereas V_d remains unaltered for naproxen, a marker drug with high protein binding.¹⁴ Diclofenac, as naproxen, is extensively bound to plasma proteins.¹⁷ Extensive protein binding reduces drug tissue distribution, as protein binding opposes extravasation.¹⁶ Therefore, it appears that high protein binding limits the impact on drug tissue distribution of blood flow redistribution occurring during spinal shock.³

It is well documented that half-life depends on both, clearance and V_d .¹⁶ Hence, an impaired clearance in absence of significant alterations in V_d yields a prolonged half-life, leading to increased bioavailability, as it was observed for intravenous diclofenac in SCI rats. Despite these features, the bioavailability of oral diclofenac was reduced by SCI. To reconcile these apparently contradictory observations, it is necessary to consider that acute SCI not only can produce changes in drug elimination and distribution, but also in absorption.^{9,10,12,13} There is evidence of at least two mechanisms by which SCI can impair oral drug absorption. As mentioned above, SCI induces hemodynamic changes yielding a redistribution of tissue blood flow to ensure function of main organs such as the brain.³ There is evidence that such redistribution can cause mucous gastrointestinal irrigation to be diminished,¹⁹ and thereby contribute to decreased diclofenac absorption. Consistently, Medeiros *et al.*¹² have described that SCI impairs the transport function of the intestinal mucosa and have suggested an association with blood pooling in the lower extremities and visceral organs, occurring during spinal shock that can alter abdominal Starling forces. Hence, it appears that hemodynamic status has a major role in drug bioavailability changes observed after SCI. On the other hand, there is also evidence that SCI diminishes gastrointestinal motility,^{4,19,20} leading to a slow gastric emptying that has been suggested to result in a reduced drug absorption at the duodenum.^{4,7–10,12} It is notable that the effect of SCI on absorption is of such magnitude that it not only compensates for reduced clearance, but also overrides it. Therefore, the net result is a reduction of bioavailability after oral diclofenac in SCI rats, despite a prolonged half-life.

The present results, as well as those reported elsewhere by our group^{4,12–15} and other researchers,^{5–10} clearly indicate that pharmacokinetics of a given drug can exhibit important differences in patients with SCI compared with able-bodied individuals. An adequate understanding of these phenomena is warranted, and therefore further research is still required. It should be noted that generalizations do not apply. The information obtained for a certain drug cannot be simply extrapolated to others.¹⁰ The results presented here contribute to a better understanding of the pharmacokinetic altera-

tions occurring after cord injury. It is now clear that SCI-induced alterations not only depend on the nature of the lesion, but also on the route of administration and the particular physiological processes involved in the absorption, distribution and elimination of a given drug. Furthermore, our results confirm the usefulness of animal studies of experimental SCI to understand and characterize pharmacokinetic changes. As it appears that hemodynamic changes have a relevant role in SCI-induced alterations, animal models can be used as tools for testing the bioavailability of different drugs and their association with the hemodynamic status at several injury levels. Moreover, animal models of SCI can also be used to determine the impact of changes in endocrine and renal function on drug bioavailability.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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