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Original Article

Acute spinal cord injury changes the disposition of some, but not all drugs given intravenously

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Study design: Experimental laboratory investigations in paraplegic rats.
Objective: In order to understand why acute spinal cord injury (SCI) changes the disposition of some, but not all drugs given intravenously (i.v.), pharmacokinetic parameters of drugs with different pharmacological properties were evaluated to determine the influence of SCI on physiological processes such as distribution, metabolism and excretion.
Setting: Mexico City, Mexico.
Methods: Rats were subjected to severe SCI (contusion) at T-9 level; pharmacokinetic studies of phenacetin, naproxen or gentamicin were performed 24 h after. These drugs were not chosen as markers because of their therapeutic properties, but because of their pharmacokinetic characteristics. Additional studies including plasma proteins, liver and renal function tests, and micro-vascular hepatic blood flow, were also performed at the same time after injury.
Results: Acute SCI significantly reduced distribution of drugs with intermediate and low binding to plasma proteins (phenacetin 30% and gentamicin 10%, respectively), but distribution did not change when naproxen – a drug highly bound to plasma proteins (99%) – was used, in absence of changes in plasma proteins. Metabolism was significantly altered only for a drug with liver blood flow – limited clearance (phenacetin) and not for a drug with liver capacity-limited clearance (naproxen). The liver function test did not change, whereas the hepatic micro-vascular blood flow significantly decreased after SCI. Renal excretion, evaluated by gentamicin clearance, was significantly reduced as a consequence of SCI, without significant changes in serum creatinine.
Conclusions: Changes in drug disposition associated to acute SCI are complex and generalization is not possible. They are highly dependent on each drug properties as well as on the altered physiological processes. Results motivate the quest for strategies to improve disposition of selective i.v. drugs during spinal shock, in an effort to avoid therapeutic failure.
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Introduction

Reports regarding pharmacokinetic alterations related to spinal cord injury (SCI) in patients treated with both oral,[1] intramuscular,[2,3] and intravenous (i.v.)[4,5] drugs started about 20 years ago. Unlike other pathological entities, such as renal and hepatic chronic dysfunction, there is a lack of correlation between altered physiological processes and changes in drug disposition in human SCI. It is difficult to perform systematic pharmacokinetic studies in SCI patients. Therefore, the use of experimental models appears to be a suitable strategy for understanding pharmacokinetic alterations due to SCI, as well as the pathophysiological mechanisms involved.[6,7]

Systemic and metabolic alterations associated to SCI can produce changes in drug disposition, which depend on variables such as injury characteristics (intensity, level, and time elapsed after injury), pharmacological properties of tested drugs and administration route.

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Mechanisms involved in pharmacokinetic alterations using drugs given orally after high and low thoracic acute SCI of different intensities have been previously explored. The oral bioavailability of paracetamol,[8] salycilates,[9] and cyclosporin[10] is reduced after experimental SCI. These alterations are more evident during acute stage and the reduction in oral bioavailability is likely due to an impaired gastric emptying.

Significant alterations in distribution and elimination of drugs given i.v. have been reported in patients with chronic (greater than 1 year duration) SCI.[5,11,12] However, physiological processes involved on i.v. bioavailability alterations associated to SCI are understudied.

In the present study, it was explored if bioavailability of drugs of different pharmacokinetic characteristics is differently altered when given i.v. after experimental acute SCI and the relationship with some functional variables, including plasma proteins, liver and renal function tests, and hepatic micro-circulation. Phenacetin, naproxen, and gentamicin were not chosen as markers because of their therapeutic properties, but because of their pharmacokinetic characteristics, the latter of which allows for the study of distribution, metabolism, and excretion of drugs.

Methods

Animals

Female Sprague–Dawley rats (240–260 g) were used. At 12 h before studies, food was withdrawn, although animals had free access to water. Local Animal Care Committee approved the study. We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were following during the course of this research.

Spinal cord injury

Animals were submitted to spinal cord contusion by the Allen weight drop method modified for rats, as previously described by García-López.[7] Briefly, rats were anaesthetized with a mixture of ketamine ($77.5\,mg\,kg^{-1}$) and xylazine hydrochloride ($12.5\,mg\,kg^{-1}$). Under aseptic conditions, a laminectomy was performed at the T-9 level. Rats were then placed on a stereotaxic device and a stainless steel cylinder weighing 15 g was dropped from a height of 10 cm through a guide tube onto the exposed dura. Sham-injured controls were only submitted to a laminectomy at the same level.

Study design

Pharmacokinetic studies of phenacetin, naproxen and gentamicin were performed 24 h after injury or sham-injury in six groups (experimental and control group for each drug; $n=5$).

For biochemical studies, nine rats were submitted to SCI and nine rats were sham injured; blood samples were withdrawn from the caudal artery 24 h after spinal surgery.

For liver micro-circulation assessment, the liver surface of injured and sham-injured rats ($n=8$) was assessed by laser Doppler flowmetry.

Pharmacokinetic study

Corresponding drug for pharmacokinetic studies was administered by i.v. route. For rats with phenacetin, the dose was $23\,mg\,kg^{-1}$ blood samples (100–150 μl) were withdrawn from the caudal artery at 0, 5, 10, 20, 30, 45, 60, 90, 120, and 180 min after drug administration. Rats with naproxen received $6\,mg\,kg^{-1}$ blood samples were withdrawn at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 600 min after drug administration. Rats with gentamicin were given $15\,mg\,kg^{-1}$ blood samples were withdrawn at 0, 5, 15, 30, 60,120, and 240 min after drug administration. Total volume of blood extracted was limited to <2 ml per rat for all drugs. Phenacetin and naproxen concentrations in whole blood were determined by HPLC as previously was described;[13,14] gentamicin concentration in plasma was determined by fluorescence polarization immunoassay using a TDx analyzer (Abbott Diagnostics, Irvin, TX, USA). Individual whole-blood phenacetin, naproxen, and gentamicin concentrations against time curves were plotted and a pharmacokinetic analysis was performed using a noncompartmental approach (Professional Win Nonlin, Scientific Consulting Inc., Lexington, KY, USA). The volume of distribution and clearance of each compound were estimated.

Blood biochemical parameters

Blood samples were taken in heparinized chilled glass tubes and immediately centrifuged at 4000 g for 10 min. Aliquots of plasma were stored at $-20^{\circ}C$ until biochemical analysis was performed. Plasma albumin, total proteins, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and creatinine were measured using a commercially available test kit (Merck, Darmstadt, Germany). Absorbance was measured using a spectrophotometer (Carry 50, Bio, UV Visible spectrophotometer Varian, USA). Results were expressed as units per litter.

Hepatic micro-circulation assessment

Liver micro-circulation was measured using a laser Doppler flowmeter (MoorLab, Moor Instruments, Devon, UK) with the method previously described and validated for rats subjected to spinal cord section.[15] Briefly, a fiber optic probe with an estimated penetration depth of 0.5 mm (MP3, Moor Instruments) was placed on the medial liver lobe through a midline abdominal laparatomy. Precautions were taken to avoid artifacts related to abdominal movements associated with breathing. Records of flow (product of average concentration

and speed of moving red blood cells), concentration (number of moving red blood cells), and speed were obtained in anesthetized rats before injury or sham-injury (basal), and 24 h after.

Statistical analysis

Results of pharmacokinetic study as well as liver and renal functional tests in injured and sham-injured animals were compared using Student's *t*-test. For the liver micro-circulation studies, the signal was recorded and analyzed using the Moorsoft software for Windows V 1.1. Arbitrary units obtained from the flowmeter were converted to percentages, assuming the baseline value for each animal as 100%. Differences between basal recordings and those obtained 24 h after were plotted and statistically analyzed using a Student's *t*-test for paired data. The significance level was set a $P<0.05$.

Drugs and reagents

Phenacetin, naproxen, and gentamicin were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other reagents were analytical grade. High-quality water, employed to prepared solutions, was obtained using a Milli-Q Reagent Water System (Continental Waters Systems, El Paso, TX, USA).

Results

All animals studied exhibited normal locomotor activity before starting the study. One day after surgical procedure, injured rats showed complete flaccid paraplegia, whereas sham-injured animals exhibited normal walk after recovery from anesthesia.

Phenacetin, naproxen, and gentamicin blood concentrations

Phenacetin whole-blood concentrations are shown in Figure 1a. SCI notably increased phenacetin blood levels compared with controls. Naproxen blood levels were similar in both injured and control animals (Figure 1b). Gentamicin plasma concentrations were moderately reduced in SCI rats compared with controls (Figure 1c).

Pharmacokinetic parameters

SCI significantly reduced phenacetin and gentamicin of volume of distribution compared with controls ($P<0.05$). Volume of distribution of naproxen was not affected comparing injured rats with controls (Table 1).

SCI significantly reduced phenacetin and gentamicin clearance compared with controls ($P<0.05$). Naproxen clearance was similar between SCI and the sham-injured group (Table 2).

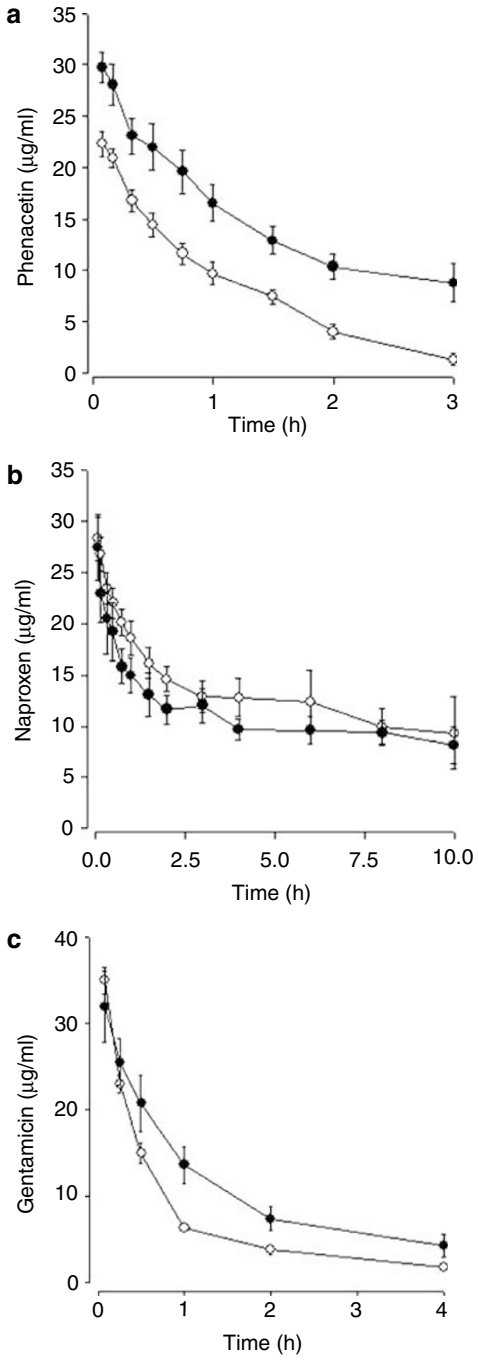

Figure 1 Whole-blood concentration of phenacetin (**a**), naproxen (**b**), gentamicin (**c**) in rats submitted to spinal cord injury (●) and in sham-injured control (○). Data represent mean ± SEM of five animals

Blood biochemical parameters

Total proteins and albumin levels were similar between injured and control rats (Table 3). No significant differences in LDH, AST, and ALT were found between the SCI and the control group (Table 4). Serum creatinine shows an insignificant trend to increase after SCI (0.90 ± 0.21 *versus* 0.66 ± 0.02 mg/dl).

Table 1 Volume of distribution (mean ± SEM) of naproxen, phenacetin and gentamicin observed after i.v. administration of a 6, 23, and 15 mg kg^{-1} dose, respectively ($n = 5$)

% Bound to plasma protein	*Volume of distribution (l/kg)*	
	Sham–lesion	*SCI*
Naproxen, 99%	0.19 ± 0.016	0.20 ± 0.024
Phenacetin, 30%	0.97 ± 0.04	0.78 ± 0.04[a]
Gentamicin, 10%	1.09 ± 0.09	0.61 ± 0.08[a]

[a]Compared with sham–lesion group, $P < 0.05$

Table 2 Clearance (mean ± SEM) of phenacetin and naproxen observed after i.v. administration of a 23 and 6 mg kg^{-1} dose, respectively to Sprague–Dawley rats submitted to spinal cord injury at the T8–T9 level and in sham-injured controls ($n = 5$)

Drug	*Clearance (ml/h kg)*	
	Sham-injured	*SCI*
Naproxen	34 ± 8	44 ± 11
Phenacetin	919 ± 115	435 ± 71[a]
Gentamicin	470 ± 39	311 ± 54[a]

[a]Compared with sham group, $P < 0.05$

Table 3 Total proteins and albumin blood concentrations

Groups	*Total proteins (g/dl)*	*Albumin (g/dl)*
Sham-injured	6.1 ± 0.13	1.38 ± 0.03
SCI	6.0 ± 0.13	1.46 ± 0.04

Values are expressed as mean ± SEM of nine animals

Table 4 Liver function biochemical parameters

Groups	*ALT (U/L)*	*AST (U/L)*	*AP (U/L)*	*LDH (U/L)*
Sham-injured	159 ± 11	900 ± 104	84 ± 8	1533 ± 158
SCI	113 ± 12	685 ± 68	76 ± 7	1318 ± 96

Values are expressed as mean ± SEM of nine animals

Hepatic micro-vascular blood flow

In rats subjected to SCI, the hepatic micro-vascular blood flow significantly decreased compared with sham-injured animals ($P = 0.002$). Flow difference is related to a significant fall in red blood cells speed in injured rats compared with sham controls ($P = 0.023$), whereas concentration was very similar between both groups ($P = 0.499$) (Figure 2).

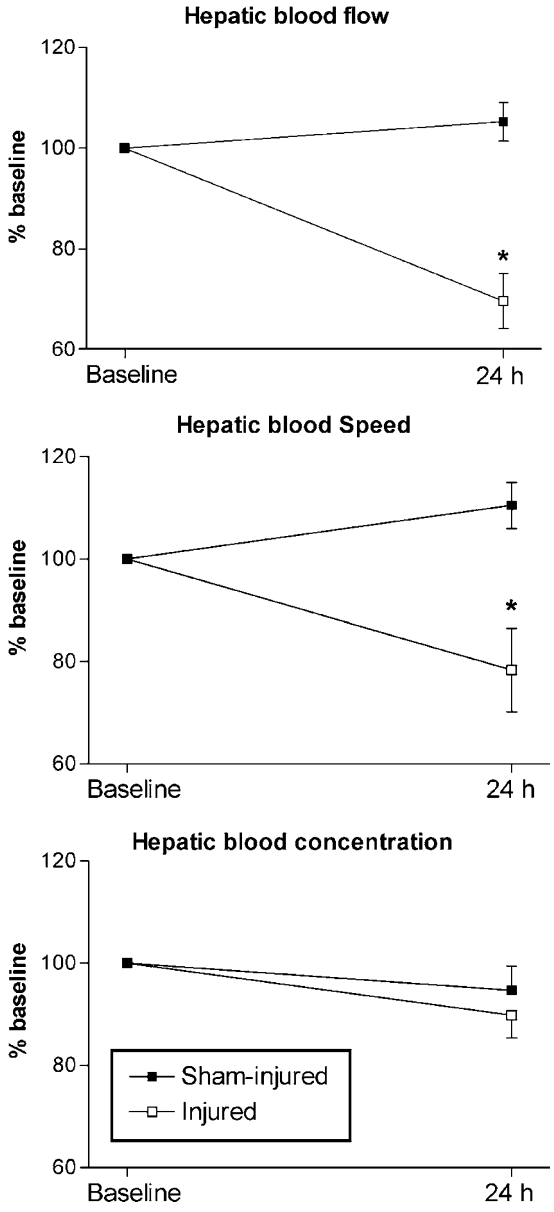

Figure 2 Hepatic micro-circulation parameters recorded immediately before spinal surgery (baseline) and 24 h after. The hepatic blood blow drops significantly in injured rats regarding to the sham-injured group. This change is mainly dependent on a drop in hepatic blood speed. The hepatic blood concentration remains similar between groups. Data represent mean ± SEM of eight animals; *$P < 0.05$

Discussion

Even though it is known that SCI changes disposition of some drugs given i.v.,[5,11,13] there is a lack of information regarding the mechanisms involved in such alterations. This topic is particularly interesting, taking into account that during spinal shock, patients receive an important number of i.v. drugs including neuroprotective agents (such as methylprednisolone), antibiotics, anticoagulants, vasoactive agents, etc.

Here, it was found that acute SCI modifies disposition of some, but not all drugs given i.v. Differences in drug disposition will be analyzed according to drug pharmacological properties and to fundamental physiologic

processes involved in the systemic bioavailability of drugs given i.v., namely distribution and elimination. These processes were assessed here by volume of distribution and clearance, respectively.

Distribution implies transporting the drug to tissues and ultimate to cells throughout the bloodstream. This process depends on several factors, including cardiac output, systemic macro- and micro-circulation, and drug protein binding.[16]

The process of elimination involves drug biotransformation and excretion. For most drugs subject to change, biotransformation is performed in the liver. Drugs which metabolic clearance depends on liver blood flow (such as phenacetin, methylprednisolone, or cyclosporine) – also known as high-extraction drugs – are subjected to an extensive hepatic metabolism, which is dependent on the amount of drug arriving to liver. In contrast, biotransformation of low-extraction drugs (such as most non-steroidal anti-inflammatory drugs), does not depend on liver blood flow. It mainly depends on liver enzymatic activity.[17–19]

During spinal shock, there is low circulation volume and low cardiac output, which importantly contribute to decrease blood flow to non-vital organs, including liver.[15,20–22] This could lead to significant changes in drug distribution and elimination, particularly for high-extraction drugs.

Disposition of drugs which elimination depends on liver blood flow, and show low to medium protein binding

Phenacetin is a prototype of drugs which elimination depends on liver blood flow and show low to medium protein binding (30%). The main pharmacokinetic parameter alterations for this drug were a significant decrease in volume of distribution and on clearance, which reflect alterations in drug distribution and elimination, respectively.

For phenacetin, distribution decrease may be related to its low to medium protein binding. Kinetically, the free (unbound) drug is diffused from plasma to interstitial fluid in such a way, that blood flow is the rate limiting step in the distribution of these drugs. Owing to a decrease in blood flow after SCI, free drug that must go to tissues remains in circulating volume, which explains the poor drug tissue distribution and high plasma concentrations that were observed in this study.

As phenacetin is a high-extraction drug, decrease on clearance observed in this study suggests that low hepatic blood flow instead of disturbances in liver enzymatic capacity occur. Experimental observations carried out in this study confirm such proposal: the hepatic blood flow decrease significantly, while no changes in hepatic functional test were observed when comparing injured *versus* sham-injured animals.

Disposition of drugs which elimination depends on liver enzymatic activity, and show high protein binding

Naproxen is an example of drugs whose elimination is not dependent on liver blood flow, but rather depends on liver enzymatic activity, and show high protein binding (99%). Results in this study show that any pharmacokinetic parameter of this drug was not altered after acute SCI, demonstrating that elimination and distribution do not change in relation to sham-injured subjects.

With naproxen, lack of alterations on distribution can be related to its high protein binding. In this case, the bound drug remains in plasma and only the free drug goes to interstitial fluid. As concentration of plasma proteins was not altered by the injury, theoretically the carry of bound- and free-drug was not modified.

As naproxen is a low-extraction rate drug, biotransformation was not affected by SCI, in agreement with the adequate enzymatic liver function observed in this study. In this case, alterations in hepatic blood flow are unimportant.

Taking into account that there were no changes in pharmacokinetic parameters of this drug, it was hypothesized that other drugs with similar pharmacological characteristics – such as most nonsteroidal anti-inflammatory drugs – could be used at recommended dosage and schedule to eligible body subjects.

Disposition of unchanged drugs which elimination depends on renal function

Owing its pharmacological properties, gentamicin is an attractive drug to be used as an excretion marker. It is not metabolized, so elimination is not dependent on hepatic blood flow or liver enzymatic activity. Around 90% is excreted without changes in urine and its elimination is by glomerular filtration.[23] In this study, it was found that gentamicin clearance was significantly reduced, which indicates that renal excretion is significantly decreased with regards to sham-injured animals. The volume of distribution was also significantly diminished.

These changes could be explained by alteration in renal blood-flow during spinal shock[15,20] and consequently the alteration in glomerular filtration.[24,25] Results on serum creatinine of this study suggest that a global renal failure is not involved.

Taking into account the alteration in excretion of gentamicin, which is ototoxic and nephrotoxic, as all aminoglucosides, it was considered fundamental to carry out a close monitoring of blood concentration of this kind of drugs.

In summary, SCI induces changes on disposition of some, but not all drugs given i.v., which depend on both pharmacological properties of drugs administered. A better understanding of the pathophysiological mechanism involved in distribution, metabolism, and excretion alterations after SCI should be very useful for an appropriate management, as well as for the optimization of pharmacological therapy in this population.

It is important to mention that not in all i.v. administered drugs the distribution changes; the factor that determines if the distribution changes depends on the pharmacological characteristics.

Drugs with decreased volume of distribution and with elimination dependent on hepatic blood flow, such as phenacetin and methylprednisolone, will not fully reach the hypoperfused tissues. As a consequence, there will be higher drug levels in blood and the drugs will remain more time in blood due to diminished elimination.

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