THE INFLUENCE OF EXPERIMENTAL SPINAL CORD INJURY ON CARBAMAZEPINE PHARMACOKINETICS

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Background: Previous studies have demonstrated that pharmacokinetic behavior of several drugs such as paracetamol, theophylline, and aminoglycosides are significantly altered in patients with spinal cord injury. So far, no study on pharmacokinetics of carbamazepine has been performed in patients or experimental models with spinal cord injury. The present study was designed to find the influence of experimental spinal cord injury on carbamazepine pharmacokinetics.

Methods: Among 12 male albino rabbits, 6 were subjected to spinal cord injury at the 8th thoracic level by knife severance method and 6 rabbits underwent laminectomy alone (sham-lesioned control group). All received a single oral dose of carbamazepine (20 mg/kg) 24 hours after the injury. Blood samplings were done at predetermined times to 96 hours after drug administration. Carbamazepine concentration in serum samples was determined by high-performance liquid chromatography. Pharmacokinetic parameters including maximum concentration, time to reach maximum concentration, half-life, and area under the curve (AUC) were directly determined from the concentration-time curve. Area under the concentration against time curve (AUC) was calculated from the real data.

Results: Maximum concentration was appeared at 2.8 hours after administration in sham-lesioned control group at a concentration of 2.3 µg/mL, whereas in spinal cord injury group it was appeared at 4.4 hours at a concentration of 2.7 µg/mL. In spinal cord-injured group, area under the curve and half-life were increased from 29.1 µg/mL.hr to 38.7 µg/mL.hr and from 7.7 hr to 14.1 hr as compared with the sham-lesioned control group, respectively. Statistical analyses of data showed that spinal cord injury does not induce significant changes in carbamazepine pharmacokinetics.

Conclusion: We concluded that pharmacokinetic behavior of carbamazepine was not significantly changed by spinal cord injury, although its subtle pharmacokinetic changes could be resulted from alteration in gastrointestinal tract motility, blood perfusion, or metabolism.

Keywords: Carbamazepine • pharmacokinetics • spinal cord injury

Introduction

Morbidity and mortality rates after both human and experimental spinal cord injury (SCI) are high. Life-threatening complications such as pneumonia, urinary tract infections, and infected pressure sores are common, mainly at the early stages of the lesion, despite the use of standard pharmacologic treatment. It is possible that such therapeutic failures are due, at least in part, to unsuitable dosing strategies, which do not consider pharmacokinetic alterations in this patient population. SCI may change the kinetics of drug absorption, distribution, and elimination.

About 66% of all SCI patients reported some types of pain; the most common variety of pain consisted of a sensation usually described as “burning” in those body parts below the level of injury. Evidence suggests that the most effective drugs in treatment of this type of pain are carbamazepine (CBZ) and phenytoin. There are evidences that pharmacokinetic behavior of several drugs such as paracetamol, theophylline, and aminoglycosides are significantly altered in patients with SCI. However, to the best of our
knowledge, no study on pharmacokinetics CBZ has been performed on patients or experimental models during the acute phase of SCI. We, therefore, considered it of interest to study whether pharmacokinetics of CBZ are altered by SCI. Clinical reports on drug kinetics in SCI are often anecdotal, because it is extremely difficult to perform systematic pharmacokinetic studies on patients with SCI. This difficulty is due to the important inter-individual variability in injury extent and location. Therefore, the use of experimental models appears to be a suitable strategy for understanding the pharmacokinetic alterations caused by SCI. We studied the pharmacokinetic behavior of CBZ after oral administration to rabbits subjected to experimental SCI. In this study, rabbit was selected as animal model because of long half-life of the drug and the need for 15 blood samples from animals during each study.

Materials and Methods

Drugs and reagents
Carbamazepine was obtained from Sobhan Pharmaceutical Company (Iran). CBZ suspension was home made. Acetonitrile, high performance liquid chromatography (HPLC) grade, and propylparaben were obtained from E. Merck (Darmstadt, Germany). All other reagents were of analytical grade. High-quality water, employed to prepare solutions, was obtained with the use of a Milli-Q Reagent Water System (Continental Water Systems, El Paso, TX, USA).

Animals
Twelve male albino rabbits weighing 2500 – 3000 g were used. Twelve hours before drug administration, food was withheld, but animals had free access to water. The study was approved by the local Animal Care Committee.

Spinal cord injury
Animals were anesthetized by intraperitoneal injection of ketamine HCl (75 mg/kg) and thiopental sodium (50 mg/kg). Under aseptic conditions, laminectomy was performed at the T8 level; the spinal cord was completely severed by a clean transverse cut with the help of a scalpel. Then, aponeurotic plane and the skin were separately sutured with 5 – 0 nylon thread. Postsurgical care was performed as described by Guizar et al.

Determination of carbamazepine pharmacokinetic parameters
A single dose of 20 mg/kg of CBZ, as suspension, was given by gavage through a nasogastric tube. Blood samples (2 mL) were drawn at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hr after drug administration. The total blood volume extracted did not exceed 30 mL. CBZ concentration in serum samples was determined by a modified HPLC method described by Bhatti et al. The HPLC system consisted of a LC 10 A DVP VP pump, a variable SPD 10 A UV-Vis detector, and Class VP chromatographic software, all from Shimadzu (Japan). Separation was achieved using a Shimpack C18 analytical column (250 mm × 4.6 mm ID, Shimpack, Japan). The isocratic mobile phase consisted of phosphate buffer (pH 4.3), acetonitrile (750:250, v/v) prepared daily and degassed by passing through a 0.45-µm filter. All chromatographic separations were performed at room temperature. The flow rate was set to 0.9 mL/min. UV detection was performed at 225 nm.

Individual serum concentration against time curves were constructed, and the maximum concentration (C max), as well as the time to reach the maximum concentration (T max), were directly determined from these plots. Half-life was estimated by linear regression of the terminal concentration decay phase plotted in semi-logarithmic coordinates. The area under the concentration against time curve (AUC) to the last concentration-time point was determined by the trapezoidal rule and extrapolated to infinity by dividing the last detectable concentration by the terminal slope. The absorption rate constant was estimated by residual method.

Study design
Animals were divided into two groups each consisting of six rabbits. Animals in group 1 were subjected only to laminectomy and served as sham-injured (control group), whereas animals in group 2 subjected to SCI. Oral CBZ by gastric gavage was given 24 hr after the surgical procedure. Comparisons between control and SCI animals were performed by Student’s t-test for unpaired data. Also, 95% confidence interval was used to determine the differences between the mean pharmacokinetic parameters in two groups. Differences were considered to reach statistical significance when P was < 0.05.

Results
All the animals exhibited locomotor activity
before the initiation of the study. After surgical procedure, injured rabbits showed complete flaccid paraplegia; five of them had watery stool after 12th hr. The sham-injured animals exhibited normal walk after recovery from anesthesia. CBZ serum concentrations observed at various times with a single oral dose of 20 mg/kg administered after SCI and under control conditions are shown in Figure 1. This figure indicates that $C_{\text{max}}$ was appeared at 2.8 ± 1.1 hr after administration in control group at a concentration of 2.3 ± 0.3 µg/mL. $C_{\text{max}}$ in SCI group was appeared at 4.2 ± 1.2 hr at a concentration of 2.7 ± 0.7 µg/mL. These pharmacokinetic parameters were higher and variable in SCI than the control group. Pharmacokinetic parameters are shown in Table 1.

The results showed that, though not statistically significant, pharmacokinetic parameters derived in this study were greater in SCI group as compared with the control group.

**Discussion**

We know that SCI results in physiologic disturbances, which alter the pharmacokinetics of several therapeutic agents. However, drug treatment in these patients is still performed on an empirical basis, since no rational strategy has yet been provided. It is hence, necessary to characterize the pharmacokinetic alterations induced by SCI in order to adequately design dosage regimens. Since it is difficult to perform systematic studies on patients, we have undertaken the characterization of SCI-induced pharmacokinetic alterations using a well-described experimental model, i.e., spinal cord severance by knife in the rabbit. Such characterization, however, is highly difficult to perform in human subjects, given the high interindividual variability observed in clinical setting. Therefore, pharmacokinetic studies using experimental model of SCI appear to be the most suitable strategy.4 CBZ was studied because it is widely used for treatment of pain in SCI patients,15 and some evidence suggest that one of the most effective agents in treatment of neuropathic pain due to SCI is CBZ.6,7 The results of present study showed that SCI at the level of T8 did not produce any significant alterations in CBZ pharmacokinetic parameters, although there was a trend toward an increase in $C_{\text{max}}$ and AUC and prolongation of $T_{\text{max}}$ and half-life. Lara et al found that SCI at the T8 level leads to reduction in $C_{\text{max}}$ and AUC of salicylic acid after oral administration of aspirin in rat, suggesting that SCI decreased the rate of aspirin absorption.11,16 Lopez et al showed that SCI at the level of T8 significantly reduced $C_{\text{max}}$ and AUC of paracetamol.4 Since CBZ is only orally administered, it seems that any changes in motility or secretion of the gastrointestinal (GI) tract induced by SCI could affect the rate and extent of its absorption. In the present study, we observed that in contrast to some of the above-mentioned water-soluble drugs, SCI did not only decrease $C_{\text{max}}$ and AUC of CBZ, it also increased these parameters to some extent. There is evidence that gastric emptying is impaired by SCI,9,17 probably by stimulation of nitric oxide release that results in inhibition of GI motility.18 This means that

![Figure 1. Carbamazepine serum concentration observed after oral administration of 20 mg/kg in rabbits subjected to spinal cord injury at the level of T8 (blue squares) and in sham controls (black squares). Data are expressed as the mean ± SEM (n = 6).](image)

**Table 1.** Pharmacokinetic parameters (mean ± SEM) observed after oral administration of 20 mg/kg of carbamazepine to rabbits subjected to SCI (n = 6) at the T8 level and in sham controls (n = 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham control</th>
<th>SCI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-24}$ (µg/mL·hr)</td>
<td>25.3 ± 1.8</td>
<td>27.8 ± 4.2</td>
<td>0.299</td>
</tr>
<tr>
<td>$AUC_{24-\infty}$ (µg/mL·hr)</td>
<td>29.1 ± 2.9</td>
<td>38.7 ± 4.6</td>
<td>0.056</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>2.3 ± 0.3</td>
<td>2.7 ± 0.7</td>
<td>0.294</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>2.8 ± 1.1</td>
<td>4.2 ± 1.4</td>
<td>0.230</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>7.7 ± 0.7</td>
<td>14.1 ± 4.6</td>
<td>0.112</td>
</tr>
</tbody>
</table>

*AUC = area under the curve; SCI = spinal cord injury; $C_{\text{max}}$ = maximum concentration; $T_{\text{max}}$ = time to reach the maximum concentration.*
probably, under these conditions, for very low water solubility of drugs such as CBZ, the ingested drug has more time to be absorbed from the GI tract. On the other hand, it has been reported that SCI results in constriction of several vascular territories including GI wall.\textsuperscript{11, 19} Therefore, other mechanisms cannot be ruled out with the information available. The results of our study showed that the half-life of CBZ is increased by SCI, though not statistically significant. A prolonged half-life in experimental animals 24 hr after SCI at the T8 level has also been reported for cyclosporine and diclofenac.\textsuperscript{20, 21} These reports suggest that SCI results not only in alterations in drug absorption, but also in drug elimination. The mechanisms in the impairment of drug elimination by SCI remain unclear.\textsuperscript{20}

It should be taken into account that SCI is not a static process; the primary lesion produced by the mechanical trauma is followed by a secondary lesion, which increases the original neuronal damage.\textsuperscript{4} This has been attributed to the presence of multiple endogenous toxic substances within the lesion area\textsuperscript{19, 22, 23} as well as to a disruption of the microcirculation.\textsuperscript{24} Overlapping the pathologic processes following SCI, there are several reparative changes, including scar formation and plasticity processes, which include both the central and autonomic nervous systems.\textsuperscript{25 – 28} Because the present findings on CBZ obtained in early phase of SCI (the first 96 hr), further investigations are necessary for the study of pharmacokinetic parameters of the drug during the subacute and chronic phases of SCI.

It should be mentioned, however, that in the present study, SCI was produced at the T8 level, and the alterations below that level could be less intense than SCI at higher levels. Therefore, in addition to the time elapsed after SCI, the location of SCI should also be considered as a covariate in determining the dosing schedule.

SCI appears to result in pharmacokinetic changes, and these alterations appear to be complex. The global effect of SCI on drug pharmacokinetics is given by the sum of the alterations in drug absorption, distribution, and elimination. These effects depend on the pertinent physiologic mechanisms, route of administration, extent and location of the injury, and time elapsed after SCI.

We concluded that pharmacokinetic behavior of CBZ was not significantly altered in early phase of SCI.

Acknowledgment

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References

256 – 302.


