CHAPTER 3

TOPICAL AND ORAL PHARMACOKINETICS OF CC-2
3.1 Introduction

CC-2 is a potent sulphur mustard (SM) decontaminant and has shown promising efficacy in laboratory animals (1, 2). It is still under development and preclinical pharmacokinetic data is needed to support its development into a clinically viable product for SM decontamination. Preclinical pharmacokinetic studies gain importance from the fact that they are necessary not only to determine rate and extent of availability of the agent under investigation but are also required by drug regulatory bodies. CC-2 is a topical decontaminating agent and hence belongs to a class termed as locally acting drug products. Such agents should have minimum systemic exposure post application as they are intended to exert their action locally. Therefore, to ensure safety, information on systemic exposure is important. The degree of systemic exposure can be determined by measurement of active moiety in blood, plasma, serum or urine employing normal pharmacokinetic techniques (3). The present chapter deals with topical and oral pharmacokinetics of CC-2 in male Sprague-Dawley rats.

3.2 Experimental

3.2.1 Materials

Pure reference standard of CC-2 (purity >99%) provided by Defence Research and Development Establishment (DRDE), Gwalior, India was used in the studies. Analytical grade gum acacia was procured from Qualigens Fine Chemicals, Bombay, India. Triple distilled water from all quartz glass apparatus was used in formulation preparation.

3.2.2 Animals

The pharmacokinetic studies of CC-2 were performed in young, healthy male Sprague-Dawley rats (250 ± 25 g) obtained from Laboratory Animal Services Division of the institute. The animals were housed in plastic cages under standard laboratory conditions with a regular 12 h day-night cycle. Standard pelleted laboratory chow (Goldmohar laboratory Animal feed, Lipton India Ltd, Chandigarh, India) and water were allowed ad libitum. Rats were acclimatized in animal room for at least two days prior to commencement of study. The study protocol was approved
by local ethical committee (Reg. No. 34/1999/CPCSEA) and experiments in rats, euthanasia and disposal of carcasses were carried out as per their guidelines.

3.2.3 Formulation

Formulation for dermal application was provided by DRDE, Gwalior (India) containing 10 g of CC-2 suspended in distilled water (q.s., 50 g). Suspension formulation of CC-2 for rat oral feeding was prepared by grinding the weighed quantity in a dry mortar. Gum acacia (1%, w/v) was used as suspending agent. The suspension was prepared by drop wise addition of water and fresh formulation was used every time.

3.2.4 Dermal Application

One day before treatment, hair from the back of rats was closely clipped using a pair of scissors and a circular test area (2.5 cm diameter, 4.91 cm²) was marked. Care was taken not to abrade the skin. The dermal formulation was applied to the circular area at an amount of 40.8 mg/cm². A nonocclusive plastic cover was then gently fixed with surgical tape to prevent the animal from disturbing the application site. Rats were individually caged and were provided with food and water *ad libitum*. Two blood samples per rat, one intracardiac and one from inferior vena cava were withdrawn. The samples were collected at 0.5, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h post dermal application. Serum was separated and samples were stored at -60°C pending analysis.

3.2.5 Oral Administration

After an overnight fast, rats were randomly divided in three groups (n=3) each containing 6 rats. On a single day, animals received CC-2 at an oral dose of 0.5 g/kg as a suspension (2 ml/kg) using a rat feeding needle and syringe. From each group of 6 rats, two blood samples were withdrawn per rat, one intracardiac (~1.2 ml) and one from inferior vena cava post dosing at a pre-decided sampling schedule of 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 h. The blood was allowed to clot; serum was
separated and samples were stored at -60°C pending analysis. Same protocol was followed at 1 and 2 g/kg oral dose.

3.2.6 Analysis of CC-2 in Rat Serum

The concentration of CC-2 in unknown serum samples after dermal and oral administration was determined using a validated HPLC assay method as described in section 2.3 (4).

3.2.7 Pharmacokinetic Analysis

The peak serum CC-2 concentration (C<sub>max</sub>) and its time of occurrence (t<sub>max</sub>) were directly read from the concentration-time data. Other pharmacokinetic parameters were determined on subjecting the concentration-time data to non-compartmental analysis using WinNonlin version 1.5 software (SCI consultants, USA). A minimum of three data points were used to calculate the terminal half-life \([t_{1/2} = \ln(2)/\lambda_z]\) where \(\lambda_z\) is elimination rate constant. The mean residence time (MRT) was calculated using formula \(\text{MRT} = \text{AUMC}_\text{inf}/\text{AUC}_\text{inf}\). The clearance (Cl/F) and the volume of distribution (V<sub>d</sub>/F) were calculated from \(\text{Cl}/\text{F} = \text{Dose}/\text{AUC}_{\text{inf}}\) and \(V_{d/F} = \text{Dose}/\lambda_z\cdot\text{AUC}_{\text{inf}},\) in which F is the fraction of the administered dose absorbed. Area under the concentration-time curve (AUC) was calculated by the trapezoidal rule (5). The relative bioavailability for dermal application relative to oral route was calculated using the equation:

\[
\%F_{\text{Relative}} = \frac{\text{AUC}_{\text{dermal}} \times \text{Dose}_{\text{oral}}}{\text{AUC}_{\text{oral}} \times \text{Dose}_{\text{dermal}}} \times 100
\]

3.3 Results and Discussion

The validated HPLC assay method was applied to determine concentration of CC-2 following dermal and oral administration in rats (4). CC-2 was monitored up to a period of 18 h after dermal application and 0.5 g/kg oral administration while it could be monitored up to 24 h following 1 g/kg and 2 g/kg oral administration. No additional peak indicative of metabolite was observed following oral and topical administration during HPLC analysis. Serum concentration-time profiles of CC-2
(mean ± SEM) following topical and oral administration are depicted in Figure 3.1 (6). Pharmacokinetic parameters of CC-2 are listed in Table 3.1.

**Figure 3.1** Mean ± SEM serum concentration-time profile of CC-2 after dermal application and oral administrations in rats.
Table 3.1 Pharmacokinetic parameters of CC-2 after oral administration and dermal application in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oral Dose</th>
<th>Dermal Application</th>
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<tbody>
<tr>
<td></td>
<td>0.5 g/kg</td>
<td>1 g/kg</td>
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<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>59.2±5.9</td>
<td>198.5±6.5</td>
</tr>
<tr>
<td>2</td>
<td>136.9±4.4</td>
<td>240.6±24.2</td>
</tr>
<tr>
<td>3</td>
<td>46.3±7.2</td>
<td>77.1±9.4</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;max&lt;/sub&gt; (h)</strong></td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng.h/ml)</strong></td>
<td>672</td>
<td>1739</td>
</tr>
<tr>
<td><strong>Elim. t&lt;sub&gt;1/2&lt;/sub&gt; (h)</strong></td>
<td>5.9</td>
<td>7.4</td>
</tr>
<tr>
<td><strong>MRT (h)</strong></td>
<td>6.1</td>
<td>8.7</td>
</tr>
<tr>
<td><strong>Vd/F (L/kg)</strong></td>
<td>5973</td>
<td>5977</td>
</tr>
<tr>
<td><strong>Cl/F (L/h/kg)</strong></td>
<td>700</td>
<td>562</td>
</tr>
</tbody>
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Concentration is represented as Mean ± SEM (n=3)

*Abbreviations: AUC<sub>0-∞</sub> = area under the concentration-time curve from time zero to infinity, C<sub>max</sub> = peak concentration, Cl/F = clearance, MRT = mean residence time, t<sub>max</sub> = time to peak concentration, Elim. t<sub>1/2</sub> = elimination half-life, V<sub>d</sub>/F = volume of distribution*

Following dermal application of CC-2, a single C<sub>max</sub> (41.7 ± 8.8 ng/ml) was observed at 2 h. Elimination t<sub>1/2</sub> was found to be 12.0 h. V<sub>d</sub>/F and Cl/F were high post dermal application (average, 3.96 L/h/kg for rat)(7). After oral administration (0.5, 1 and 2 g/kg) of CC-2, three concentration maxima (C<sub>max</sub>) at each dose were observed with t<sub>max</sub> ranging from 0.75-1, 1.5-3 and 8-12 h for C<sub>max1</sub>, C<sub>max2</sub> and C<sub>max3</sub> respectively. The C<sub>max</sub> values did not follow a dose dependent increase. Area under
the curve (AUC), which indicates the extent of absorption, showed a proportional increase at 0.5 and 1 g/kg dose but not at 2 g/kg. Elimination t_{1/2} after oral dosing was similar at three doses ranging between 5.9-7.4 h. After oral administration V_d/F and Cl/F were higher in comparison to oral administration. Relative bioavailability (mean ± SEM) for dermal route in comparison to oral route was found to be 43.2 ± 6.1 %.

CC-2 is a lipophillic (logP 3.5 ± 0.2) molecule (8). It is poorly soluble in solvents like water, ethanol, dimethylsulphoxide, polyethyleneglycol and propyleneglycol used commonly for i.v. administration. Therefore, its absolute systemic exposure following dermal application could not be determined. Systemic exposure of CC-2 following dermal application was determined relative to oral administration. Oral pharmacokinetic studies were also performed at three different doses in increasing order.

The rate and extent at which a chemical moiety passes through skin is influenced by its physiochemical properties such as molecular mass, ionization of drug at physiological pH, the lipid/water partition coefficient etc.(9). Drugs with preferable logP value of ≤ 2 are considered to be a potential candidate for transdermal delivery (10). NSAIDs with logP > 2.5 exhibited lower rate of absorption than those with logP < 2.5 due to unfavourable solubility properties (11). The Log P of CC-2 is > 2.5 and hence it may not be an ideal molecule to pass across dermal layers (10). The inherent lipophilicity of CC-2 should minimize its diffusion into the vascularized dermis and minimal uptake into the systemic circulation after dermal application. This was reflected in concentration-time profile of CC-2 as lower concentration at each time point was observed following dermal and oral administration at comparable dose. Time taken to reach C_{max} (t_{max}) after dermal application was higher than that after oral administration. This phenomenon is typical for dermal route of application. The stratum corneum provides an effective barrier that retards penetration of topically applied formulations. This would result in a lag time, followed by a broad peak concentration profile. Half-life of CC-2 was found to be higher (t_{1/2} = 12 h) via dermal route than that after oral administrations (mean t_{1/2} = 6.7 h). This could be because drug may continue to diffuse into the skin, becoming systemic, even after C_{max} is attained. Thus, longer than expected elimination half-lives may be found if these values are based on first 24 h after dermal application (12).
Following oral administration, CC-2 exhibited multiple peaks and there was no dose proportionate increase in $C_{\text{max}}$ and AUC indicating that it follows non-linear pharmacokinetics. Multiple peak phenomena observed with CC-2 is not unusual as the same have been documented for a number of drugs (13-15). Several mechanisms have been proposed for the phenomenon: (i) enterohepatic recycling (16), (ii) presence of absorption window along the gastrointestinal tract (17), and (iii) variable gastric emptying (18). It is proposed that lower aqueous solubility of CC-2 can be a contributing factor for such profile. Although high values of $V_d/F$ and $Cl/F$ are observed, not much can be said about these parameters in absence of i.v. data (19). At three oral doses, no observable adverse effect was seen in rats during study period. Relative bioavailability of CC-2 averaged to 43.2 %. However, it is likely that still lower levels of CC-2 will be observed in systemic circulation in practical situations as most of the topically applied CC-2 will be used in decontaminating SM resulting in formation of products known to be non-toxic. Thus, lower amounts of CC-2 will be available for absorption.

3.4 Conclusion

CC-2 is a lipophilic compound and is readily but not appreciably absorbed after dermal application or oral administration. Irregular concentration-time profile following oral administration can be attributed to high lipophilicity and poor aqueous solubility of CC-2. Although mean relative bioavailability after dermal application is 43.2 %, it is likely that actual bioavailability will be still lesser supporting CC-2 as a future decontaminant against SM.
References


