DIURNAL VARIATION IN THE METABOLISM OF S-CARBOXYMETHYL-L-CYSTEINE IN HUMANS

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ABSTRACT:

The routes of metabolism of S-carboxymethyl-L-cysteine in humans are dependent on the time of dosing. Administration of 750 mg of S-carboxymethyl-L-cysteine (Day 1) during the day at 8:00 AM followed by a 8:00 AM to 4:00 PM urine collection revealed that S-carboxymethyl-L-cysteine S-oxide was the major urinary metabolite produced. The 4:00 PM to midnight urine collection resulted in S-(carboxymethylthio)-L-cysteine being identified as the major urinary metabolite. However, the administration of 750 mg of S-carboxymethyl-L-cysteine (day 15) during the night at midnight and analysis of the midnight to 8:00 AM urine collection found that thiodiglycolic acid was the major urinary metabolite, whereas thiodiglycolic S-oxide was identified as the major urinary metabolite in the 8:00 AM to 4:00 PM urine collection. A diurnal variation in the metabolism of S-carboxymethyl-L-cysteine was seen and, in particular, the timing of S-carboxymethyl-L-cysteine administration had a profound effect on the identity of urinary S-oxide metabolites produced. After administration at 8:00 AM the urinary S-oxides produced were S-carboxymethyl-L-cysteine S-oxide and S-methyl-L-cysteine S-oxide but at midnight the major urinary S-oxide metabolite produced was thiodiglycolic acid S-oxide.

The metabolism of the mucolytic agent and substituted L-cysteine analog, S-carboxymethyl-L-cysteine (SCMC)\(^1\) is complex (Mitchell et al., 1984), with S-oxidation, \(\alpha\)-amino group \(N\)-acetylation and deamination/transamination, and \(\alpha\)-carboxyl group decarboxylation (Brand et al., 1936; Berk Pharmaceuticals, 1973), together with side chain decarboxylation and \(\beta\)-C-S bond cleavage (Brand et al., 1936; Blood and Lewis, 1941; Binkley, 1950) being reported. However, after the suggestion that metabolism in humans is variable (Mitchell et al., 1984) and that differences in metabolism are linked to a number of adverse drug reactions (Emery et al., 1984; Ayesh et al., 1987) and disease states (Olomo et al., 1988; Scadding et al., 1988; Bradley et al., 1994; Steventon et al., 1999), a steady stream of reports in the literature in favor (Mitchell et al., 1984; Haley et al., 1985; Ayesh et al., 1988) or against (Specht et al., 1990; Hofmann et al., 1991; Brockmoller et al., 1991) the production of S-carboxymethyl-L-cysteine S-oxide (SCMC SO) have appeared. In addition, the major urinary metabolite of SCMC was believed to be in fact a mixed disulfide [S-(carboxymethylthio)-l-cysteine (CMT C)] that was mistaken for the SCMC SO metabolite by investigators using descending paper chromatography (Price-Evans, 1993), but this has now been found to be incorrect (S teven ton, 1998). It is possible that the differences in quantities and actual metabolites reported by various groups may be due to differences in the diurnal handling of the drug, and this article reports on the effects of chronobiological factors on the metabolism of SCMC in humans.

Materials and Methods

Chemicals. SCMC, S-methyl-L-cysteine (SMC), and thiodiglycolic acid (TDA) were obtained from Sigma Chemical Co. (Poole, Dorset, UK). SCMC SO, S-methyl-L-cysteine S-oxide (SMCSO), thiodiglycolic acid S-oxide (TDASO), and CMT C were synthesized by the methods of Schobler and Grafje (1958), Meese et al. (1990), and Staffeldt et al. (1991). All compounds had melting points, \(^1\)H-NMR and mass spectrometry which was in agreement with the literature (Meese, 1987; Meese et al., 1990, 1991; Staffeldt et al., 1991).

Volunteers. Five healthy male volunteers (age, 20.3 \(\pm\) 1.2 years; means \(\pm\) S.D.) were recruited from the students at the Department of Biological Sciences, University of the West of England, Bristol, UK. The time interval between the two separate studies was 14 days. All the individuals gave informed consent and the studies were approved by the appropriate university ethics committee. No volunteer was taking any medication and all had normal hepatic and renal function tests.

Daytime administration (day 1). The volunteers fasted from midnight and emptied their bladders before taking 750 mg SCMC at 8:00 AM. Urine was collected from 8:00 AM to 4:00 PM and 4:00 AM to 4:00 PM. The total urine volume of each collection was recorded and two 20-ml aliquots were stored at \(-20^\circ\)C until analyzed.

Nighttime administration (day 15). The volunteers fasted from 6:00 PM and emptied their bladders before taking 750 mg SCMC at midnight. Urine was collected from midnight to 8:00 AM and 8:00 AM to 4:00 PM. The total urine volume of each collection was recorded and two 20-ml aliquots were stored at \(-20^\circ\)C until analyzed.

HPLC and Thin-Layer Chromatograph (TLC) Analysis of SCMC and Metabolites in Urine Samples. The HPLC analysis of the urine samples was carried out by the method of Staffeldt et al. (1991) as reported by Steventon (1998). The TLC analysis of the urine samples was carried out by the method of Gregory et al. (1993) as reported by Steventon (1998). The results reported are from the HPLC analysis of urine samples, however TLC analysis gave similar results and both methods can be used to provide a “double check” on the analysis of SCMC and its metabolites in urine (Steventon, 1998).

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\(^1\) Abbreviations used are: SCMC, S-carboxymethyl-L-cysteine; SCMC SO, S-carboxymethyl-L-cysteine S-oxide; SMC, S-methyl-L-cysteine; SMCSO, S-methyl-L-cysteine S-oxide; TDA, thiodiglycolic acid; TDASO, thiodiglycolic acid S-oxide; CMT C, S-(carboxymethylthio)-l-cysteine.

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### TABLE 1

**SCMC metabolism after dosing at 8:00 AM**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Metabolites (% of Dose Administered)</th>
<th>8:00 AM–4:00 PM urine collection after 750 mg SCMC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 PM–midnight urine collection after 750 mg SCMC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>8:00 AM–midnight urine collection after 750 mg SCMC&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCMC</td>
<td>SCMC&lt;sub&gt;O&lt;/sub&gt;</td>
<td>SMC</td>
<td>SMCSO</td>
</tr>
<tr>
<td>1</td>
<td>20.2</td>
<td>14.1</td>
<td>2.0</td>
<td>3.4</td>
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<td>2</td>
<td>30.5</td>
<td>10.0</td>
<td>5.3</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>15.3</td>
<td>1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>4</td>
<td>15.1</td>
<td>4.7</td>
<td>10.8</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>21.4</td>
<td>18.2</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>21.4</td>
<td>18.2</td>
<td>3.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

### TABLE 2

**SCMC metabolism after dosing at midnight**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Metabolites (% of Dose Administered)</th>
<th>Midnight–8:00 AM urine collection after 750 mg SCMC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>8:00 AM–4:00 PM urine collection after 750 mg SCMC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Midnight–4:00 PM urine collection after 750 mg SCMC&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SCMC</td>
<td>SCMC&lt;sub&gt;O&lt;/sub&gt;</td>
<td>SMC</td>
<td>SMCSO</td>
</tr>
<tr>
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<td>17.2</td>
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<td>1.1</td>
<td>N.D.</td>
</tr>
<tr>
<td>2</td>
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<td>4.1</td>
<td>3.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>3</td>
<td>19.6</td>
<td>7.4</td>
<td>2.1</td>
<td>N.D.</td>
</tr>
<tr>
<td>4</td>
<td>24.1</td>
<td>11.8</td>
<td>1.0</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>26.7</td>
<td>18.2</td>
<td>4.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Mean ± S.D. | 28.2 ± 6.8 | 13.3 ± 4.8 | 5.3 ± 3.6 | 3.8 ± 2.4 | 1.3 ± 0.4 | 3.6 ± 1.0 | 55.5 ± 8.1 | 6.7 ± 2.6 | 5.2 ± 1.0 | 8.3 ± 1.8 |

Range | 19.6–37.9 | 5.7–11.8 | 1.0–11.8 | 0.0–2.0 | 2.1–5.1 | 45.4–65.9 | 6.7–21.6 | 5.2–10.8 |

%Total, % total drug-related compounds recovered in urine collection; %SO, % S-oxides recovered in urine collection; N.D., not detected.

<sup>a</sup> Analysis of 8:00 AM to 4:00 PM urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.

<sup>b</sup> Analysis of 4:00 PM to midnight urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.

<sup>c</sup> Analysis of 8:00 AM to midnight urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.

### TABLE 3

**Diurnal variation in metabolism**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Metabolites (% of Dose Administered)</th>
<th>8:00 AM–4:00 PM urine collection after 750 mg SCMC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 PM–midnight urine collection after 750 mg SCMC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>8:00 AM–midnight urine collection after 750 mg SCMC&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
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<td>SCMC&lt;sub&gt;O&lt;/sub&gt;</td>
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</tr>
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<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>30.5</td>
<td>10.0</td>
<td>5.3</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>15.3</td>
<td>1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>4</td>
<td>15.1</td>
<td>4.7</td>
<td>10.8</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>21.4</td>
<td>18.2</td>
<td>3.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Mean ± S.D. | 20.2 ± 6.8 | 14.1 ± 4.8 | 2.0 ± 3.6 | 3.4 ± 2.4 | 1.0 ± 0.4 | 3.6 ± 1.0 | 55.5 ± 8.1 | 6.7 ± 2.6 | 5.2 ± 1.0 | 8.3 ± 1.8 |

Range | 19.6–37.9 | 5.7–11.8 | 1.0–11.8 | 0.0–2.0 | 2.1–5.1 | 45.4–65.9 | 6.7–21.6 | 5.2–10.8 |

%Total, % total drug-related compounds recovered in urine collection; %SO, % S-oxides recovered in urine collection; N.D., not detected.

<sup>a</sup> Analysis of 8:00 AM to 4:00 PM urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.

<sup>b</sup> Analysis of 4:00 PM to midnight urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.

<sup>c</sup> Analysis of 8:00 AM to midnight urine collection after administration of 750 mg SCMC to five volunteers at 8:00 AM.
Results

Daytime Administration. The results obtained after daytime dosing (8:00 AM) are shown in Table 1. The major compounds recovered in the first 0- to 8-h urine collection (8:00 AM–4:00 PM) were the unchanged drug, SCMC (range, 11.4–30.5% of dose) and its S-oxide, SCMCSO (range, 4.7–18.2% of dose). Both SMC (range, 1.0–10.8% of dose) and SMCSO (range, 1.0–8.2% of dose) were minor urinary metabolites. No TDA, TDASO, or CMTC were detected (Table 1). During the 8- to 16-h urine collection period (4:00 PM–midnight) the major urinary compound recovered was again the parent drug SCMC (range, 5.3–15.1% of dose), with minor amounts of CMTC (range, 2.1–5.1% of dose), SCMCSO (range, 0.0–2.1% of dose), SMC (range, 0.0–1.1% of dose), and TDA (range, 0.0–2.0% of dose). SMCSO or TDASO were not detected (Table 1). From the combined results (0–16 h of urine collection), it can be seen that the quantified metabolites accounted for 55.5 ± 8.1% (mean ± S.D.; range, 45.4–65.9%) of the administered dose, indicating that a substantial proportion is still to be excreted or that it has already been voided in the form of previously unknown metabolites (Table 1). The production of CMTC was found to be independent of SCMCSO and TDASO production (results not shown).

Nightime Administration. A different metabolic profile was observed after the nighttime administration of the drug (Tables 2). During the initial 0- to 8-h urine collection period (4:00 PM–8:00 AM), the major compound excreted in the urine was again the unchanged drug SCMC (range, 5.1–33.4% of dose), but TDA was also prominent (range, 8.4–23.4% of dose). Minor urinary metabolites were SCMCSO (range, 0.0–4.1% of dose) and SMC (range, 0.0–1.0% of dose). TDA or
CMTC were not found. The most noticeable features within the initial 0–8 h of urine collection after nighttime administration when compared with daytime dosing were the production of TDA and the dramatic decrease (4.4- to 8.8-fold) in urinary S-oxide metabolite recovery (Table 2). Within the 8- to 16-h urine collection (8:00 AM–4:00 PM), TDASO was evident (range, 15.4–23.7% of dose) together with TDA (range, 5.7–12.3% of dose) and unchanged SCMC (range, 4.0–12.1% of dose). Smaller amounts of CMTC (range, 1.0–5.2% of dose) and SCMCSO (range, 0.0–2.0% of dose) were found with only one volunteer producing SMC (1.0% of dose). No SMCSO metabolite was detected. The most evident differences observed between the 8- to 16-h urine collections are the presence of large amounts of TDA and TDASO after nighttime administration (TDASO was absent after daytime ingestion, Table 1). It can be seen from the combined results (0–16 h) obtained after nighttime administration that the quantitated metabolites account for a large proportion (77.9 ± 6.7%, mean ± S.D.; range, 68.7–86.2%) of the administered dose and subsequent overall recovery was significantly greater after nighttime administration than when compared with daytime intake (P < .05, Student’s t test, Table 2). The production of CMTC was found to be independent of SCMCSO and TDASO production (results not shown).

Discussion

For a drug that has a multitude of metabolic options available, the time of drug administration had a pronounced effect on the actual metabolite profile obtained. After daytime ingestion, S-oxidation appears to be the predominant route during the first 8 h, with formation of SCMCSO as the major urinary metabolite (Fig. 1A), followed by

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Fig. 2. Nighttime dosing.

A, metabolism of SCMC midnight to 8:00 AM when administered at midnight. B, metabolism of SCMC 8:00 AM to 4:00 PM when administered at midnight.
mixed disulphide (CMTC) formation (Fig. 1B). However, administration at nighttime results initially in the formation of TDA via \( \alpha \)-amino group deamination/transamination and subsequent decarboxylation (Fig. 2A), followed by disulphide formation (CMTC) and \( S \)-oxidation of TDA to form TDASO (Fig. 2B).

Mixed disulphide (CMTC) formation occurred in the 8- to 16-h period irrespective of the time of dosing, suggesting the requirement for extensive degradation of the drug before CMTC can be formed. This observation is in agreement with other reports that indicate that the CMTC metabolite was not found in the first 0- to 8-h urine collection (Kuper and Idle, 1990; Gregory et al., 1993; Steventon, 1998). \( S \)-oxidation appears to be most active during the daytime (daylight) hours (8:00 AM–4:00 PM), permitting the formation of \( S \)-oxide metabolites from whatever sulphide precursor is presented, be it unchanged drug (daytime administration) producing SCMC SO or a metabolite (nighttime administration) producing TDASO. Decreased \( S \)-oxidation of this drug overnight has been observed previously (Mitchell and Waring, 1989; Mitchell et al., 1992) and may reflect endogenous variation in the metabolism of cysteine-like sulfides by this cytosolic enzyme(s). During the nighttime hours, deamination and decarboxylation seem to take priority.

Although the number of subjects studied was small, close inspection of the results showed a marked variation in the quantitative excretion of individual metabolites between different volunteers (Tables 1 and 2). After the daytime dosing, subject 4 produced less urinary SCMC SO (\( S \)-oxidation) (5.7%) than the other four subjects (15.2 ± 3.7%, mean ± S.D.; range, 10.0–18.2%) and more urinary SMC (decarboxylation) (11.8% compared with 3.7 ± 2.0%; range, 2.0–6.3% of dose). After nighttime ingestion, subject 2 produced less urinary CMTC (\( \beta \)-C-S cleavage) (1.0%) than the remaining four volunteers (3.7 ± 1.0%; range, 3.1–5.2% of dose).

Attempts to reconcile such differences when found in larger groups have evoked the possibility of genetic control of metabolism. It has been suggested that a polymorphism exists within a population in the ability to form the mixed disulphide metabolite CMTC. Thus a metabolic ratio ([SCMC]/[CMTC]) with an antimode value of 17–18 has been constructed to discriminate between “poor” and “extensive” mixed disulphide formers (Kuper and Idle, 1990; Gregory et al., 1993; Steventon, 1998). Thus employing the antimode value of 18 (the antimode in this case was the metabolic ratio that separated the bimodal frequency distribution for the mixed disulphide metabolic ratio) the present five volunteers were all (100%) extensive disulphide formers irrespective of whether dosing was daytime or nighttime. This should be compared with values of 90 to 94% reported by others (Kuper et al., 1991; Gregory et al., 1993). Similarly, variation in \( S \)-oxidation has been proposed using a “14.3% total recovery as \( S \)-oxides” as an antimode value (Mitchell et al., 1984). Using this criterion, the present results suggest that one individual (1/5; 20%) would be a “poor \( S \)-oxidizer” based on the 0- to 8-h (8:00 AM–4:00 PM) urine results in daytime dosing (Mitchell et al., 1984), but all five (5/5; 100%) would be “poor \( S \)-oxidizers” after nighttime dosing. Conversely, if the 8- to 16-h (4:00 PM–midnight) urine data were to be used, all subjects would be poor \( S \)-oxidizers after the daytime dosing but extensive \( S \)-oxidizers after nighttime dosing! In addition, the metabolite profile has been shown by other workers to be dose dependent (Waring, 1980; Mitchell et al., 1984; Meese et al., 1990; Brocknmoller et al., 1991). It is relatively easy to understand why differences have appeared in the literature and why there is confusion regarding the metabolism of SCMC. To successfully reproduce results, strict adherence to a given protocol is essential (Mitchell and Waring, 1989; Waring and Mitchell, 1990).

Relatively little is understood concerning the impact of circadian variation on the biochemical and physiological processes that influence the stages of a drug’s journey through the body. Day/night differences in gastric emptying rates and body blood flow, especially mesenteric blood flow, could greatly modify gastrointestinal drug absorption and subsequent distribution have been reported previously (Labrecque and Belanger, 1991), as have diurnal variations in glomerular filtration rate, active tubular secretion, and tubular reabsorption, all of which may affect drug excretion (Waterhouse and Minors, 1989).

These factors could all be involved in the quantitative differences seen between daytime (8:00 AM) and nighttime (midnight) administration of SCMC and its subsequent metabolite profile. One possible explanation for the qualitative differences seen between daytime (8:00 AM–midnight) and nighttime (midnight–4:00 PM) metabolite profiles would be a diurnal variation in drug metabolism. Cytochrome P-450 metabolism of aniline, benzphetamine, benzo(\( \alpha \))pyrene, biphenyl, imipramine, and steroids has been reported to show diurnal variation in experimental animals (Labrecque and Belanger, 1991). Diurnal variations in metabolism in animals were not only confined to the phase I reactions but sulfation and glucuronidation of phenol and \( p \)-nitrophenol were also affected (Labrecque and Belanger, 1991).

In the rat, drug metabolism was found to be greater at night (awake hours) than during the day (sleep hours), which is akin to the humans data reported here, \( S \)-oxidation was greatest during daytime (awake hours) and decreased during the night (sleep hours). This resulted in alternative metabolic routes for SCMC being employed by the body during the nighttime hours (\( \alpha \)-amino group deamination/transamination and oxidative decarboxylation) compared with the daytime hours (\( S \)-oxidation). This could possibly be due to circadian rhythms in the endocrine system, which may modulate the \( S \)-oxidation of sulfides within the mammalian body.

The metabolism of SCMC in terms of the enzymes involved, diurnal and or genetic control are poorly understood at present. There are a number of unresolved issues. 1) Is the diurnal metabolism of SCMC reversible (can the metabolism be day-night cycled? ) 2) Are those individuals who produce low amounts of \( S \)-oxide metabolites during the day on a reverse diurnal cycle? This has implications for the reports of the association of the poor \( S \)-oxidation phenotype with adverse drug reactions and clinical disease states. 3) What are the enzymes that are involved in the metabolism of SCMC? All of these problems as yet remain to be addressed.

References


