ABSORPTION, DISPOSITION, AND METABOLISM OF TRANS-METHYL STYRYL KETONE IN FEMALE B6C3F1 MICE

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ABSTRACT:

trans-Methyl styryl ketone (MSK; trans-4-phenyl-3-buten-2-one) is a β-unsaturated ketone that has a wide range of uses in industry, as well as consumer products. MSK does not appear to be overtly toxic in animal models, however, it has been shown to be mutagenic in several in vitro assays after S-9 activation. In this study experiments were conducted to characterize MSK absorption, distribution, metabolism, and elimination after iv, oral, and topical administration to female B6C3F1, mice. After iv administration, [14C]MSK (20 mg/kg; 120 μCi/kg) was rapidly cleared from the blood as evidenced by the following pharmacokinetic values (mean ± SD): terminal disposition half-life (t1/2), 7.98 ± 1.72 min; mean residence time, 5.6 ± 1.7 min; steady-state apparent volume of distribution (Vss), 3.33 ± 0.75 liters/kg; and systemic body clearance (CLsys), 0.53 ± 0.05 liters/min/kg. Within 48 hr, 92.4% of the dose was excreted in the urine and 3.5% in the feces. The major blood metabolites after iv administration were identified by GC-MS as the 4-phenyl-3-buten-2-ol (methyl styryl carbinol), 4-hydroxy-4-phenyl-2-butanoate, and benzyl alcohol. After oral administration of [14C]MSK (200 mg/kg; 100 μCi/kg), 95% of the dosed radioactivity was recovered in the urine and 1.2% in the feces within 48 hr. Major urinary metabolites were identified by LC-MS/MS as N-phenylacetyl-L-glycine (35.1% of dose) and N-benzyl-L-glycine (19.1% of dose). Only a small amount of MSK was detected in the blood after oral administration (0.073 μg/ml at 10 min), and [14C]-equivalents in the blood never exceeded 2.8% of the dose. After topical application of [14C]MSK (250 mg/kg; 50 μCi/kg), approximately 40% of the dose was absorbed and 84.5% of the absorbed dose was excreted into the urine (36% of the total dose). Urinary metabolites were similar to those described for oral administration. Importantly, [14C]-equivalents were not detected in the blood at any time after dermal administration. These results indicate that the rate of MSK clearance is equivalent to its rate of absorption, and tissue exposure to intact MSK is expected to be limited.

MSK1 (3-buten-2-one, 4-phenyl-(8CI, 9CI); CAS registry no. 1896 – 62-4) has a wide range of uses in industrial and commercial uses. It is a reactive carbonyl compound used in many types of organic syntheses. MSK is known to occur naturally, but its extensive use as a fragrance additive in soaps, detergents, lotions, creams, and perfumes (1). Although MSK has been used extensively in several consumer products, it is known to be mutagenic in several short term in vitro assays (1). As a reactive carbonyl compound used in many types of organic syntheses, MSK is known to occur naturally, but its extensive use as a fragrance additive in soaps, detergents, lotions, creams, and perfumes (1). Although MSK has been used extensively in several consumer products, it is known to be mutagenic in several short term assays. In the Ames Salmonella typhimurium assay, MSK produced a positive mutagenic response in strain TA100 with S-9 activation, but not in strain TA98 regardless of activation (2). In the mouse lymphoma L5178Y, TK ± assay MSK was positive but only with S-9 activation (3). Thus, it is apparent that MSK requires metabolic activation to exert its mutagenic effects. However, MSK does not appear to be overtly toxic in either rats or mice (4).

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1 Abbreviations used are: MSK, trans-methyl styryl ketone; HPLC, high pressure liquid chromatography.

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We have recently examined and reported on the metabolism, pharmacokinetics, and disposition of MSK in male Fischer 344 rats (5). After both oral and topical administration of MSK, the majority of the absorbed dose was readily absorbed, as evidenced by greater than 80% of the absorbed dose appearing in the urine within 12 to 24 hr. Absorption of MSK through the derma was significantly slower than from the gastrointestinal tract. However, after each route of administration, MSK could not be detected in the blood. At the end of the experiments (48 to 120 hr), no significant amount of radioactivity was retained by the tissues. These findings are likely owing to the rapid hepatic clearance of MSK and the excretion of its water soluble metabolites (principally N-phenylacetyl-L-glycine) into the urine. A small portion of the dose escaped metabolism and was excreted into the urine intact (0.8–4.8%). Thus, in the rat the rate of clearance for MSK appears to be equivalent to or exceeds its rate of absorption, and the internal doses of MSK are exceedingly low. To determine whether other species or other sexes metabolize MSK in a manner similar to that of the rat, the studies reported here describe the distribution and metabolism of MSK after iv, oral, and dermal exposure in the female B6C3F1 mouse.

Materials and Methods

Chemicals. Universally ring labeled [14C]MSK (trans-4-phenyl-3-buten-2-one) was obtained from Chemsyn Science Laboratories (Lenexa, KS).
MSK was obtained from Acros Organics (Pittsburgh, PA), and its chemical purity was greater than 99%. All other reagents were either of analytical or HPLC grade.

Animal Studies. Animals. Female B6C3F1 mice (21–27 g) were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). Upon arrival, the animals were acclimated for 5 to 7 days in a temperature controlled (25°C) 12 hr light/12 hr dark cycle facility before any treatment. The animals were provided food (Teklad 4% Mouse-Rat Diet, Harlan Teklad, Madison, WI) and water ad libitum.

Intravenous administration. [14C]MSK (20 mg/kg, 120 μCi/kg), in emulphor:ethanol:water:saline, 3:4:12 (v/v/v) was administered via the tail vein (4 ml/kg). Mice were killed at selected times (0, 1, 2, 3, 5, 7, 10, 15, 25, 30, 45, and 60 min) by carbon dioxide inhalation, and blood was collected from the posterior vena cava. A separate set of animals was placed in glass metabolism cages for collection of urine (6, 12, 24, and 48 hr), feces (24 and 48 hr), and exhaled radioactivity over a 48 hr period. [14C]-Equivalents associated with exhaled organics were trapped in 2-methoxyethyl ether (Mallinkrodt Chemical, Paris, KY) and 14CO2 was trapped in a CarboSorb (Packard, Meriden, CT) and ethylene glycol (Mallinkrodt Chemical, Paris, KY) solution 2:1 (v/v). Trapping solvents were changed and measured for total radioactivity by scintillation counting at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 hr after administration of [14C]MSK. Feces were analyzed for total radioactivity by liquid scintillation counting, while blood and urine samples were analyzed directly by scintillation counting.

Oral administration. [14C]MSK (200 mg/kg, 100 μCi/kg) in 0.5% methyl cellulose (5 ml/kg) was administered orally by gavage to female B6C3F1 mice. Mice were killed at selected times (0, 5, 10, 15, 30, and 45 min, as well as 1, 3, 6, 24, and 48 hr) by carbon dioxide inhalation, and blood was collected from the posterior vena cava. The animals in the 48-hr time group were placed in glass metabolism cages after dosing to allow for collection of urine (6, 12, 24, and 48 hr) and feces (24 and 48 hr), as well as exhaled radioactivity (0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 hr).

Topical administration. The in vivo percutaneous absorption of [14C]MSK after topical application was determined using the method of Winter and Sipes (6). Following topical application of [14C]MSK (250 mg/kg, 50 μCi/kg) in 10 μl acetone, mice were killed (1, 2, 3, 6, 24, 48, and 72 hr) by carbon dioxide inhalation, and blood was collected from the posterior vena cava. The animals in the 72-hr time group were placed in glass metabolism cages after dosing to allow for collection of urine (6, 12, 24, 48, and 72 hr) and feces (24, 48, and 72 hr), as well as exhaled radioactivity (0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, and 72 hr). After euthanasia the skin at the treatment site was washed with 25 ml of methanol and aliquots of the wash were analyzed using liquid scintillation counting. Furthermore, the skin site was analyzed for radioactivity by tissue oxidation (7). Radioactivity adsorbed to the activated charcoal was desorbed by exhaustive extraction with methanol followed by dichloromethane. The metalumin traps were washed in acetone (3 × 100 ml). Aliquots of the desorbing solvent, as well as trap washings, were analyzed using liquid scintillation counting.

Data analysis. The blood concentration-time data after iv bolus dosing were analyzed by both compartmental and noncompartmental methods. The methods of analysis performed on these data have been previously described (5).

Analytical Methods. HPLC analysis of MSK and its metabolites. MSK and its metabolites were extracted from the blood with dichloromethane (DCM). Samples were dried and reconstituted with hexane/ethanol acetate, 90:10 (v/v). The reconstituted samples were injected onto a 250 × 4.6 mm silica (10 μm) column (Phenomenex, Torrance, CA) and eluted with a mobile phase of methanol and ethyl acetate, 90:10 (v/v) at a flow rate of 1 ml/min with a run time of 50 min as previously described (5). For analysis of urinary metabolites, urine samples were diluted 1:1 (v/v) with 1.0% acetic acid and centrifuged to remove any precipitate. Prepared urine samples (pH 5) were injected onto a 250 × 4.6 mm Whatman Partisil ODS-2 (10 μm) column (Whatman, Hillsboro, OR), and metabolites were eluted with a mobile phase of water and acetonitrile both containing 0.1% acetic acid at a flow rate of 1 ml/min with a total run time of 80 min as previously described (5).

Metabolite identification using MS and MS/MS analysis. For metabolite isolation and identification, urine samples from the oral, topical, and iv administration study (pooled from 6, 12, and 24 hr collection) were subjected to reversed phase HPLC as described above. Samples were analyzed on a Finnigan TSQ 7000 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an atmospheric pressure source as previously described (5). Volatile urine and blood metabolites were separated and analyzed using a Fisons GC-8000 gas chromatograph coupled to a Fisons MD800 quadrupole mass spectrometer (Fisons Instruments, Beverly, MA) as previously described (5). The mass spectrum of putative metabolites in the samples were compared with the mass spectrum of authentic standards to verify compound identity.

Results

Intravenous Administration. After iv administration the blood concentrations of MSK declined rapidly and were below the limit of detection after 30 min (fig. 1). The concentration-time profile could be adequately described by a biexponential equation, consistent with a linear two-compartmental model. The average (± SD) terminal half life (t1/2), apparent volume of distribution at steady-state (Vss), and systemic body clearance (CL) values were 7.98 ± 1.72 min, 3.34 ± 0.75 liters/kg, and 0.54 ± 0.05 liters/min/kg, respectively. The mean residence time (MRT) for MSK was 5.6 ± 1.7 min. The major blood metabolites (for identification see below), 4-phenyl-3-buten-2-ol and 4-hydroxy-4-phenyl-2-butanoic acid, like the parent compound were rapidly cleared. The MRT of these metabolites were estimated to be 5.91 and 15.1 min for 4-phenyl-3-buten-2-ol and 4-hydroxy-4-phenyl-2-butanoic acid, respectively. These data suggest that the elimination of 4-phenyl-3-buten-2-ol is formation rate limited. For 4-hydroxy-4-phenyl-2-butanoic acid elimination seems to be controlled by further metabolism (fig. 2).

[14C]MSK was rapidly eliminated and by 6 hr greater than 88% of the dose had been excreted into the urine. Only a small percentage of the dose appeared in the feces (3.5%) and little was exhaled (1.2%). Cumulative excretion of radioactivity in urine and feces is shown in fig. 3A and recovery of total radioactivity is shown in table 1.

Oral Administration. When administered orally [14C]equivalent were rapidly excreted into the urine (table 1). By 6 hr, greater than 84% of the dose was found in the collected urine. Cumulative excretion of radioactivity in the urine and feces is shown in fig. 3B. The radioactivity associated with the exhaled carbon dioxide and organic vapors was minimal (0.3%). Whole blood samples after oral dosing with [14C]MSK were analyzed for parent compound and metabolites. Parent compound was detected in the blood between 10 and 30 min.
after oral administration (table 2). At peak concentration (10 min), 
0.73 mg MSK/ml of blood was detected, while only 2.6% of the 
total dose was observed in the blood.

**Topical Administration.** After topical application of [14C]MSK, 
57.1% of the dose was associated with the activated charcoal, skin-
trap, and site of application, while the remainder of the dose was 
absorbed (40.1%). Elimination of total radioactivity after topical 
exposure is shown in table 1. Of the amount absorbed, 84.2% was 
excreted and recovered in urine while 8.5% was recovered in feces. 
The rate of excretion was slower than that observed after oral or iv 
administration of MSK (fig. 3C). Although greater than 40% of dose 
was absorbed, neither MSK or [14C]-equivalents were detected in the 
blood at any time.

**Determination of Blood and Urine Metabolites.** Blood metabo-
lites. Normal phase HPLC analysis of blood obtained after iv admin-
istration revealed five major radioactive peaks representing four me-
tabolites and MSK (RT 10, 12, 23, 28, and 61 min) (fig. 4 and table 
3). Because of the volatility of the analytes, these metabolites were 
analyzed using GC-MS. The retention time and mass spectra of 
putative metabolites in the samples were compared with the mass 
spectra of authentic standards to verify compound identity. The mass 
spectrum for many of the metabolites were identical to those previ-
ously described by Sauer et al. (5). Peak A<sub>blood</sub>, B<sub>blood</sub>, and D<sub>blood</sub> 
determined to be 4-phenyl-3-buten-2-ol (III; fig. 5), methyl 
styryl ketone (I), and benzyl alcohol (IV), respectively. Peak C<sub>blood</sub> 
could not be identified likely owing to its limited blood concentra-
tion. Metabolite peak E<sub>blood</sub> was unique to the mouse and its mass spec-
trum for possessed a molecular ion corresponding to m/z 164 (fig. 6). 
The EI spectrum of the m/z 164 molecular ion corresponded to 
4-hydroxy-4-phenyl-2-butanone (II). The retention times and mass 
spectra of putative metabolites in the samples were compared with the 
mass spectra of authentic standards to verify compound identity.

**Urinary metabolites.** After reversed phase HPLC analysis of urine 
from mice treated orally, topically, and intravenously with [14C]MSK, 
several major radioactive peaks were detected. Each of the different 
routes of administration showed similar but not identical metabo-
litte profiles in the urine (fig. 7). A quantitative accounting of the urinary 
metabolites after each route of administration are shown in table 4. 
The predominant metabolite (F) was N-phenylacetyl-l-glycine (VI) 
and the secondary metabolite (D) was N-benzyl-l-glycine (V). Meta-
oblitte H was determined to be N-acetyl-S-(4-phenyl-2-butanoate)-l-
cysteine (VII). Because of its volatility, metabolite peak K was 
separated and analyzed using a GC-MS. The mass spectrum for 
metabolite K contained two metabolites; 4-phenyl-3-buten-2-one (I) 
and 4-phenyl-3-buten-2-ol (III). The mass spectra of these com-

![Fig. 2. Whole blood concentration of major blood metabolites of methyl styryl ketone (expressed as percentage of dose) as a function of time after iv administration of [14C]MSK (20 mg/kg, 120 μCi/kg) to female B6C3F<sub>1</sub> mice. Data represent mean ± SD, (N = 3 per time).](image)

![Fig. 3. Cumulative recovery of total radioactivity in the urine and feces following (A) iv (20 mg/kg, 120 μCi/kg), (B) oral (200 mg/kg, 100 μCi/kg), and (C) topical (250 mg/kg, 50 μCi/kg) administration of [14C]MSK to female B6C3F<sub>1</sub> mice. Data expressed as mean percentage of dose ± SD, (N = 3).](image)
DISPOSITION AND METABOLISM OF trans-METHYL STYRYL KETONE IN THE MOUSE

TABLE 1

Recovery of [14C]-equivalents from female B6C3F1 mice following administration of [14C]trans-methyl styryl ketone

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Percentage of Radioactive Dose Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Intravenous (20 mg/kg)</td>
<td>92.4 ± 6.0</td>
</tr>
<tr>
<td>Oral (200 mg/kg)</td>
<td>94.4 ± 3.4</td>
</tr>
<tr>
<td>Topical (250 mg/kg)</td>
<td>36.1 ± 10.8</td>
</tr>
</tbody>
</table>

† Mean % of dose ± SD, N = 3; oral and intravenous studies were conducted for 48 hr, while the dermal study was conducted for 72 hr.
‡ HPLC retention time.
§ Includes percutaneous skin trap, activated charcoal, and skin at site of MSK application.

TABLE 2

Summary of time course of total radioactivity and trans-methyl styryl ketone in blood following oral administration

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total Radioactivity in Blood (%) of dose</th>
<th>Amount of MSK in Blood (µg MSK/ml of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.0 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>2.6 ± 1.1</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>15</td>
<td>2.9 ± 0.7</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>2.9 ± 0.7</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>45</td>
<td>2.8 ± 1.1</td>
<td>ND</td>
</tr>
<tr>
<td>60</td>
<td>1.8 ± 0.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected, mean ± SD, N = 3.

TABLE 3

Percentage of parent and metabolites in the blood following intravenous administration of [14C]trans-methyl styryl ketone

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HPLC R T†</th>
<th>Peak Identification‡</th>
<th>Time of Peak Blood Concentration (min)</th>
<th>Amount at peak Blood Concentration (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl styryl carbinol</td>
<td>10</td>
<td>A_blood</td>
<td>3</td>
<td>5.4 ± 1.7</td>
</tr>
<tr>
<td>Methyl styryl ketone</td>
<td>12</td>
<td>B_blood</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Undefined</td>
<td>23</td>
<td>C_blood</td>
<td>3</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>28</td>
<td>D_blood</td>
<td>3</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>4-hydroxy-4-phenyl-2-butanone</td>
<td>61</td>
<td>E_blood</td>
<td>5–10</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

Mean ± SD, N = 3.
† HPLC retention time.
‡ Fig. 4.

Fig. 4. Representative HPLC radiochromatogram of an extracted blood sample taken from a female B6C3F1 mouse 5 min after iv administration of [14C]MSK (20 mg/kg, 120 µCi/kg). The peaks are identified by letters that correspond to those listed in table 3.

cleared from the body, primarily by excretion into the urine. When administered topically, MSK was absorbed significantly more slowly and to a lesser degree than when given orally. After oral administration 98% of the dose was absorbed, but only 43% of the dose was absorbed after topical administration. Although MSK was not detected in the systemic circulation to a significant degree, intact MSK was found in the urine after each route of administration. Thus, it is likely that the parent compound is absorbed intact, and at least a small portion escapes metabolism gaining access to the systemic circulation. Although tissue exposure to parent compound is likely to be low, the amount and number of metabolites that specific tissues are exposed to is significant.

The dispositional as well as metabolic data obtained from this study were similar to those reported for the Fischer 344 rat by Sauer et al. (5). For both the rat and mouse the blood concentration-time profile of MSK after iv administration could be adequately described by a biexponential equation, consistent with a linear two-compartmental model. Not surprisingly, the clearance of MSK from the blood by the mouse was significantly faster than the rat. A comparison of the average terminal half-life (t½), apparent volume of distribution at steady-state (Vss), systemic body clearance (CLs), and mean residence time (MRT) support this conclusion (7.98 vs. 17.7 min; 3.34 vs. 0.89 liters/kg; 0.54 vs. 0.07 liters/min/kg; and 5.6 vs. 13.1 min; mouse vs. rat, respectively). A surprising aspect of this comparison is with regard to the values of systemic clearance, especially the magnitude in the mouse. The value of 540 ml/min/kg for CLs in the mouse is in excess of estimation of hepatic blood flow (ca. 110 ml/min/kg) and cardiac output (ca. 400 ml/min/kg). This would suggest very efficient extra-hepatic clearing mechanisms, but at this time we cannot suggest what they might be. Furthermore, in the mouse the large apparent volume of distribution at steady state for MSK suggests a significant distribution to tissues. This value was approximately 3-fold greater...
than the that observed for the rat. Thus, it seems that tissue exposure to parent compound is significantly greater in the mouse than in the rat. Following oral administration, MSK (\(0.73\, \text{mg/ml}\)) was observed in the blood of mice, but not in the blood of rats receiving a similar (200 mg/kg) or significantly larger (2000 mg/kg) dose of MSK. This difference in blood profiles following oral administration could be related to a higher rate of intestinal absorption of MSK or a lower intrinsic hepatic clearance in the mouse. Thus, there are some differences between the rat and mouse in the absorption, metabolism, and excretion of MSK. However, quantitative differences between species become even more evident when comparing the metabolism of MSK (see below).

In the mouse, the principle blood metabolites of MSK are methyl styryl carbinol and 4-hydroxy-4-phenyl-2-butanone which represented approximately 5.4% (\(\approx 6.5\, \text{µg/ml}\)) and 2.3% (\(\approx 3.0\, \text{µg/ml}\)) of the dose, respectively, at peak blood concentrations following iv administration. Similar to the parent compound, these metabolites were rapidly metabolized after being formed (MRT; 5.9 to 15.1 min). The formation of these metabolites indicates that MSK can be simultaneously reduced or oxidized following administration. Thus, the keto functional group of MSK is reduced to a carbinol, or the \(\alpha,\beta\)-unsaturated bond is oxidized. Although these metabolites represent the major blood metabolites following iv administration, they comprised only a minor fraction of the dose (<0.1%) excreted in the urine. These metabolites serve as substrates in other metabolic pathways which result in the formation of the ultimate urinary metabolites. The formation of methyl styryl carbinol and 4-hydroxy-4-phenyl-2-butanone appears to represent two divergent metabolic pathways which result in the eventual formation of \(N\)-phenylacetyl-l-glycine and \(N\)-benzyl-l-glycine, respectively.

A scheme for the metabolism of MSK in the B6C3F₁ mouse consistent with the data reported here is shown in fig. 5. After administration, MSK appears to follow three primary metabolic pathways: reduction, oxidation, and conjugation with glutathione. These pathways subsequently result in the formation of \(N\)-phenylacetyl-l-glycine (phenaceturic acid), \(N\)-benzyl-l-glycine (hippuric acid), and \(N\)-acetyl-S-(4-phenyl-2-butanone)-l-cysteine as the major terminal metabolites of MSK. Benzylc \(\beta\)-unsaturated ketones are metabolized
to either benzoic acid or phenylacetic acid according to the number of carbon atoms associated with their alkene side chain (10). Interestingly, both benzoic acid and phenylacetic acid are formed from MSK. Nucleophilic attack from glutathione also accounted for a major metabolic pathway for MSK. This glutathione conjugate undergoes further metabolism resulting in the urinary excretion of N-acetyl-L-cysteine conjugate which accounted for 1.7 to 9.9% of the dose, depending on the route of administration.

A principal difference in the blood metabolite profile between Fischer 344 rats and B6C3F1 mice was the presence of an oxidized metabolite of MSK, 4-hydroxy-4-phenyl-2-butanone. This metabolite was present in the mouse, but was not detected in the blood of rats. Interestingly, the formation of this metabolite by the mouse did not result in any unique terminal metabolites. However, the distribution of terminal metabolites differs between mice and rats. For example, after oral administration N-phenylacetyl-L-glycine comprised 64.5% of the total dose in the rat, while it only accounted for 35.1% of the total dose in the mouse. On the other hand, N-benzyl-L-glycine comprised 9.9% of the total dose in the rat, but 19.1% of the total dose in the mouse. Thus, the mouse produces significantly more benzoic acid than phenylacetic acid when compared with the rat (6:1 vs. 2:1 [phenylacetic acid : benzoic acid]; rat vs. mouse). A possible explanation for this species difference is the formation of 4-hydroxy-4-phenyl-2-butanone and its further metabolism to benzyl alcohol. The mouse produces approximately 2-fold more benzyl alcohol than the rat, which is likely converted to benzoic acid and conjugated to glycine. Incubation of MSK with primary culture of hepatocytes obtained from livers of rats and mice results in conjugated metabolites (unpublished data). Both rat and mouse hepatocytes produce methyl styryl carbinol and 4-hydroxy-4-phenyl-2-butanone. Although not detected in the whole animal, it is likely that the rat forms 4-hydroxy-4-phenyl-2-butanone. This metabolite is conceivably formed via a retrograde aldol reaction to benzyl alcohol (or more likely benzaldehyde), which is subsequently oxidized and conjugated with glycine. From these data it appears that the reduction or oxidation of MSK results in the formation of the principal terminal metabolites (N-phenylacetyl-L-glycine or N-benzyl-L-glycine). Furthermore, the initial metabolic conversion (reduction, oxidation, or immediate conjugation) not only dictates the fate of a given MSK molecule, but any alteration of these processes could dramatically affect the distribution of terminal metabolites.

Thus, these differences in the metabolic conversion of MSK may be responsible for the dissimilar distribution of terminal metabolites observed in the rat and mouse.

In summary, results presented here indicate that after oral and topical administration, MSK is relatively well absorbed. Although the rate and completeness of absorption for these routes of administration were significantly different, in each case MSK was absorbed intact as evidenced by a small fraction of the parent compound present in the urine. The rapid and extensive metabolism of MSK resulted in very low levels of circulation parent compound. The ultimate urinary metabolites (glycine conjugates) of MSK were similar between routes of administration. Further studies are required to assess the ability of individual tissues (especially between species) to biotransform MSK and determine specific tissue differences in its toxicity.

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TABLE 4
Percentage of metabolites excreted into the urine following administration of $[^{14}{}C]$trans-methyl styryl ketone

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HPLC R_T</th>
<th>Peak</th>
<th>Percentage of Total Dose in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>Identification‡</td>
<td>Oral (200 mg/kg)</td>
</tr>
<tr>
<td>Undefined</td>
<td>11</td>
<td>A</td>
<td>4.5</td>
</tr>
<tr>
<td>Undefined</td>
<td>16</td>
<td>B</td>
<td>8.5</td>
</tr>
<tr>
<td>N-benzyll-L-glycine</td>
<td>24</td>
<td>D</td>
<td>19.1</td>
</tr>
<tr>
<td>Undefined</td>
<td>25</td>
<td>E</td>
<td>9.2</td>
</tr>
<tr>
<td>N-phenylacetyl-L-glycine</td>
<td>26</td>
<td>F</td>
<td>35.1</td>
</tr>
<tr>
<td>Undefined</td>
<td>32</td>
<td>G</td>
<td>4.1</td>
</tr>
<tr>
<td>N-acetyl-S-(4-phenyl-2-butanone)-L-cysteine</td>
<td>34</td>
<td>H</td>
<td>6.7</td>
</tr>
<tr>
<td>Undefined</td>
<td>41</td>
<td>J</td>
<td>2.3</td>
</tr>
<tr>
<td>Methyl styryl ketone</td>
<td>52</td>
<td>K_1</td>
<td>8.6</td>
</tr>
<tr>
<td>Methyl styryl carbinol</td>
<td>52</td>
<td>K_2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total % dose in urine</td>
<td></td>
<td></td>
<td>86.6%</td>
</tr>
</tbody>
</table>

Pooled urine samples (0 to 24 hr) from three rats per route of administration. † HPLC retention time.
‡ Fig. 7.

References

3. National Cancer Institute: “NCI Short-Term Test Results” National Cancer Institute, Division of Cancer Etiology Short-Term Test Program, Bethesda, MD (1994).