Methaqualone Metabolites in Human Urine after Therapeutic Doses

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We measured five principal metabolites of methaqualone in the urine of seven volunteers after single and multiple doses of the drug. Urine, collected for up to 72 hours after the last dose, was analyzed for methaqualone and its principal metabolites by high-resolution capillary-column gas chromatography. The major biotransformation of methaqualone under therapeutic conditions occurred through benzylic and para-hydroxylation of the o-tolyl moiety. Methaqualone itself was present in concentrations of no more than 1 mg/liter, if it could be detected at all. The observed physiological effects and total urinary excretion of metabolites reflected the cumulative nature of the parent drug when it was administered in multiple doses. No clear relationship was found between appearance of a specific metabolite and time after ingestion of the drug, although higher amounts of 2-methyl-3-(2'-hydroxymethylphenyl)-4(3H)-quinazolinone were noted in those individuals who tolerated the drug less well.

Additional Keyphrases: capillary-column gas chromatography · drug metabolism · excretion and effect relation · cumulative effect · glucuronide hydrolysis

The identification of methaqualone metabolites in urine after ingestion of the drug has been the subject of several papers (1–7), but in only a few have attempts been made to quantify them. Bonnichsen et al. reported the relative percentages of conjugated metabolites as determined in several cases of methaqualone overdose (2, 3). Reavey et al.6 cautioned against the use of methaqualone concentrations in urine as evidence of overdose or recent abuse because concentrations of the drug in blood, as well as excretion patterns in urine, are so erratic. With the many isomeric metabolite possibilities that exist for methaqualone, it seemed likely that the different excretion patterns might reflect a particular physiological state of the individual or even metabolic anomalies. Because the drug is cumulative physiologically, and tolerance appears to develop rapidly in some individuals, it was reasonable to expect that further studies, especially those that included quantitative data, would help elucidate the metabolism of the drug. The possibility that certain metabolites might be intermediates, or undergo rearrangements, was suggested by Stillwell et al. (8), and Bonnichsen and coworkers (1–3) likewise found artifacts and differences in urinary metabolites of methaqualone after acid hydrolysis as compared to hydrolysis by use of β-glucuronidase (EC 3.2.1.31). These analytical considerations provided an additional inducement to proceed with the metabolite quantification in the hope that we might be able to clarify some of the metabolic relationships and so contribute to more precise interpretations of methaqualone results for clinical purposes.

Methods

Seven apparently normal, healthy volunteers were given methaqualone (2-methyl-3-o-tolyl-4(3H)-quinazolinone) as follows: three individuals received a single 300-mg dose of Quaalude (Rorer, Inc.); two others received 300-mg doses on two consecutive days; and two persons received 150-mg doses on four consecutive days. Nobody received a total dose of more than 600 mg, and no other drugs were administered or taken during the entire period of urine collections. Specimen collections were casual rather than scheduled, and all urine excreted was collected for the period beginning 24 h before the first dose until 72 h after the final dose.

The urine samples were prepared and extracted as described by Permisohn et al. (7), except that instead of an acid hydrolysis, 5-ml aliquots of urine were hydrolyzed with “glusulase” (Endo Laboratories, Inc., Garden City, N.Y. 11530) for 18 h at 37 °C in a Dubnoff shaking incubator. The equivalent of 50 μl of glusulase

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[5870 Fishman units of aryl sulfatase (EC 3.1.6.1) and
9730 Fishman units of β-glucuronidase] in 3 ml of so-
dium acetate buffer, pH 4.6, was added to each 5 ml of
urine specimen. The hydrolyzed urines were adjusted
to a pH of 8.0 ± 0.5 with a few drops of saturated po-
tassium hydroxide, and each 5-ml aliquot was extracted
with 50 ml of chloroform.

Some specimens were also extracted at pH 3.0, in the
manner suggested by Bonnichsen et al. (1), to determine
whether pH made any difference. Insofar as the prin-
cipal metabolites were concerned, the observed differ-
ences were negligible except that alkaline extraction was
consistently “cleaner” than acidic extraction in terms
of extraneous substances.

Analytical recovery of the synthetic monohydroxyl-
ated metabolites I, III, IV, and V averaged 91.2% (SD,
2.1) for the concentration range 0.1–1.0 mg/liter of hy-
drolyzed urine, while the recovery for methaqualone
itself was 95.3% (SD, 6.0; n = 19) for the same concen-
tration range. A 40-ml aliquot of the solvent extract,
filtered through Whatman 41 filter paper to remove
traces of the aqueous supernate, was evaporated in a
stream of nitrogen gas at 50 °C. Each residue was dis-
solved in 25 μl of “silylation-grade” dimethylformamide,
and an equal volume of N,O-bis(trimethylsilyl)acetae-
mide was added to form the silyl derivatives of the
metabolites.

One microliter of the derivatized mixture was chro-
matographed on a 50-m support-coated open tubular
glass capillary column (0.76 mm i.d.) that contained
16801) for a liquid phase. Details for the preparation of
these columns and the operation of the column without
inlet stream splitting have been published (9, 10). The
column was maintained isothermally at 205 °C, because
temperature programming did not improve resolution
of the metabolites and only increased the analysis
time.

Mass-spectrometric analyses were done with a LKB
9000 gas chromatograph–mass spectrometer system
that had been modified for dual-column operation with
either a packed or capillary column (10). The following
mass-spectrometric operating conditions were em-
ployed: ion source, 250 °C; ionizing current, 3.6 A;
electron energy, 70 eV; accelerating voltage, 3500 V; and
Ryhage two-stage jet separator, 250 °C. Selected ion
monitoring for unchanged methaqualone was done with
a 1.00 mm × 4 mm (i.d.) glass column packed with 5%
SE-30 on 80/100 mesh Gas Chrom Q (Applied Science
Labs. Inc.) operated isothermally at 250 °C. Masses 250
(M⁺) and 235 (M-15) were monitored by periodically
alternating the accelerating voltage with an accelerating
voltage alternator accessory.

Samples of the specimens were interspersed (“bracketed”) with samples of standardized concen-
trations of a mixture of methaqualone and four of the
metabolites that had been prepared synthetically (5, 11)—see Figure 1, metabolites I, III, IV, and V. Peak
areas were integrated by means of a Hewlett-Packard
(Avondale, Pa. 19311) Model 3370B electronic inte-
grator, and a linear calibration curve was maintained
by computer, on-line to the system, as determined from
the first three standardized mixtures of synthetic met-
abolites in concentrations ranging from 0.1 to 1.0 μg/μl
(g/liter). Metabolite concentrations for the specimen
chromatograms were then calculated by computer from
peak areas in relation to the calibration curve. Subse-
quent samples of the standardized synthetic-metabolite
mixtures were interspersed at intervals of every third
or fourth specimen, to check the calibration curve for
quantitative precision.

Because we could not find an appropriate internal
standard that would not interfere with the separation,
an alternative method of computation was devised to
confirm the accuracy of the results as well as to deter-
mine the concentration of metabolite II, for which we
had no synthetic counterpart. In this alternative com-
putational approach we utilized methaqualone as an
internal standard, adding it to specimens that we had
previously found to contain very little or no unchanged
drug. With the high ratio of metabolite to methaqua-
alone, the latter was usually undetectable in the neces-
sarily small injection volume used for capillary columns;
because of this, most of the specimens could be checked
by this technique. Because the detector response factor
was virtually the same for methaqualone and for each
of the metabolites, the total metabolite concentration
was calculated from the peak areas in relation to that of
methaqualone, and the concentration of each metabo-
lite was then determined as its relative percentage of
the total peak area. The coefficient of variation for values obtained by both methods of com-
putation for the same metabolite and specimen were
within the 10% accepted as criterion for all replicate
determinations.

Results

The physiological response to the drug appeared to
be quite similar in all subjects who were given a 300-mg
dose. Drowsiness began about 45 min after ingestion
of the drug, and persisted for at least 3 h thereafter.
Slurred speech was apparent in some of the individuals,
especially after a second 300-mg dose administered on
the second day. Both of the volunteers (D and E) who
received this second dose said that the effect of the
second dose of the drug was more intense and came
upon them more rapidly than the first dose. The peak
effect, in all instances, was reached at about 2 h after
the dose, and those who received a second dose of the drug
complained of an unpleasant sensation and some que-
asiness at the time. A few of the participants napped
briefly (60–90 min) and awoke with no ill after-
effets.

In contrast to those who received the higher dose, the
volunteers (F and G) who were given 150 mg of metha-
qualone for four consecutive days did not manifest
drowsiness until after the dose on the third day. On the
fourth day, one of the participants (F) appeared to be
responding very similarly to subject D, who had received
two consecutive 300-mg doses. His speech became
slightly slurred after the dose, and he managed to stay awake only with great difficulty. Moreover, he complained of an unpleasant feeling during the height of the drug effect. The other volunteer (G), whose weight and physical stature was greater than that of the other participants, displayed only a slight drowsiness after the fourth 150-mg dose. However, the effect was noticeable, whereas he had appeared to be quite normal on the previous days. This would be consistent with the cumulative nature of methaqualone described by Alvan and coworkers (12, 13).

Our analysis revealed five major monohydroxylated metabolites (see Figures 1 and 2). The mass spectra of these trimethylsilylated metabolites were identical to those reported by Bonnichsen et al. (1). These metabolites were measured by gas chromatography for each volunteer for all urine collections throughout the period under study. Table 1 shows the amounts of each metabolite excreted by each individual during the three consecutive 8-h periods immediately after the last dose of methaqualone. The 8-h intervals were chosen for convenience of comparisons and to compensate for the irregular time intervals of the various urine collections. The cumulative excretion of each monohydroxy metabolite is depicted graphically in Figure 3 for all volunteers receiving a single 300-mg dose and in Figure 4 for those individuals on a multiple-dose regimen. In all cases, 2-methyl-3(2'-hydroxymethylphenyl)-4(3H)-quinazolinone (I) and 2-methyl-3(4'-hydroxy-2'-methylphenyl)-4(3H)-quinazolinone (IV) were the

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**Table 1. Metabolite Output for Excretion during the 24 h after the Last Dose**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time h</th>
<th>Urine vol</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A b</td>
<td>0-8</td>
<td>420</td>
<td>2.16</td>
<td>—</td>
<td>0.84</td>
<td>11.26</td>
<td>1.23</td>
<td>15.49</td>
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<tr>
<td>A</td>
<td>8-16</td>
<td>410</td>
<td>2.46</td>
<td>—</td>
<td>1.06</td>
<td>5.86</td>
<td>0.99</td>
<td>10.37</td>
</tr>
<tr>
<td>A</td>
<td>16-24</td>
<td>270</td>
<td>1.86</td>
<td>—</td>
<td>0.68</td>
<td>5.97</td>
<td>0.64</td>
<td>9.15</td>
</tr>
<tr>
<td>B b</td>
<td>0-8</td>
<td>495</td>
<td>5.01</td>
<td>1.87</td>
<td>6.71</td>
<td>15.37</td>
<td>3.16</td>
<td>32.12</td>
</tr>
<tr>
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<td>8-16</td>
<td>180</td>
<td>2.10</td>
<td>0.09</td>
<td>1.51</td>
<td>7.22</td>
<td>0.32</td>
<td>11.24</td>
</tr>
<tr>
<td>B</td>
<td>16-24</td>
<td>545</td>
<td>6.57</td>
<td>0.54</td>
<td>6.02</td>
<td>8.59</td>
<td>2.09</td>
<td>23.81</td>
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<tr>
<td>C b</td>
<td>0-16</td>
<td>280</td>
<td>3.98</td>
<td>1.99</td>
<td>0.83</td>
<td>10.58</td>
<td>2.03</td>
<td>19.41</td>
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<tr>
<td>C</td>
<td>16-24</td>
<td>255</td>
<td>2.32</td>
<td>1.41</td>
<td>0.85</td>
<td>9.83</td>
<td>1.46</td>
<td>15.87</td>
</tr>
<tr>
<td>D c</td>
<td>24-32</td>
<td>1415</td>
<td>16.55</td>
<td>8.52</td>
<td>9.52</td>
<td>8.68</td>
<td>0.88</td>
<td>44.15</td>
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<tr>
<td>D</td>
<td>32-40</td>
<td>400</td>
<td>11.28</td>
<td>3.50</td>
<td>4.07</td>
<td>1.74</td>
<td>0.62</td>
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<tr>
<td>D</td>
<td>40-48</td>
<td>320</td>
<td>6.54</td>
<td>1.35</td>
<td>2.62</td>
<td>7.81</td>
<td>0.52</td>
<td>18.84</td>
</tr>
<tr>
<td>E c</td>
<td>24-32</td>
<td>1125</td>
<td>8.23</td>
<td>2.11</td>
<td>2.33</td>
<td>15.48</td>
<td>0.41</td>
<td>28.56</td>
</tr>
<tr>
<td>E</td>
<td>32-40</td>
<td>175</td>
<td>7.02</td>
<td>1.41</td>
<td>3.05</td>
<td>7.27</td>
<td>0.40</td>
<td>19.15</td>
</tr>
<tr>
<td>E</td>
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<td>330</td>
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<td>1.57</td>
<td>5.95</td>
<td>10.91</td>
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<td>32.79</td>
</tr>
</tbody>
</table>

* Sum of all casual collections for that time period, in milliliters.
* Single 300-mg tablet; zero hours is the time when the first dose was taken.
* Two 300-mg tablets, taken 24 h apart.
* No urine voided first 8 h.
* Not detected.

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Fig. 1. Principal monohydroxylated methaqualone metabolites

Fig. 2. Capillary-column gas-chromatographic separation of trimethylsilyl ethers of the five principal methaqualone metabolites

Subject D, 9 h after initial 300-mg dose
Fig. 3. Cumulative excretion of each of the five major methaqualone metabolites vs. the logarithm of time for three volunteers receiving a single 300-mg dose

Time of ingestion is time zero. Data points correspond to the times of casual urine collections

principal metabolites. A sixth, but unidentified, monohydroxylated metabolite that corresponded to metabolite X₁ of Bonnichsen et al. (3) appeared in some of the specimen chromatograms (see Figure 2). Since the concentration of this metabolite was low (less than 1 mg total) and the identity was uncertain, no data have been included at this time.

Discussion

Most of the methaqualone metabolites have been reported to be excreted in the form of glucuronide conjugates (2, 8). Moreover, hydrolysis with a mineral acid destroys much of the sample (2, 7), so we used enzymatic hydrolysis with glusulase to cleave these conjugates more gently. Our experience with acid hydrolysis suggested that metabolite II was particularly susceptible to acid degradation; indeed, enzymatic hydrolysis gave consistently higher yields for this metabolite than acid hydrolysis. Enzymatic hydrolysis may not be without artifacts, however, since Stillwell et al. (8) suggested that metabolite X₁ is formed by a dehydration rearrangement of a dihydrodiol during incubation with glusulase.

The isomeric metabolites III, IV, and V were not completely resolved on packed columns containing either non-polar (SE-30) or moderately polar (OV-17, OV-210) liquid phases (7). The separation of these metabolites as their trimethylsilyl derivatives with sufficient resolution for quantification was possible only with a high-resolution capillary column of the type described. The high capacity and stability of this column also allowed use of a splitless injection technique for increased sensitivity and quantitative precision (10).

A packed column that could accommodate a larger sample injection volume was initially used for methaqualone determinations, because the urinary concentrations of the parent drug were consistently too low to be detected with the 1.0 μl normally injected on the capillary column. However, the packed column did not separate methaqualone completely from extraneous substances and the resulting matrix effects impaired quantitative accuracy. Selected ion monitoring with a gas chromatography–mass spectrometry system eventually solved the problem, but not before many of the samples had been depleted. Consequently, these data are not included in the tables or figures because they are incomplete. As well as we could estimate, the highest concentration of methaqualone for a therapeutic dose of 300 mg did not exceed 1.0 mg/liter of urine. Over half of the urines (58.5%) that showed any evidence of free, unchanged methaqualone being present contained less than 50 μg of it in the total collection; and at least half of these specimens were estimated to contain no more than 10–20 μg in the total volume of a casual (i.e., un timed) urine collection.

Table 1 allows a detailed comparison of metabolite excretion immediately after a single dose or upon completion of a multiple-dose regimen. Individual response and metabolism evidently vary considerably within each therapeutic dosage category, although this variation may be partly due to such straightforward factors as differences in body fat and weight. The cumulative effect of the drug is indicated by the increased amounts of metabolites in subjects D and E after two 300-mg doses 24 h apart as compared to subjects A, B, and C, who ingested only a single 300-mg dose.

Figures 3 and 4 graphically compare the cumulative excretion of each monohydroxylated metabolite in subjects on single and multiple doses, respectively. Of the five major metabolites measured, I and IV exceeded the amounts of the others. Therefore, hydroxylation of methaqualone tends to occur predominantly on the o-tolyl ring at the 4-position, which is the most susceptible locus to electrophilic substitution, or at the reactive benzylic carbon. The relationship of these two metabolites is not clear, but it is noteworthy that where metabolite I is predominant, the effects were more pronounced and those individuals (D and F, Figure 4) were less tolerant of the drug than were those who manifested the greater concentrations overall of me-
tabolite IV (e.g., subject B, Figure 3). Subjects E and G only experienced a pronounced effect after their final dose, and it is interesting to note that the curves for metabolites I and IV for these individuals tend to converge. Bonnichsen et al. (2, 3) reported that metabolite I was present, unconjugated, together with methaqualone in blood from cases of suspected drug overdose, and Stillwell et al. (8) found the same in urine for a similar situation. Since these data were obtained from cases of intoxication by methaqualone, several questions arise as to the significance of metabolite I and its relation to acute intoxication. Unfortunately, our data from therapeutic doses cannot resolve these questions, but the pattern is consistent for the few examples that have been studied. At the very least, this compound, which is synthetically available (II), should be examined for sedative and hypnotic activity as well as toxicity. It may well be that this metabolite is active whether in the same or in a different manner from the parent drug.

The data in Table 1 for subjects B, D, and E suggest that metabolite II might relate to the time of last ingestion of the drug. The concentration of this metabolite is initially equivalent to that of metabolite I or IV and then declines so that it is barely detectable 10–24 h after ingestion. This ingestion-time relationship of metabolite II is also suggested in Figures 3 and 4, where it shows a sharp increase on the graph while the drug is being taken, which rapidly declines once administration ceases. However, the low concentrations of this metabolite and the variation in this pattern from one individual to another indicates that this trend might be too subtle in some instances for one to be able to draw
such inferences. In addition, care must be exercised to distinguish metabolite II from extraneous substances, or the conclusion could be very misleading.

Attempts to draw quantitative comparisons with the findings of Bonnichsen et al. (2, 3) were difficult, because they do not specify the total amounts of metabolite. Quantities were reported either in units of concentration or as relative percentages of total metabolite, as determined by ultraviolet spectrophotometry, so some of the data included unchanged methaqualone as well as metabolite. Some of the monohydroxy metabolite concentrations measured in our study were higher than those reported by Bonnichsen et al. for suicide cases that involved methaqualone overdose. Values for metabolite I alone were as high as 70–90 mg/liter for a collected volume of 125–200 ml of urine within 8–12 h after a single 300-mg dose. Our experience was that metabolite concentrations tended to fluctuate greatly from one specimen to another, and that some of the fluctuation was due to differences in the volume of urine excreted. However, the total quantity of metabolite in each collection tended to be more uniform, and gave results that were consistent.

The irregular amounts of metabolite excreted confirm the observations of Reavey et al.6 and leave the interpretation of a single determination open to question. Our study has focused on the five principal metabolites that could be identified and quantified. Other investigators (4, 5, 8) have reported at least five additional metabolites that could be important in establishing links between a state of overdose and ordinary manifestations of therapeutic concentrations of the metabolites. Information derived from one dose regimen can only suggest areas for further investigation, and establish some basis for comparisons. More quantitative data from cases of intoxication are needed before the significance of metabolite determinations can be completely evaluated.

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References