Fertility and General Reproduction Studies in Rats with the HMG-CoA Reductase Inhibitor, Atorvastatin

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Fertility and reproduction studies were conducted in rats with the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, atorvastatin. Male rats received vehicle (0.5% methylcellulose) or atorvastatin at 20, 100, or 175 mg/kg by oral gavage for 11 weeks prior to mating with untreated females; treatment continued throughout mating and until necropsy on Day 115. An untreated control group of males was also included in the same procedures. Dose-related body weight gain suppressions of 17 and 25%, and food consumption suppressions of 7 and 16%, occurred during the 11-week premating treatment period at 100 and 175 mg/kg, respectively, compared with vehicle controls. There were no treatment-related effects on testes, epididymides, or accessory organs weights, testicular or epididymal sperm counts, sperm motility, or sperm morphology during Week 15 of treatment. Plasma drug concentrations during Week 15 increased with dose to a Cmax of 1820 ± 1020 ng eq/ml at 175 mg/kg. There were no effects on copulation or fertility indices, number of days to mating, or female reproductive parameters (number of implants, live fetuses, or pre- and postimplantation loss). In the female fertility study, female rats received vehicle (0.5% methylcellulose) or atorvastatin at 20, 100, or 225 mg/kg by oral gavage for 2 weeks prior to mating with untreated males; treatment continued throughout mating and until Gestation Day 7. Sperm-positive females were sacrificed on presumed Gestation Day 13 to 15 for evaluation of reproductive parameters. Body weight gain in atorvastatin groups was comparable to controls during the premating period, but was suppressed by 35% at 225 mg/kg during the treatment period of gestation (Days 0–8), and was significantly increased at 225 mg/kg during the posttreatment period of gestation (Days 8–13). Plasma drug concentrations on premating treatment Day 14 increased with dose to a Cmax of 7030 ± 3680 ng eq/ml at 225 mg/kg. The mean number of estrous cycles, copulation and fertility indices, number of days to mating, and number of viable litters were comparable between groups. In addition, term sacrifice parameters (number of corpora lutea, implants, live fetuses, pre- and postimplantation loss) were not significantly different between groups. Thus, these studies demonstrate no adverse effects of atorvastatin on fertility and reproduction in rats at doses up to 175 and 225 mg/kg in males and females, respectively, and 20 mg/kg was a no-effect dose.

One of the primary aims in the development of therapeutic agents to reduce the risk of atherosclerosis and cardiovascular disease is the regulation and lowering of serum lipids. The inhibition of microsome 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the biosynthesis of cholesterol, has been shown to be an effective mechanism for lowering serum cholesterol in experimental animals and man. Several inhibitors of HMG-CoA reductase have been developed and used clinically for the treatment of hyperlipidemia. Atorvastatin has been shown to be a highly efficacious inhibitor of HMG-CoA reductase that produces greater reductions in LDL-cholesterol in hyperlipidemic patients than other lipid-regulating drugs (Nawrocki et al., 1995). Due to their cholesterol-lowering properties, this class of inhibitors might be expected to have adverse effects on reproduction by reducing the supply of circulating cholesterol which is required for steriodogenesis (Dobs et al., 1993). Most HMG-CoA reductase inhibitors are effective cholesterol-lowering agents in rabbits, guinea pigs, dogs, and humans (Tsujita et al., 1986; Gerson et al., 1989; MacDonald et al., 1988; Alberts et al., 1989; Purvis et al., 1992; Frishman et al., 1989). However, these reductase inhibitors, including atorvastatin, do not significantly lower serum cholesterol levels in chow-fed rats (Endo et al., 1979; MacDonald et al., 1988; Tsujita et al., 1986; Krause and Newton, 1995), and several have been shown to have no adverse effects on reproduction in rats (FDA, 1987, 1993; Tanase and Hirose, 1987; Wise et al., 1990). Nevertheless, HMG-CoA reductase inhibitors are considered teratogenic due to studies conducted with lovastatin (Minsk et al., 1983), and sporadic testicular effects have been observed in dogs (MacDonald et al., 1988; Gerson et al., 1989). Therefore, in the course of safety evaluation of atorvastatin for registration as a new drug, fertility studies were conducted according to ICH guidelines (ICH, 1994), and the present results demonstrate the lack of effects on fertility and reproduction in male and female rats.
MATERIALS AND METHODS

Atorvastatin (CI-981, PD 134298-38A) is described chemically as \([R- (R^*, R^*)]-2-(4-nuorophenyl)-8,6-dihydroxy-5-(1-methylethyl)-3-phenyl-4-\[(phenylamino)carbonyl]-1H-pyrole-1-heptanoic acid calcium salt (2:1).\) Dosing suspensions of atorvastatin were prepared weekly in 0.5% aqueous methylcellulose, and concentrations were determined to be within 10% of intended values with only minor exceptions. Homogeneity and stability of suspensions were within acceptable limits (within ±10%) over the concentration ranges used.

**Animals.** Male and female Sprague-Dawley rats [Crl:CDBR VAF/Plus] were obtained from Charles River Breeding Laboratories (Portage, MI). Males were 11 weeks of age at the initiation of treatment (male study) or at the time of mating (female study). Females were 12 to 13 weeks of age at the initiation of treatment (female study) or the time of mating (male study). Purina Certified Rodent Chow 5002 and water were provided \(ad libitum.\) Animals were housed individually except during mating in stainless steel cages with wire bottoms under controlled environmental conditions of light, temperature, and humidity (NIH, 1985). Animals were acclimated for at least 1 week and were individually identified. Rats were randomly assigned to dose groups by body weight. Insemination was determined by the occurrence of sperm in a vaginal smear and was considered Day 0 of gestation.

**Male fertility study.** Atorvastatin was administered by gavage to groups of 30 male rats at 0 (0.5% methylcellulose), 20, 100, and 175 mg/kg. An additional group of 25 untreated males was included in all procedures for comparison with vehicle controls. The high dose, intended to induce at least some evidence of general toxicity, was selected based on a previous study in which 225 mg/kg caused adverse clinical signs and 5 females were euthanized in moribund condition whereas a dose of 175 mg/kg resulted in only one moribund sacrifice and fewer clinical signs. Current ICH guidelines recommend a study of fertility and early embryonic development to implantation in females which should detect effects on estrous cycle, embryo transport, implantation, and development of preimplantation stages of the embryo (ICH, 1994). Therefore, females were treated for 2 weeks prior to mating, during mating, and until implantation (Gestation Day 7). Mating was with untreated males in a 1:1 ratio for a maximum of 19 days. Vaginal smears were evaluated daily for stage of estrus for 15 days prior to treatment, during treatment, and during the mating period. At the time of insemination, females were separated from the male, body weights were determined every other day during gestation, and females were sacrificed between Gestation Days 13 and 15. At maternal sacrifice, the number of corpora lutea and the location and status of each implant site were recorded (live/dead embryo, early/late resorption), and a gross necropsy was conducted.

**Male reproductive parameters.** During Treatment Week 15, 10 males per group were sacrificed by carbon dioxide asphyxiation. Immediately after euthanasia, a section (approximately 1 cm) of the left vas deferens was removed for evaluation of sperm motility. The tissue was incubated for 3 min in 10 ml of medium containing 10 mg/ml bovine serum albumin (Fraction V, Sigma) in Dulbecco’s phosphate-buffered saline (D-PBS) (Gibco) in a 10-cm plastic petri dish. The temperature of the medium, microscope, and sample chambers was maintained at 37 ± 2°C in a custom-made Plexiglas box using an ASI 400 airstream incubator (Nicholson Precision Instruments). During the 3-min incubation the vas deferens tissue was removed from the medium when it was visually determined that a sufficient amount of sperm had diffused from the end of the tubule into the medium, and then the sample was gently mixed. After the 3-min incubation, a sample of sperm suspension was removed by capillary action using a 7-in. tube and evaporated to dryness at 37°C under nitrogen. The residue was reconstituted in 50 μl of distilled water and then 50 μl of a buffer prepared by dissolving 52 mg NADPH and 26 mg d,l-dithiothreitol in 0.7 ml of 2 M phosphate buffer (pH 7.4), 1.4 ml of 0.1 M EDTA, and 5 ml of water and 100 μl of rat liver microsomes (0.75 to 1.0 mg protein/ml) were added.
as the source of HMG-CoA reductase. The reaction mixture was incubated
for 30 min in a 37°C shaking water bath. The tubes were removed from
the water bath and 50 μl of a 1 M phosphate buffer (pH 7.4) containing
0.2 mg/ml of [14C]HMG-CoA (approximately 100,000 dpm/ml) was added.
The mixture was vortexed and incubated for 35 min at 37°C in a shaking
water bath. Then 25 μl of concentrated HCl was added, the tubes were
vortexed, and tubes were incubated 25 min at 37°C to lactonize [14C]-
mevalonic acid to [14C]mevalonolactone. The tubes were removed from
the shaking water bath, cooled to room temperature, and vortexed, and a 200-
μl aliquot of the mixture was transferred to a wet AG 1X8 anion-exchange
column. Each column was eluted with five 1-ml volumes of water and all
five eluates were collected in a scintillation vial. Ten milliliters of scintilla-
tion cocktail was added to each vial, the vial was vortexed, and [14C]
radioactivity was measured in a Packard TriCarb 250TR counter, using a
Transformed Spectral Index (tSIE) standard method of quench correction.
The relationship between percentage inhibition of HMG-CoA reductase and
log atorvastatin concentration in calibration standards was used to construct
a calibration curve. The assay measures atorvastatin and atorvastatin metab-
ilites in plasma capable of inhibiting HMG-CoA reductase. Therefore,
atorvastatin concentration is expressed in terms of atorvastatin equivalents.
Mean total recovery of [14C]atorvastatin from rat plasma by protein precipi-
tation was 95.1%. The lower limit of quantitation was 0.36 ng eq/ml.
For purposes of statistical analysis, one concentration which was below this
limit (a 20 mg/kg female at Time 0) was assigned a value of zero. The
relative standard deviation (%RSD) of calibration standards (range 0.36 to
16 ng/ml) ranged from 1.19 to 3.47% and relative error (%RE) of back-
calculated values was within ±1.67%. Assay precision and accuracy, based
on quality control sample %RSD and %RE values, ranged from 2.68 to
8.62% and were within ±5%, respectively.

Pharmacokinetic and statistical methods. Statistical comparisons be-
tween the treated groups and the vehicle control used p < 0.05 as the level
of significance. Pairwise comparisons of the untreated control group with
the vehicle control group in the male study were conducted using the
Wilcoxon rank sum test for continuous data and Fisher’s exact test for
dichotomous data. To control for the multiplicity of statistical comparisons
(i.e., to reduce the likelihood of false-positive conclusions), the parameters
were divided into distinct classes of related parameters (e.g., body weights
in one class, food consumption in another). The classwise significance level
was then allocated to each parameter proportionally by the inverse of the
square root of the number of parameters in a class (Tukey et al., 1985).

Continuous data were analyzed by Tukey’s sequential trend test using
the rank-dose scale and rank-transformed data (Tukey et al., 1985; Park,
1985) at the 5% classwise significance level. A trend reversal test was
performed at the 1% classwise significance level. Dichotomous data were
analyzed by sequential application of a weighted Cochran–Armitage test
for linear trend in proportions at the 5% classwise significance level. To
ensure that the linear dose–response relationship was realistic, a nonlinear-
ity test was performed at the 1% classwise significance level.

Atrorvastatin-equivalent pharmacokinetic parameters were obtained by
compartmental analysis of plasma concentration–time data (Gibaldi and
Perrier, 1982). Maximum plasma atorvastatin-equivalent concentrations
(Cmax) and their corresponding times (tmax) were recorded as observed.
Area under the plasma concentration–time curve from Time 0 to 24 hr
[AUC(0-24)] was estimated using the linear trapezoidal rule. The apparent
elimination rate constant (λz) was estimated as the absolute value of the
slope of a least-squares linear regression of natural logarithm of plasma
atorvastatin-equivalent concentration versus time during the terminal phase
of the plasma concentration–time profile. Typically the 12- and 24-hr
plasma concentration–time data were used to estimate λz. Apparent elimina-
tion half-life values (t1/2) were calculated as 0.693/λz.

Comparison of atorvastatin-equivalent Cmax, tmax, AUC(0-24), and
apparent half-life values between male and female rats were made using a
two-way analysis of variance. Cmax and AUC(0-24) values for all dose
groups were normalized for dose before analysis of variance was performed.

Bonferroni’s method was used for all pairwise multiple comparisons. Statistical
comparisons were made using the p < 0.05 level of significance. Dose
proportionality was assessed by visual inspection of pharmacokinetic par-

ter values.

These studies were conducted in accordance with U.S. FDA Good Labora-
tory Practice regulations and current guidelines for animal welfare (NIH, 1985).

RESULTS

Male Fertility Study

No treatment-related deaths, clinical signs, or gross pathological
changes were observed. There were no differences between untreated and vehicle control groups, with the ex-
ception of accessory sex organ weights which were significant-
ly greater in untreated controls. Statistically significant
dose-related body weight gain suppressions of 17 and 25%
occurred during the 11-week premating treatment period at
100 and 175 mg/kg, respectively, compared with vehicle
controls (Table 1). Weight gain was most affected during
the first 4 weeks of treatment at those doses. Food consump-
tion was statistically significantly decreased by 7 to 16% at
100 and 175 mg/kg during the first 2 to 3 weeks of treatment
and was decreased by 5% at 175 mg/kg during the 11-week
premating period compared with vehicle controls.

There were no treatment-related differences in absolute
weights of the testes, epididymides, or accessory sex organs
(Table 1). Relative testes weights were statistically signifi-
cantly increased at 175 mg/kg compared with vehicle con-
trons, which was primarily due to the decrease in body
weight. There were no effects on epididymal sperm or testic-
ular spermatid counts and no effects on sperm motility or
morphology. No histopathological abnormalities were ob-
erved in testes or epididymides.

There were no significant differences between groups in
body weight gain during gestation for pregnant females.
There were also no biologically significant differences be-
tween groups in copulation or fertility indices or in number
of days to mating or number of viable litters. The group
mean numbers of corpora lutea, implants, live fetuses, and
pre- and postimplantation losses were comparable in all
groups (Table 2).

Female Fertility Study

One pregnant female at 225 mg/kg had clinical signs of
dehydration, emaciation, salivation, red material around
mouth, urinary stain, and diarrhea on Gestation Days 7 and
8 and was found dead on Gestation Day 9 with additional
necropsy findings of decreased body fat, watery cecal con-
tents, gas-filled small intestine, and small thymus. The rela-
tionship of the death to treatment was considered equivocal
since no other animals died at this dose and due to the
non-specific nature of the clinical signs and pathological
findings. An increased incidence of alopecia and sporadic
salivation occurred at 225 mg/kg. No other treatment-related
TABLE 1
Body Weight, Food Consumption, and Reproductive Parameters in Male Rats Given Atorvastatin (Male Fertility Study)

<table>
<thead>
<tr>
<th>Male dose (mg/kg)</th>
<th>Untreated</th>
<th>0</th>
<th>20</th>
<th>100</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight gain (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0–77</td>
<td>185.3 ± 8.73</td>
<td>180.7 ± 6.76</td>
<td>194.4 ± 7.92</td>
<td>150.2 ± 5.91*</td>
<td>134.9 ± 7.30*</td>
</tr>
<tr>
<td><strong>Food consumption (g)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0–77</td>
<td>1958 ± 34.5</td>
<td>1955 ± 32.2</td>
<td>1997 ± 28.9</td>
<td>1885 ± 26.9</td>
<td>1861 ± 30.0</td>
</tr>
<tr>
<td><strong>Week 15 body weight (g)</strong></td>
<td>568 ± 16.2</td>
<td>565 ± 17.5</td>
<td>581 ± 11.3</td>
<td>557 ± 13.6</td>
<td>533 ± 13.2*</td>
</tr>
<tr>
<td><strong>Testes weight (g)</strong></td>
<td>3.54 ± 0.072</td>
<td>3.59 ± 0.076</td>
<td>3.72 ± 0.071</td>
<td>3.59 ± 0.060</td>
<td>3.81 ± 0.059</td>
</tr>
<tr>
<td><strong>Epididymides weight (g)</strong></td>
<td>1.52 ± 0.031</td>
<td>1.50 ± 0.026</td>
<td>1.56 ± 0.030</td>
<td>1.50 ± 0.028</td>
<td>1.55 ± 0.031</td>
</tr>
<tr>
<td><strong>Accessory organs weight (g)</strong></td>
<td>3.62 ± 0.114**</td>
<td>3.18 ± 0.102</td>
<td>3.15 ± 0.124</td>
<td>3.09 ± 0.151</td>
<td>3.20 ± 0.073</td>
</tr>
<tr>
<td><strong>Sperm (10^6)</strong></td>
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<td></td>
</tr>
<tr>
<td>Per gram cauda epididymis</td>
<td>1506 ± 38.3</td>
<td>1738 ± 111.5</td>
<td>1529 ± 67.2</td>
<td>1541 ± 77.9</td>
<td>1418 ± 79.0</td>
</tr>
<tr>
<td>Per cauda epididymis</td>
<td>496 ± 17.4</td>
<td>502 ± 38.7</td>
<td>480 ± 22.7</td>
<td>457 ± 22.5</td>
<td>464 ± 26.1</td>
</tr>
<tr>
<td><strong>Spermatids (10^6)</strong></td>
<td></td>
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<tr>
<td>Per gram testis</td>
<td>156 ± 5.1</td>
<td>160 ± 9.9</td>
<td>157 ± 4.6</td>
<td>160 ± 5.5</td>
<td>164 ± 4.6</td>
</tr>
<tr>
<td>Per testis</td>
<td>251 ± 10.0</td>
<td>264 ± 16.4</td>
<td>263 ± 11.0</td>
<td>255 ± 7.6</td>
<td>285 ± 9.7</td>
</tr>
<tr>
<td><strong>Sperm motility (%)</strong></td>
<td>93.8 ± 0.94</td>
<td>94.1 ± 1.01</td>
<td>97.5 ± 0.55</td>
<td>95.3 ± 1.07</td>
<td>94.0 ± 1.65</td>
</tr>
<tr>
<td><strong>Sperm morphology (%)</strong></td>
<td>96.3 ± 1.75</td>
<td>98.9 ± 0.49</td>
<td>97.2 ± 0.85</td>
<td>97.0 ± 1.11</td>
<td>98.3 ± 0.63</td>
</tr>
</tbody>
</table>

Note. Male rats (mean body weight 378–381 g) were given daily oral doses of atorvastatin or 0.5% methylcellulose vehicle for 11 weeks prior to mating, during mating, and until necropsy. Ten males per group were sacrificed during Week 15 and male reproductive parameters were determined. Values represent the mean ± SE for N = 14–30 (body weight and food consumption) and N = 10 for Week 15 reproductive organ weights and sperm parameters.

* Significantly different from vehicle control at p < 0.0500
** Significantly different from vehicle control at p < 0.0204.

clinical signs or gross necropsy findings were observed in any treatment group. No biologically significant differences in body weight gain occurred during the 2-week premating treatment period (Table 3). However, group mean body weight gain was statistically significantly reduced by 35% at 225 mg/kg during the treatment period of gestation (Days 0–8) compared with vehicle controls. During the posttreatment period of gestation (Days 8–13), body weight gain at 225 mg/kg was increased by 25% and food consumption was increased by 19% compared with vehicle controls.

TABLE 2
Female Body Weights and Reproductive Parameters (Male Fertility Study)

<table>
<thead>
<tr>
<th>Male dose (mg/kg)</th>
<th>Untreated</th>
<th>0</th>
<th>20</th>
<th>100</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight gain (g)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gestation Days 0–7</td>
<td>40.1 ± 2.19</td>
<td>42.6 ± 3.05</td>
<td>39.8 ± 1.43</td>
<td>38.0 ± 1.50</td>
<td>36.9 ± 1.68</td>
</tr>
<tr>
<td>Gestation Days 7–13</td>
<td>35.0 ± 2.48</td>
<td>30.2 ± 5.42</td>
<td>36.3 ± 1.63</td>
<td>35.7 ± 1.06</td>
<td>39.6 ± 2.08</td>
</tr>
<tr>
<td><strong>Reproduction data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copulation index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>100</td>
<td>96.7</td>
<td>93.3</td>
<td>93.3</td>
<td>96.7</td>
</tr>
<tr>
<td>No. days to mating</td>
<td>3.4 ± 0.48</td>
<td>3.6 ± 0.72</td>
<td>3.5 ± 0.50</td>
<td>3.0 ± 0.39</td>
<td>3.2 ± 0.43</td>
</tr>
<tr>
<td>No. with viable litters</td>
<td>24</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td><strong>Term sacrifice parameters</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No. implant sites</td>
<td>16.9 ± 0.30</td>
<td>16.3 ± 0.62</td>
<td>16.4 ± 0.59</td>
<td>16.1 ± 0.44</td>
<td>16.3 ± 0.54</td>
</tr>
<tr>
<td>No. live fetuses</td>
<td>15.9 ± 0.37</td>
<td>15.2 ± 0.66</td>
<td>15.2 ± 0.55</td>
<td>15.0 ± 0.48</td>
<td>14.9 ± 0.57</td>
</tr>
<tr>
<td>No. resorptions</td>
<td>1.0 ± 0.18</td>
<td>1.0 ± 0.23</td>
<td>1.2 ± 0.18</td>
<td>1.1 ± 0.23</td>
<td>1.4 ± 0.23</td>
</tr>
<tr>
<td>Preimplantation loss (%)</td>
<td>6.7 ± 1.67</td>
<td>7.8 ± 2.01</td>
<td>8.2 ± 2.71</td>
<td>5.9 ± 2.10</td>
<td>6.2 ± 2.46</td>
</tr>
<tr>
<td>Postimplantation loss (%)</td>
<td>5.8 ± 1.07</td>
<td>6.8 ± 1.65</td>
<td>7.1 ± 1.05</td>
<td>7.2 ± 1.64</td>
<td>9.0 ± 1.65</td>
</tr>
</tbody>
</table>

Note. Untreated female rats (mean body weight 275–277 g) were mated with male rats treated with atorvastatin for 11 weeks prior to mating and during mating. Females were sacrificed on Gestation Days 13–15 and reproductive parameters were determined. Copulation index = [No. sperm positive/No. cohabitated] × 100%; Fertility index = [No. pregnant/No. sperm positive] × 100%. Values are the mean ± SE for N = 18–29, where appropriate.
TABLE 3

Body Weights and Reproductive Parameters of Female Rats Treated with Atorvastatin (Female Fertility Study)

<table>
<thead>
<tr>
<th>Female dose (mg/kg)</th>
<th>0</th>
<th>20</th>
<th>100</th>
<th>225</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premating Days 0-15</td>
<td>21.5 ± 1.81</td>
<td>21.6 ± 1.64</td>
<td>28.9 ± 2.12</td>
<td>23.6 ± 2.14</td>
</tr>
<tr>
<td>Gestation Days 0-8</td>
<td>48.3 ± 2.17</td>
<td>45.6 ± 2.28</td>
<td>47.2 ± 1.66</td>
<td>31.4 ± 4.52*</td>
</tr>
<tr>
<td>Gestation Days 8-13</td>
<td>29.4 ± 1.37</td>
<td>30.3 ± 1.90</td>
<td>30.8 ± 1.75</td>
<td>36.8 ± 2.11*</td>
</tr>
<tr>
<td>Food consumption (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premating Days 0-6</td>
<td>115 ± 1.7</td>
<td>114 ± 1.8</td>
<td>114 ± 2.2</td>
<td>104 ± 2.3*</td>
</tr>
<tr>
<td>Premating Days 6-15</td>
<td>182 ± 2.8</td>
<td>181 ± 3.8</td>
<td>198 ± 3.4*</td>
<td>192 ± 2.8*</td>
</tr>
<tr>
<td>Gestation Days 0-8</td>
<td>212 ± 4.0</td>
<td>216 ± 5.1</td>
<td>218 ± 4.0</td>
<td>198 ± 9.4</td>
</tr>
<tr>
<td>Gestation Days 8-13</td>
<td>133 ± 6.3</td>
<td>141 ± 5.1</td>
<td>150 ± 3.3*</td>
<td>158 ± 3.3*</td>
</tr>
<tr>
<td>Reproduction data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copulation index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>96.0</td>
<td>100</td>
<td>92.0</td>
<td>96.0</td>
</tr>
<tr>
<td>No. days to mating</td>
<td>2.9 ± 0.48</td>
<td>3.4 ± 0.68</td>
<td>2.4 ± 0.20</td>
<td>3.1 ± 0.65</td>
</tr>
<tr>
<td>No. with viable litters</td>
<td>24</td>
<td>25</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Term sacrifice parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. corpora lutea</td>
<td>18.0 ± 0.55</td>
<td>19.2 ± 0.71</td>
<td>19.2 ± 0.50</td>
<td>19.5 ± 0.70</td>
</tr>
<tr>
<td>No. implant sites</td>
<td>15.9 ± 0.97</td>
<td>17.3 ± 0.65</td>
<td>17.3 ± 0.62</td>
<td>16.8 ± 1.00</td>
</tr>
<tr>
<td>No. live fetuses</td>
<td>15.0 ± 1.00</td>
<td>15.8 ± 0.55</td>
<td>16.0 ± 0.52</td>
<td>14.7 ± 1.15</td>
</tr>
<tr>
<td>No. resorptions</td>
<td>1.0 ± 0.19</td>
<td>1.5 ± 0.32</td>
<td>1.3 ± 0.27</td>
<td>2.0 ± 0.64</td>
</tr>
<tr>
<td>Preimplantation loss (%)</td>
<td>12.6 ± 4.91</td>
<td>9.0 ± 2.99</td>
<td>9.1 ± 2.85</td>
<td>14.9 ± 4.38</td>
</tr>
<tr>
<td>Postimplantation loss (%)</td>
<td>7.0 ± 1.83</td>
<td>7.9 ± 1.51</td>
<td>7.2 ± 1.36</td>
<td>12.9 ± 4.14</td>
</tr>
</tbody>
</table>

Note. Groups of 30 female rats (mean body weight 275–279 g) were treated with atorvastatin for 2 weeks prior to mating; 25 continued treatment during mating with untreated males and until Gestation Day 7. Females were sacrificed on Gestation Days 13–15 and reproductive parameters were determined. Copulation index = [No. sperm positive/No. cohabitated] × 100%; Fertility index = [No. pregnant/No. sperm positive] × 100%. Values are the mean ± SE for N = 23–30, where appropriate.

* Significantly different from vehicle control at p < 0.0224.

There were no differences between groups in the mean number of estrous cycles completed or the number of animals with abnormal estrous cycles (not shown). Copulation and fertility indices, number of days to mating, and number of viable litters were comparable between groups (Table 3). In addition, term sacrifice parameters (corpora lutea, implants, live fetuses, and pre- and postimplantation loss) were not significantly different between groups.

Pharmacokinetics

For male rats, Cmax and AUC(0-24) values for atorvastatin equivalents during Treatment Week 15 generally increased with increasing dose (Table 4; Figs. 1 and 2). Mean Cmax value (±SE) was 1820 ± 456 ng eq/ml at 175 mg/kg and the corresponding AUC(0-24) value was 14,500 ± 4338 ng eq • hr/ml. Mean tmax ranged from 2.4 to 4.6 hr in all groups, and mean apparent half-life values ranged from 5.53 to 9.95 hr.

For female rats, increases in Cmax and AUC(0-24) values on prematting Treatment Day 14 were generally greater than proportional to increasing dose (Table 4; Figs. 1 and 2). The mean Cmax value was 7030 ± 1646 ng eq/ml at 225 mg/kg. The corresponding mean AUC(0-24) value was 56,200 ± 20,751 ng eq • hr/ml. Mean tmax and apparent half-life values ranged from 1.00 to 5.20 hr and 2.42 to 4.85 hr, respectively. The mean Cmax for females was statistically greater than for males at 175/225 mg/kg based on dose-normalized values.

Atorvastatin-equivalent concentrations were below the detection limit in controls.

DISCUSSION

Atorvastatin, administered to rats prior to and during mating at doses which induced toxicity in the parental animals (175 mg/kg in males, 225 mg/kg in females), had no adverse effects on fertility and reproduction. In the male fertility study, significant body weight gain suppression and decreased food consumption occurred at 100 and/or 175 mg/kg, doses which are at least 60-fold greater than the maximum therapeutic dose of 80 mg per day in humans (Nawrocki et al., 1995). Despite the toxicity, there were no effects on copulation, fertility, number of days to mating, number of offspring or live litters, male reproductive organ weights, sperm counts, sperm motility, or sperm morphology. In the female fertility study, significant body weight gain suppression occurred at 225 mg/kg with no effects on estrous cycles, copulation, fertility, number of days to mating, or number of offspring or live litters. A dose of 20 mg/kg was a no-effect dose in males and females.
## TABLE 4

Atorvastatin Pharmacokinetic Parameters in Male and Female Rats

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng eq/ml)</td>
<td>110 ± 13.3</td>
<td>80.7 ± 12.5</td>
<td>1,320 ± 279</td>
<td>2,030 ± 470</td>
<td>1,820 ± 456</td>
<td>7,030 ± 1,646*</td>
</tr>
<tr>
<td>tmax (hr)</td>
<td>4.60 ± 2.29</td>
<td>1.00 ± 0.0</td>
<td>2.40 ± 1.40</td>
<td>2.40 ± 1.40</td>
<td>3.80 ± 1.71</td>
<td>5.20 ± 1.71</td>
</tr>
<tr>
<td>AUC (0-24) (ng eq • hr/ml)</td>
<td>926 ± 155</td>
<td>480 ± 42.6</td>
<td>7,240 ± 1851</td>
<td>9,760 ± 2,462</td>
<td>14,500 ± 4,338</td>
<td>56,200 ± 20,751</td>
</tr>
<tr>
<td>t1/2 (hr)</td>
<td>5.53 ± 1.29</td>
<td>3.62 ± 0.53</td>
<td>6.33 ± 1.92</td>
<td>4.85 ± 1.23</td>
<td>9.95 ± 5.59</td>
<td>2.42 ± 0.44</td>
</tr>
</tbody>
</table>

Note. Values are means ± SE, n = 5. Cmax, maximum plasma concentration, tmax, time of maximum plasma concentration; AUC(0-24), area under the plasma concentration–time curve from Time 0 to 24 hr; t1/2, apparent half-life.
* Male and female rats received 175 and 225 mg/kg doses, respectively. All Cmax and AUC(0-24) comparisons were based on dose-normalized values.
* Male versus female values significantly different at p < 0.05.

The results of this study show that atorvastatin-equivalent pharmacokinetics are highly variable and differ between male and female rats. Regarding variability, a coefficient of variation in excess of 50% was common for many mean pharmacokinetic parameter values (data not shown). Variability appeared to increase with increasing dose. Mean atorvastatin Cmax and AUC(0-24) values for male rats generally increased with increasing dose. In female rats, mean Cmax and AUC(0-24) values generally increased in a greater than proportional manner with increasing dose. Apparent half-life and tmax values did not appear to change as a function of dose for either male or female rats. Because of the high pharmacokinetic variability and discrepant results between male and female rats, it is not clear whether atorvastatin pharmacokinetics are dose proportional in rats. Gender differences in the pharmacokinetics of atorvastatin equivalents in rats are supported by AUC(0-24) values generally being greater in female rats than male rats. The increase in AUC(0-24) with increasing dose was more pronounced in female rats than in male rats.

![FIG. 1. Mean ± SE (n = 5) atorvastatin plasma concentrations in male (open circles) and female (solid circles) rats after receiving 20, 100, and 175 or 225 mg/kg daily oral doses for 15 weeks for males and 14 days for females. Male and female rats received the same doses with the exception that high-dose male rats received 175 mg/kg and female rats received 225 mg/kg.](image)

![FIG. 2. Relationship between atorvastatin dose and AUC(0-24) in male and female rats. Mean ± SE for males (open circles) and females (solid circles) after receiving 20, 100, and 175 or 225 mg/kg daily oral doses for 15 weeks for males and 14 days for females.](image)
had no effects on sperm quality, seminal plasma concentration of familial hypercholesterolemic patients with simvastatin count, morphology, and serum androgen concentration) were unaffected (Farnsworth et al., 1987). Short-term treatment of familial hypercholesterolemic patients with simvastatin had no effects on sperm quality, seminal plasma concentration of various sex gland products, or the serum concentrations of cortisol, testosterone, LH, FSH, or prolactin (Purvis et al., 1992). No indications of adverse effects on steroidogenesis in females have been reported. Despite these sporadic reports of rather minor changes in dogs and humans, there has not been any conclusive evidence of adverse reproductive effects in the large numbers of patients who have taken HMG-CoA reductase inhibitors.

In addition to the lack of effects of atorvastatin on fertility and reproduction in the present studies, previous studies have shown atorvastatin to be nonteratogenic in rats and rabbits (Dostal et al., 1994), whereas other HMG-CoA reductase inhibitors, lovastatin (Minsker et al., 1983) and fluvastatin (FDA, 1993), were shown to produce malformations in rats. Thus, these previous studies and the present studies demonstrate that atorvastatin, a potent HMG-CoA reductase inhibitor, is not teratogenic in rats or rabbits and has no effects on fertility and reproduction in rats.

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REFERENCES


