Developmental Toxicity Study in Rats and Rabbits Administered an Emulsion Containing Medium Chain Triglycerides as an Alternative Caloric Source

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A 20% lipid emulsion was designed as an intravenous caloric source for hypermetabolic patients whose nutritional needs cannot be satisfied by conventional parenteral glucose/long chain triglyceride/amino acid therapies. The test emulsion contained a 3:1 ratio of medium chain triglycerides (MCT) to long chain triglycerides (LCT) and had a total caloric value of approximately 2 kcal/mL.

MCT (6 to 12 carbon fatty acids), unlike LCT (greater than 12-carbon fatty acids), are not stored as fat within the body; they are rapidly and completely oxidized (Johnson and Cotter, 1985). When administered in combination with LCT, the rate of metabolism of MCT may be varied by adjusting the proportion of MCT to LCT, thereby controlling the rate of energy delivery to the patient. Due to rapid hydrolysis of MCTs in the intestine, absorption of free fatty acids is twice as fast as LCTs; therefore, MCTs are more readily utilized for caloric energy, but are less effectively incorporated into tissue lipids (Crowe et al., 1985).

Recent studies using animal models simulating various clinical conditions have shown MCTs to be an efficient and unique caloric source. The potential benefits of using MCT-containing emulsions in clinical conditions were demonstrated in various animal models of hepatic dysfunction, thermal injury, and bacterial infection. Experiments designed to evaluate lipid emulsions in hepatic insufficiency included administration of total parenteral nutrition (TPN) to four groups of rats for 4 days (Pomposelli et al., 1984). Two groups received either pure LCTs or 50% MCT–50% LCT with lipids providing 50% of the calories. The remaining 50% of the calories was provided as glucose; 12.5 g amino acids/kg were also supplied in excess of the caloric load. The positive control received 100% of nonprotein calories as glucose. The negative control consisted of a glucose group without any nitrogen source. The glucose-plus-nitrogen group and the pure LCT group developed fatty livers while the livers of the MCT–LCT group were morphologically normal.

Metabolism of radioactively labeled lipid emulsion administered as a bolus injection in rats, which had been subjected to 25% scald burns (Hamawy et al., 1985), indicated that MCTs were cleared from the blood more rapidly and delivered to oxidation rather than tissue storage then LCTs. Additional studies using a similar thermal-injury rat model assessing protein metabolism using several caloric sources

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including MCTs and structural lipids containing 40% MCTs and 60% triglycerides indicated that when dosing regimens were supplemented with additional calories supplied by glucose, LCTs, or MCTs, the results showed a positive nitrogen balance (Maiz et al., 1984).

MCT-containing intravenous emulsions have been used in Europe since the mid-1970s (Ball, 1991; Clarke et al., 1987; and Crowe et al., 1985; Dawes et al., 1986; Eckart et al., 1980; Nordenström et al., 1991), but few clinical investigations have been performed with such emulsions in the United States. Recent studies conducted on hospitalized total parenteral nutrition (TPN) patients (Dawes et al., 1986; Jeeravandan et al., 1995; Randall et al., 1985) and morbidly obese patients (Mascioli et al., 1985) using combined MCT–LCT emulsions (50% MCT/50% LCT or 75% MCT/25% LCT) showed the combined MCT–LCT emulsions to be safe and readily available oxidizable fuel.

The 20% lipid emulsion is designed for use as a caloric source for adult critical care patients. While not specifically indicated for use in pregnant women, the emulsion may be used if parenteral nutrition is clearly needed. There are no published reports to date on developmental toxicity in animals or humans treated with an intravenous MCT-containing emulsion. Developmental toxicity studies were done in rats and rabbits to assess the developmental toxicity, including teratogenic potential, of a 20% lipid emulsion containing a 3:1 ratio of medium chain triglycerides:long chain triglycerides (3MCT:1LCT) when administered by intravenous infusion to pregnant rats and rabbits during organogenesis.

MATERIALS AND METHODS

Test article. A 20% lipid emulsion containing a 3:1 ratio of MCT:LCT from soybean oil was prepared as a sterile nonglycerine emulsion for intravenous administration. Medium chain fatty acids are 6 to 12 carbons in length. The 20% lipid emulsion is composed primarily of 8-10 carbon fatty acids and a trace of 6-12 carbon fatty acids. The same lot of test article was used throughout the studies, and was analytically determined to be stable for the duration of both studies. Saline (0.9% NaCl for injection, USP) was used as a nonlipid control article.

Animals. Time-mated Crl:CD BR rats were obtained from Charles River Laboratories (Portage, MI). The rats were approximately 150 to 200 g on Gestation Day (GD) 0 (defined as the day females had sperm present in the vaginal smear). Mated rats were shipped by the supplier on GD 4. Mated female Hns:NZW)SPF rabbits were obtained from HRP, Inc. (Denver, PA). Animals were approximately 5.5 to 6.5 months old and weighed 3300 to 4446 g on GD 0. Mated rabbits were received on GD 2 or 3 in two consecutive weekly shipments and were observed at arrival for abnormalities indicative of health problems.

Environmental controls in the animal rooms were set to maintain temperature, relative humidity, and light/dark cycle. Animals were housed individually in suspended stainless steel cages. Feed and water were offered ad libitum except during dose administration. Certified Rodent Chow 5002 meal (PMI Feeds, Inc.) and Certified rabbit diet 5322 (PMI Feeds, Inc.) were fed to rats and rabbits, respectively (feed was offered as a restricted ration to rabbits on the day of arrival).

Experimental design. The intravenous route of administration was used because the lipid emulsion is intended for intravenous human administration as a component of parenteral nutrition. Mated rats (25 or 29/group) and rabbits (15/group) were assigned at random to one of three groups with animals from each breeding date being distributed among the groups. Animals in Group 1 received 0.9% saline at a dose volume of 21.4 mL/kg. Animals in Groups 2 and 3 received the test article at a lipid dose of 1 or 4.28 g/kg at dose volumes of 5 or 21.4 mL/kg, respectively. The 1 g/kg dose approximates the proposed clinical dosage. The 4.28 g/kg dose is the highest dose administered in preclinical studies that did not produce narcosis (unpublished). The dose was administered daily to rats by intravenous infusion for approximately 4 h immediately following GD 15 via a marginal ear vein using a Quick-Cath Teflon catheter connected to a syringe using an extension set. The dose was administered daily to rabbits via a marginal ear vein for approximately 5 h/day on GD 7 through 19 using an indwelling catheter [Insyte-W (24-gauge, 3/4-inch intravenous catheter and needle unit attached to a 30-inch extension set, Abbott Laboratories)]. Doses for rats and rabbits were delivered using syringe pumps. A 4- or 5-h infusion was used to approximate longer-term infusion in humans and to avoid an acute toxic effect resulting from shorter term infusion at faster rates. The dose rate was based on previous preclinical studies (unpublished). The rabbits were acclimated to the dose restraint apparatus for 2.5 and 5 h on the 2 days preceding initiation of dosing.

Animals were observed twice daily (AM and PM) for mortality and morbidity. In addition, rabbits were observed once daily for clinical observations. On dosing days, animals were observed predose, immediately (within 5 min) postdose, and approximately 1 h after completion of dosing. Body weights were recorded daily on GD 5 through 20 for rats and on GD 7 through 29 for rabbits; GD 0 body weights for rabbits were provided by the animal suppliers. Feed consumption data were collected daily beginning on the day of receipt for rats and on GD 6 for rabbits.

On GD 20 for rats and 29 for rabbits, animals were euthanized with carbon dioxide inhalation (rats) or with Beuthanasia-D Special euthanasia solution via marginal ear vein (rabbits) and subjected to an internal examination for macroscopic abnormalities. Lesions were preserved in 10% phosphate-buffered formalin. In addition, tails from control rats and unaffected treated rats were collected and preserved for reference. The ovaries were removed and examined, and the number of corpora lutea recorded. The uterus was excised, weighed, and the number and placement of implantation sites (live and dead fetuses and early and late resorptions) were recorded. Animals that died or were euthanized at an unscheduled interval were examined similarly.

Conceputuses were removed, and live fetuses were weighed, examined externally, sexed (rats only), and euthanized. The internal organs of the rabbit fetuses were examined in the fresh state for variations and malformations, and the sex was determined (Staples, 1974). A mid-cranial slice of the head was made to expose the internal structure of the brain for examination; the eyes were excised and examined. Viscera were removed and discarded, and fetuses were processed and examined (Staples and Schnell, 1963) for skeletal variations and malformations. For rats, approximately half of all live fetuses from each litter were processed for visceral examination by a modification of the Wilson technique for assessing soft tissue development (Wilson, 1965). The remaining fetuses were etched and processed for skeletal examination (Staples and Schnell, 1963). Following completion of fetal examinations, soft tissue malformations were preserved in 10% phosphat-bufereformalin; skeletal specimens were retained in glycerine.

Statistical analyses. The litter was the experimental unit for evaluation. All comparisons were made with the control group (Group 1). For rats, feed consumption, dam body weights, and fetal body weights were summarized with means and standard deviations calculated using an Excel spreadsheet program (Microsoft Corporation). For rabbit data, Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at p < 0.05, rank transformation was used to stabilize the variance. Analysis of variance [ANOVA (Winer, 1971a)] was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnet's t test (Dunnott, 1964) was used for pairwise comparisons.
between groups. One-way ANOVA was used to analyze body weights, body weight changes, feed consumption, and cesarean section data. As appropriate, rat and rabbit fetal abnormality data were analyzed by the Cochran-Armangi test (Thakur et al., 1985) for trend and departure and by the Fisher-Irwin exact test (Thakur et al., 1985). One-way analysis of covariance [ANCOVA (Winer, 1971b)] was used to analyze fetal body weights (males, females, and combined) with the number of fetuses in the litter as the covariate. As appropriate, for values calculated to analyze litter data or mean fetal weight data, values were first derived within the litter, and the group mean values were derived as a mean of individual litter values. Group comparisons were analyzed at the 5.0 and 1.0% two-tailed probability levels.

RESULTS

Rat Study Results

Maternal observations. The only test article-related findings observed were associated with tail lesions in both test article groups and the occasional occurrence of red-tinted urine (8 of 29) and vaginal bleeding (1 of 29) in the high-dose group. The tail findings were predominantly those of discoloration and ulceration. Incidences were 1/25, 14/25, and 23/29 for these tail lesions in the control, low-, and high-dose groups, respectively, and ranged from mild to severe with some necrosis and partial loss of the tail. These findings were generally considered to be related to occurrences of observed extravasation of the MCT:LCT lipid test article into perivascular areas. Evaluation of urine collected from one high-dose animal suggested a bacterial infection of the urogenital tract. Clinical observations for this animal included occasional red-tinted urine and vaginal bleeding. This rat was noted as having a large urinary bladder stone and kidney hydronephrosis at necropsy.

There were no marked differences in mean body weights or feed consumption for the low-dose group compared with those of the control group. However, the high-dose group consistently exhibited lower body weights beginning 1 day after dose administration throughout the remainder of the study. Feed consumption was also notably lower for nine of the ten days during dosing, with an increase in feed consumption after completion of dose administration (GD 15). The decrease in feed consumption at the high-dose level was expected based on the high-caloric nature of the test article.

Necropsy findings were primarily related to tail effects and were observed for most rats in the high-dose group and some rats in the low-dose group. In addition to tail effects, there was a trend toward an increasing incidence of necropsy findings in the high-dose group, including enlarged lymph nodes, enlarged spleen, hydronephrosis/enlarged renal pelvis, small thymus, and small red lung foci. These changes indicated that the high-dose group was likely exhibiting test article effects. There was a slight trend toward decreasing mean gravid uterine weights (Table 1) in proportion to increasing test article dose; however, due to the large variability between groups, group mean uterine weights appeared to be similar. With the exception of one control dam, all females were pregnant and had at least one viable fetus/litter (i.e., no dams had total resorptions).

Fetal observations. There were no significant group differences in preimplantation or postimplantation loss or in the mean percentage of live or resorbed fetuses; no dead fetuses were present (Table 1). Mean fetal sex ratios of the test article-treated groups were comparable with those of controls. There were no apparent effects on mean fetal body weight (combined, males, or females). There were no test article-related fetal external, soft tissue, or skeletal observations (Table 1). A high incidence of folded retina in control and test article-treated groups was attributed to shrinkage of the retina during storage in alcohol prior to being transferred to Bouin's fixative.

Omphalocele and cleft palate were observed in one fetus each in the control and high-dose groups, respectively. The only fetal skeletal malformation observed (malformed/misshapen skull bones) was present in one fetus each from two control litters. Fetal skeletal variations were present in control and test article-treated groups in a nondose-related pattern.

Rabbit Study Results

Maternal observations. Survival was 100% for the 1 g lipid/kg group. One animal in the control group died on GD

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Rat Cesarean Section and Fetal Abnormality Data (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose level (g lipid/kg)</td>
<td>Mean fetal weight (mg)</td>
</tr>
<tr>
<td>Control (N = 24)</td>
<td>67.88 ± 13.16*</td>
</tr>
<tr>
<td>1 (N = 25)</td>
<td>3.74 ± 0.26</td>
</tr>
<tr>
<td>4.28 (N = 28)</td>
<td>3.74 ± 0.26</td>
</tr>
</tbody>
</table>

Note. N, No. of litters examined.
* Mean ± standard deviation.
* Statistically significant at p = 0.05.
Feed Consumption and Body Weight Gain Data for Rabbits during Gestation

| Dose level (g lipid/kg) | GD* 6-7 | GD 12-13 | GD 19-20 | GD 20-24 | GD 24-29 | Body weight gain (g), GD 7-20*
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>175.2 ± 38.7*</td>
<td>151.0 ± 31.7</td>
<td>168.0 ± 38.2</td>
<td>166.8 ± 21.7</td>
<td>123.0 ± 39.9</td>
<td>95.4 ± 112.2</td>
</tr>
<tr>
<td>1</td>
<td>186.0 ± 33.1</td>
<td>N = 15</td>
<td>N = 12</td>
<td>N = 11</td>
<td>N = 10</td>
<td>N = 14</td>
</tr>
<tr>
<td>4.28</td>
<td>185.3 ± 46.5</td>
<td>N = 15</td>
<td>N = 14</td>
<td>N = 12</td>
<td>N = 14</td>
<td>N = 15</td>
</tr>
</tbody>
</table>

Note. N, No. of animals examined.
* GD, gestation day.
* Dosing interval.
* Mean ± standard deviation.
** Statistically significant at p < 0.01.

11 and one animal given 4.28 g lipid/kg was sacrificed after aborting on GD 20. The animal that aborted had the lowest feed consumption in the group. Decreased feed consumption, resulting in a decline in the health of this animal, may have contributed to the abortion. The abortion was not considered to be test article-related as it was in the range of historical control incidence. There were no remarkable necropsy findings for either animal.

Clinical observations in the test article-treated groups were limited to fecal findings (i.e., increased incidence of few or no feces). Three animals each at 4.28 g lipid/kg had no fecal output for 1 day during the dosing period. There were no significant differences in mean body weights for the test article-treated groups. However, mean body weight changes (represented as mean body weight losses) and feed consumption (Table 2) were significantly lower for the 4.28 g lipid/kg group during the dosing interval (GD 7 to 20). Feed consumption continued to be significantly lower for this group during the early posttreatment period (GD 20 to 24), but recovery was noted at a later interval (GD 24 to 29). The decreased feed consumption observed in this study was an expected occurrence based on the high-caloric nature of the test article. There were no test article-related findings at necropsy. All pregnant animals had at least one viable fetus at scheduled cesarean section on GD 29 (i.e., no dams had total resorptions).

Fetal observations. The mean percentage of total resorptions/litter (postimplantation loss) was significantly higher and the mean percentage of live fetuses/litter was correspondingly lower in the 4.28 g lipid/kg group (Table 3). Mean covariate-adjusted fetal body weights (males, females, and combined) for the 4.28 g lipid/kg group were significantly lower (Table 3).

The proportion of fetuses and litters in the 4.28 g lipid/kg group with external morphological abnormalities was significantly higher than that of the control group (Table 3). The most notable findings were rachischisis and short tail seen in three and two high-dose litters, respectively. Single incidences of the following malformations were also present in the high-dose group: exencephaly, ablepharia, exophthalmus, and ectrodactyly. Single litter incidences of carpal

**TABLE 3**
Rabbit Cesarean Section and Fetal Abnormalities Data

<table>
<thead>
<tr>
<th>Dose level (g lipid/kg)</th>
<th>N</th>
<th>% Resorptions</th>
<th>% Fetuses</th>
<th>% Postimplantation loss</th>
<th>Mean fetal weight (mg)</th>
<th>External</th>
<th>Litter incidence</th>
<th>Soft tissue</th>
<th>Skeletal</th>
<th>Fetal incidence</th>
<th>Litter incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>0.9</td>
<td>0.0</td>
<td>2.3</td>
<td>44.01</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>0.8</td>
<td>4.5</td>
<td>5.3</td>
<td>40.65</td>
<td>1.5</td>
<td>6.7</td>
<td>67</td>
<td>11</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>4.28</td>
<td>13</td>
<td>12.2</td>
<td>6.7</td>
<td>82.9**</td>
<td>33.54**</td>
<td>6.5**</td>
<td>38**</td>
<td>46</td>
<td>92**</td>
<td>92**</td>
<td>100</td>
</tr>
</tbody>
</table>

Note. N, No. of litters examined.
* Statistically significant at p < 0.05.
** Statistically significant at p < 0.01 by covariate analysis using the number of live fetuses/litter as the covariate.
### Table 4

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Dose level (g lipid/kg)</th>
<th>Control</th>
<th>1</th>
<th>4.28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misaligned sternebrae</td>
<td><em>Fetal incidence</em></td>
<td>0.0</td>
<td>0.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td><em>Litter incidence</em></td>
<td>0.0</td>
<td>0.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Unossified sternebrae</td>
<td><em>Fetal incidence</em></td>
<td>0.0</td>
<td>0.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td><em>Litter incidence</em></td>
<td>0.0</td>
<td>0.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Greater than 26 presacral vertebrae</td>
<td><em>Fetal incidence</em></td>
<td>9.0</td>
<td>7.7</td>
<td>46**</td>
</tr>
<tr>
<td></td>
<td><em>Litter incidence</em></td>
<td>36</td>
<td>40</td>
<td>77</td>
</tr>
<tr>
<td>Greater than 12 full pairs of ribs</td>
<td><em>Fetal incidence</em></td>
<td>19</td>
<td>20</td>
<td>57**</td>
</tr>
<tr>
<td></td>
<td><em>Litter incidence</em></td>
<td>50</td>
<td>87</td>
<td>100**</td>
</tr>
</tbody>
</table>

* Statistically significant at \( p \leq 0.05 \).
** Statistically significant at \( p \leq 0.01 \).

Though not statistically significant, there was a notable increase in the number of high-dose fetuses/litter with malformations of the vertebral column. The anomalies were varied in location (cervical, thoracic, sacral, and caudal) and type (malformed, misaligned, absent, and fused). The incidence of any one of these findings was not markedly higher than that of the controls (usually only one litter was affected with a given abnormality); however, when combined, the incidence of fetuses/litters from the high-dose group with vertebral column malformations was notably higher than that of the control group.

### DISCUSSION

Infusion of the test article, a high-calorie emulsion, resulted in an expected lower feed consumption for rats and rabbits at the 4.28 g lipid/kg level. In rabbits, this resulted in reduced fecal output. Mean maternal body weights were lower for high-dose rats during the dose administration period. Although the mean body weights for rabbits in this group were not notably different from those of the controls, a slight loss in mean body weight was sustained during the overall treatment period (GD 7 through 19).

Cesarean section examinations of rabbits revealed higher postimplantation loss (i.e., increased incidence of resorptions) and, correspondingly, fewer live fetuses at the 4.28 g lipid/kg level. Mean fetal weights were lower for this group as well, and more litters in the group tended to have fetuses with morphological anomalies than were seen in the control group. Reduced fetal weights may have been secondary to the decreased maternal feed consumption observed at this dose level. Similar findings were not present for rats.

Because treated females were consuming significantly less feed than control females, the fetal effects noted for rabbits (especially the postimplantation loss and decreased fetal weights) may have been due to dietary deprivation, as opposed to a direct effect by the test article. Increased incidence of abortion and implant resorption among pregnant rabbits subjected to dietary deprivation have been reported (Matsuzawa et al., 1980). There were no related patterns of major malformations for fetuses. Skeletal variations were present in all litters, including control and treated groups. Diverse abnormalities were seen that may have been associated with maternal toxicity (Khera, 1984; Manson, 1986). In our study, rabbit maternal weight gain in the 4.28 g lipid/kg group was 84% of that of the control group during gestation.

When the test article, a 20% lipid emulsion containing a 3:1 ratio of MCT:LCT, was administered to rats, the no-observable-effect level (NOEL) for developmental toxicity was greater than or equal to 4.28 g lipid/kg. Test article-related effects observed for the dams included decreased feed consumption and tail lesions which were attributed to perivascular extravasation of the test article. Necropsy obser-
vations also were indicative of a mild test article related effect at the high-dose level.

Administration of the test article to rabbits at 1 or 4.28 g lipid/kg resulted in a NOEL for developmental toxicity greater than or equal to 1 g lipid/kg but less than 4.28 g lipid/kg based on the adverse fetal findings (increased post-implantation loss, lower fetal body weights, and higher incidence of morphological anomalies) seen at the 4.28 g lipid/kg level. Administration of the test article also resulted in lower maternal feed consumption and slight body weight loss during treatment at this dose level. Therefore, the fetal effects (i.e., increased resorptions, decreased fetal body weights, and increased incidence of morphological anomalies) were probably the result of dietary deprivation, maternal toxicity, or both, rather than a direct teratogenic effect of the test article.

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