TERATOGENIC AND REPRODUCTIVE EFFECTS OF ETHANOL IN LONG-EVANS RATS

R. F. Mankes, I. Rosenblum, K.-F. Benitz, R. LeFevre, R. Abraham

Institute of Experimental Pathology and Toxicology,
The Albany Medical College of Union University,
Albany, New York

The effects of ethanol alone or ethanol and dieldrin given orally were investigated on the reproductive processes of female Long-Evans rats. Three studies were undertaken: effects on reproduction (phase I), perinatal and postnatal effects (phase III), and teratological effects (phase II). Female Long-Evans rats were divided into five groups for all studies: group I-distilled water; group II-corn oil; group III-4 mg Dieldrin/kg; group IV-500 mg aspirin/kg; and group V-0.4 ml ethanol/kg. For phase II, two additional groups were added to the experimental design: group VI-4.0 ml ethanol/kg and group VII-4 mg Dieldrin/kg and 0.4 ml ethanol/kg. In the phase I study, ethanol produced a significant increase in the number of malformed pups at birth: microphthalmia and paralysis were the defects noted. Aspirin caused significant teratogenic effects as well as maternal deaths from gastrointestinal hemorrhage. Dieldrin alone caused no adverse effects. In the phase III study, except for maternal toxicity among aspirin-dosed dams, no adverse findings were elicited. In the phase II study, 0.4 ml ethanol/kg induced a significant increase in the frequency of soft tissue malformations such as microphthalmia and hydronephrosis. A 10-fold increase in the ethanol dosage (4.0 ml/kg) caused embryolethality as well as soft tissue teratology. Aspirin (500 mg/kg) caused skeletal (cranial bone) defects and soft tissue anomalies in pups; Dieldrin was without untoward effects. Dams given a combination of Dieldrin and 0.4 ml ethanol/kg displayed increased embryolethality but no teratogenic effect was noted. These results suggest that the Long-Evans rat is a sensitive model for the induction of FAS at a dose level equivalent to one alcoholic drink per day in human females. The reported embryolethal effect of concurrent exposure to Dieldrin (an organochlorine pesticide) and ethanol deserves further evaluation.

INTRODUCTION

The multiple pattern of skeletal, visceral and behavioral anomalies in infants born to alcoholic mothers is known as fetal alcohol syndrome (FAS). These anomalies occur in up to one child per thousand live births and are caused by as few as two alcoholic drinks per day (Smith, 1980). Experimental induction of the FAS using the mouse as a model system...
has been achieved with dose levels that were toxic to the pregnant dams (Chernoff, 1977). Other investigators have attempted to induce FAS in mice, rats, rabbits and in vitro cultures of rat embryos (Brown et al., 1979; Oisund et al., 1978; Kennedy and Persaud, 1978; Randall and Taylor, 1979; Schwetz et al., 1978; Varma and Persaud, 1979). However, the results of these experiments have been controversial and equivocal.

The present study deals with two aspects of ethanol teratogenesis: the teratogenic potential of low doses of ethanol in Long-Evans rats, and interactions between ethanol and Dieldrin during the “critical period” of organogenesis.

METHODS

A total of 420 Long-Evans rats (360 females and 60 males, 180-250 g) were obtained from Blue Spruce Farms in Altamont, N.Y. Sixty male were untreated and served as breeders in a 1:2 ratio for all three phases of the experiment. The females were obtained in lots of 120 each, at least 30 d prior to the start of each phase. All animals were acclimated to laboratory conditions for at least 2 wk. Vaginal washings were used for the determination of the estrus cycle in females for an additional 2 wk. One hundred mature, healthy females were selected for each experimental phase. Food (Wayne Lab Blox, Chicago, Ill.) and drinking water were supplied ad libitum. During the premating period, either two males or five females were housed in wire-topped plastic cages. After mating, the females were housed individually and provided with additional bedding material (Betta Chip hardwood bedding). Based on exfoliative cytology, females predicted to enter estrus were housed overnight with a male, using a ratio of two females to one male. The presence of sperm in the vagina detectable on the following morning, was defined as d 0 of gestation.

Solutions for oral administration were prepared at weekly intervals and consecutive daily doses were given by gavage using a volume of 2 ml/kg, based on the current body weights. The following solutions were used: distilled water, Mazola corn oil, Dieldrin (dissolved in corn oil at concentration of 20 mg/100 ml), and 2 or 20% v/v aqueous solutions of 100% U.S.P. ethyl alcohol (obtained from Commercial Solvents, Inc. Aspirin tablets (E. R. Squibb & Sons, Inc., Princeton, N.J.) were obtained from the Albany Medical Center Hospital Pharmacy, ground with a mortar and pestle, suspended in distilled water at a concentration of 2500 mg/10 ml, and resuspended prior to use.

Five groups of 20 female rats were used in phase I (reproductive segment) and phase III (perinatal-postnatal segment). In addition to the five groups used for phases I and III, two additional groups were used for...
ETHANOL TERATOLOGY IN RATS

Phase II (teratology segment). Seven groups of female rats consisted of: group I-distilled water; group II-corn oil; group III-4 ml Dieldrin/kg; group IV-500 mg aspirin/kg; group V-0.4 ml ethanol/kg; group VI-4.0 ml ethanol/kg; and group VII-4 mg Dieldrin/kg and 0.4 ml ethanol/kg.

Phase I

To evaluate reproductive effects, animals were given their respective regimen 3 d before predicted conception to d 21 of lactation, and observed daily for signs of toxicity and lethality. Maternal body weights were recorded on d 3, 2, and 1 before mating; on d 0, 7, 14, 18, 19, 20, 21 of gestation; and on d 0, 1, 14, and 21 of lactation. Immediately after birth, the numbers of live or stillborn offspring, total litter weights, gestation lengths, sex distribution of offspring and number of litters were recorded. During lactation, offspring body weights and numbers of litters and pups were recorded on d 0, 7, 14, and 21. At weaning, the numbers of live pups, total litter weights, sex distribution, and deaths during lactation were recorded. All animals found dead during the study were necropsied and examined grossly.

Phase II

For the evaluation of teratogenic effects, 100 inseminated female rats were divided into 7 groups. The first 6 groups had 15 rats each, while group VII had 10. The rats were given their respective regimens from 6 to 15 d of gestation. Dams were observed daily for signs of toxicity and lethality. Maternal body weights were recorded on d 0 to 15 and 20 of gestation. All dams were overdosed with ether on d 20, and their fetuses were delivered by Caesarean section. Numbers of live pups, stillbirths, resorptions, total implantation sites, sex distribution, and numbers of corpora lutea were recorded. Approximately one-third of the fetuses were fixed in Bouin's fluid and examined for visceral and soft-tissue anomalies according to Wilson (1965). The remaining pups were fixed directly in absolute ethanol, eviscerated and examined for defects in ossification according to Staples and Schnell (1964).

Phase III

To evaluate late gestation and lactation effects, another lot of 100 inseminated female rats were divided into 5 groups of 20 rats each and given their respective regimens from d 15 of gestation to 21 of lactation. All animals were observed daily for signs of toxicity and lethality. Maternal body weights were recorded on d 0 and 15 to birth, and birth to d 7, 14, and 21 of lactation. At birth, the numbers and weights of live and stillborn offspring, total litter weights, gestation lengths, sex distribution of offspring, and numbers of litters were recorded. During lactation, offspring body weights and numbers of pups and litters were recorded on d 0, 4, and 21. At weaning, the numbers of live pups, total litter weights,
sex distribution, numbers of litters, and numbers of deaths during lactation were recorded. All animals found dead during the study were necropsied and examined grossly.

All data generated in the course of the study were entered, archived, and statistically analyzed on a DEC System 10 computer of the Computer Center of Albany Medical College in Albany, N.Y. Statistical analyses of the data were performed according to the methods of Dixon and Brown (1977). All applicable state (New York) and federal (U.S.) laws and regulations pertaining to the humane use and care of laboratory animals for medical research were followed.

RESULTS

Phase I (Reproductive Segment)

Of the 100 dams selected for the study, 48 did not mate and were discarded. Ethanol (group V) caused no maternal toxicity, and reproduction was not inhibited (Table 1). Four dams given aspirin (group IV) died of internal bleeding, one during gestation and three during lactation. Body weights were depressed in survivors.

Stillbirths (20%) and lactation deaths (41%) were increased in group IV as compared to controls. Pups of groups IV or V were significantly heavier than controls, due to the smaller numbers of offspring (Table 1).

Ethanol significantly increased the incidence of grossly abnormal or malformed pups (22%) as compared to controls (Table 2). The offspring from group V were hydrocephalic (2%), microphthalmic (13%), and exhibited partial or complete paralysis (7%). This latter "malformation" is considered a functional manifestation of anomalous brain development. Aspirin caused (7%) malformations in the offspring, notably hydrocephalus (5%) and paralysis (7%).

Phase III (Perinatal/Postnatal Segment)

Two dams given aspirin died of internal bleeding; one during gestation and one during lactation. No changes in the parameters of fecundity were detected (Table 1). Aspirin decreased pup weights at birth and weaning, but growth was not impaired (Table 1). Only three pups were malformed; two from group V had hydrocephalus and/or spina bifida, and one from group II had hydrocephalus and paralysis (Table 2).

Phase II (Teratology Segment)

One dam from group VI died on d 14 due to accidental intratracheal intubation. Significant changes in the parameters of fecundity (Table 1), indicative of embryolethality, were observed in groups IV and VII. Fewer live fetuses (74–87%) and more embryonic deaths (13–26%) than controls occurred in these animals. When group VI was compared to group V,
## TABLE 1: Reproductive Lactational, and Survival of Female Long-vans Rats Given to Female Long-vans Rats during Various Phases of Gestation

| Dose: | Duration (phase I)  | Gestation (day, g) | Dams with multiple implants (%), g | Live body weight (mean ± SD) | Lactation deaths (%) | Total body weight (Mean ± SD) | Live births (Mean ± SD) | Stillborn (%) | Lactation weights (Mean ± SD) | Weaning weights (Mean ± SD) | Lactation deaths (%) | Weaning weights (Mean ± SD) | Duration (phase II) 6 to 15 (g) | Total body weight (Mean ± SD) | Live births (Mean ± SD) | Lactation deaths (%) | Weaning weights (Mean ± SD) | Lactation deaths (%) |
|-------|--------------------|--------------------|-----------------------------------|-------------------------------|---------------------------|------------------------|----------------------------|----------------|-----------------------------|--------------------------|---------------------------|--------------------------|----------------------------|----------------------------|-------------------|----------------|-----------------------|------------------------|-----------------------|
| Control | 20 (m/kg) | 1 to 21 (g) | 10 (11) | 3.8 ± 0.7 | 40 ± 1.5 | 54 ± 2.0 | 60 ± 2.6 | 46 (93) | 3 | 45 ± 2.0 | 2 | 3 | | | | | | |
| Ethanol | 200 (m/kg) | 1 to 21 (g) | 10 (11) | 3.8 ± 0.7 | 40 ± 1.5 | 54 ± 2.0 | 60 ± 2.6 | 46 (93) | 3 | 45 ± 2.0 | 2 | 3 | | | | | | | | |
| Aspirin | 4 mg/kg | 1 to 21 (g) | 10 (11) | 3.8 ± 0.7 | 40 ± 1.5 | 54 ± 2.0 | 60 ± 2.6 | 46 (93) | 3 | 45 ± 2.0 | 2 | 3 | | | | | | | | |
| Ethanol and dieldrin | 500 mg/kg | 1 to 21 (g) | 10 (11) | 3.8 ± 0.7 | 40 ± 1.5 | 54 ± 2.0 | 60 ± 2.6 | 46 (93) | 3 | 45 ± 2.0 | 2 | 3 | | | | | | | | |
| Dieldrin | 0.4 m/kg and dieldrin | 1 to 21 (g) | 10 (11) | 3.8 ± 0.7 | 40 ± 1.5 | 54 ± 2.0 | 60 ± 2.6 | 46 (93) | 3 | 45 ± 2.0 | 2 | 3 | | | | | | | | |

All body weights in g. All lactation day, 2: lactation day, 1.

All body weights in g. All lactation day, 2: lactation day, 1.

A difference from control (group I) significant at p < 0.05 by the Kruskal-Wallis test.

A difference from group V significant at p < 0.05 by the Pearson's chi-square test.

A difference from group V significant at p < 0.05 by the Yates corrected chi-square test.

A difference from group V significant at p < 0.05 by both the Pearson and the Yates corrected chi-square tests.
TABLE 2. Teratogenic Effects of Dieldrin, Aspirin, Ethanol, or a Combination of Dieldrin and Ethanol Given to Female Long-Evans Rats during Various Phases of Gestation

<table>
<thead>
<tr>
<th></th>
<th>I—Control (water)</th>
<th>II—Corn oil</th>
<th>III—Dieldrin</th>
<th>IV—Aspirin</th>
<th>V—Ethanol</th>
<th>VI—Ethanol</th>
<th>VII—Ethanol and Dieldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>20 ml/kg</td>
<td>20 ml/kg</td>
<td>4 mg/kg</td>
<td>500 mg/kg</td>
<td>0.4 ml/kg</td>
<td>4.0 ml/kg</td>
<td>0.4 ml and 4 mg/kg</td>
</tr>
<tr>
<td>Duration (phase I)</td>
<td>-3(g) to 21(l)</td>
<td>-3(g) to 21(l)</td>
<td>-3(g) to 21(l)</td>
<td>-3(g) to 21(l)</td>
<td>-3(g) to 21(l)</td>
<td>-3(g) to 21(l)</td>
<td>12 [22]b</td>
</tr>
<tr>
<td>Malformed pups (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (7)</td>
<td>1 [10]</td>
<td>4 [13]b</td>
<td></td>
</tr>
<tr>
<td>Duration (phase II)</td>
<td>6(g) to 15(g)</td>
<td>6(g) to 15(g)</td>
<td>6(g) to 15(g)</td>
<td>6(g) to 15(g)</td>
<td>6(g) to 15(g)</td>
<td>6(g) to 15(g)</td>
<td></td>
</tr>
</tbody>
</table>

a) Gestation days, g; lactation days, l. 

b) Difference from control (group I) significant at \( p < 0.05 \) by both the Pearson and the Yates chi-square tests.

c) Difference from group IV significant at \( p < 0.05 \) by both the Pearson and the Yates chi-square tests.
dose-related decreases in live pups (86 versus 93%) and an increase in embryonic deaths (14 versus 7%) were detected.

Teratogenic effects were elicited by both levels of ethanol (groups V and VI) and aspirin (group IV) (Table 2). Controls (group I) had malformation rates of 6.7% for litters and 0.5% for pups. Ethanol (group V) caused a sevenfold increase in the malformation rate for litters (43%) and a 12-fold increase in the malformation rate for pups (6.3%). The embryolethal ethanol dose (group VI) caused more dams (69%) to bear malformed pups (6.9%) than either group I or V. Of dams given aspirin (group IV), 83% gave birth to 22% malformed pups. The combination of ethanol and Dieldrin (group VII), although embryolethal, was not teratogenic.

Ethanol exerted a dose-dependent teratogenic effect on the developing brain, eye, and kidney (Table 3). Ethanol (0.4 ml/kg) induced microphthalmia (8%) and hydronephrosis (7%), while the higher embryolethal level of ethanol (4.0 ml/kg) caused brain malformations (hydrocephalus and microcephalus), eye defects (anophthalmia and microphthalmia), and hydronephrosis (groups V and VI). Eye and kidney malformations in pups of group VI were not significantly different from controls (group I), probably due to the increase in fetal deaths (Tables 1 and 3). Ethanol, at either level, had no effects on fetal ossification when compared to controls (group I). Aspirin (500 mg/kg) adversely affected fetal development. Both soft and bony tissues showed changes (Table 3). Hydrocephalus, subcutaneous hemorrhage, micrognathia, and skull and rib defects in pups from this group showed increased incidence over controls.

Dieldrin (4 mg/kg) caused no significant increase in any malformation when compared to the appropriate controls. Two offspring exposed to a combination of Dieldrin and ethanol (group VIII) were observed to be hydrocephalic. Low incidences of hydrocephalus, anophthalmia, and fused vertebrae were seen in controls.

**DISCUSSION**

Pregnant Long-Evans rats given 0.4 ml/kg of ethanol produced offspring with significant teratologic defects without causing either maternal toxicity or embryolethality (phases I and II). The anomalies observed in the surviving progeny were defects of the eye, brain, and kidney (phase II), as well as hindlimb paralysis in progeny surviving to weaning (phase I). These results suggest the Long-Evans rat as a model for investigating the FAS.

As described by Smith (1980), FAS occur in women whose history shows consumption of alcoholic beverages in the range of 2 to 10 or more drinks per day and which results in the delivery of live but malformed children. Aborted fetuses (embryolethality) are not associated with the FAS, although spontaneous abortions are associated with maternal
TABLE 3. Malformations of Live Offspring from Rats Given Dieldrin, Aspirin, Ethanol, or a Combination of Dieldrin and Ethanol Orally from Day 6 to 15 of Gestation (Phase II)

<table>
<thead>
<tr>
<th>Dose</th>
<th>I-Control (water)</th>
<th>II-Corn oil</th>
<th>III-Dieldrin</th>
<th>IV-Aspirin</th>
<th>V-Ethanol</th>
<th>VI-Ethanol and Dieldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 ml/kg</td>
<td>20 ml/kg</td>
<td>4 mg/kg</td>
<td>500 mg/kg</td>
<td>0.4 ml/kg</td>
<td>4.0 ml/kg</td>
</tr>
<tr>
<td>Percent incidence of malformed fetuses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total soft tissue</td>
<td>2 (1-1)</td>
<td>5 (3-2)</td>
<td>13 (7-4)</td>
<td>29 (15.9)^a</td>
<td>16 (10.5)^a</td>
<td>15 (8.8)^a</td>
</tr>
<tr>
<td>Total skeletal</td>
<td>0</td>
<td>1 (1-1)</td>
<td>0</td>
<td>15 (13.5)^a</td>
<td>1 (1-1)</td>
<td>2 (2-2)</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>2 (1-1)</td>
<td>3 (2-2)</td>
<td>11 (6-4)</td>
<td>26 (13.7)^a</td>
<td>2 (1-1)</td>
<td>6 (3-3)^a</td>
</tr>
<tr>
<td>Microcephalus</td>
<td>2 (1-1)</td>
<td>2 (1-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anophthalmia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophthalmia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microphthalmia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>2 (1-1)</td>
<td></td>
<td></td>
<td>4 (2-1)</td>
<td>7 (4.1)^a</td>
<td>4 (2-2)</td>
</tr>
<tr>
<td>Other^b soft tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 (3-1)^b</td>
<td></td>
</tr>
<tr>
<td>Micrognathia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 (3-2)</td>
<td></td>
</tr>
<tr>
<td>Variant ossification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skull</td>
<td>15 (13-5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rib</td>
<td>8 (7-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral</td>
<td>1 (1-1)</td>
<td></td>
<td></td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
</tr>
<tr>
<td>Other^c</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Significantly different from control (group I) at p < 0.05, in Pearson and Yates chi-square tests. Numbers in parentheses are numbers of pups—numbers of litters.
^b Subcutaneous hemorrhages, reduced size of diencephalon (hypothalamic aplasia)—single occurrences.
^c Retarded limb, pelvic, and sacral ossifications—single occurrences.
alcoholism (Kline et al., 1980). Consumption of large amounts of ethanol is not requisite for the FAS (Abel et al., 1981) and is not necessary in order to produce malformed rat progeny. When the dose of ethanol was increased in our studies to 4.0 ml/kg (phase II, group VI), embryolethality as well as teratology was observed. Reports dealing with rodents and guinea pigs (Papara-Nicholson and Telford, 1957) given large amounts of ethanol produced similar changes (Hanson, 1980) but fetal deaths are inconsistent with the FAS. We also observed an increase in the number of dams delivering malformed pups as the dose of ethanol was increased which can reflect individual differences in susceptibility, perhaps having a genetic basis. Genetic factors may also be part of the FAS (Chernoff, 1981).

In the present studies, neural and renal anomalies are caused by 0.4 to 4.0 ml ethanol/kg body weight. Ethanol alone directly inhibits fetal growth and neural and renal differentiation, while acetaldehyde inhibitors (pyrazole) and ethanol increase resorptions and malformations in rats (Brown et al., 1979; Boggan and Randall, 1979; Bartolome et al., 1981; Elis et al., 1978; Waziri et al., 1981; Varma and Persaud, 1979). Thus, it appears that ethanol is a direct mammalian teratogen at low doses, acting on the developing fetal brain and kidney.

Another result of these experiments was to confirm the teratogenic effect of aspirin (Koshakji and Schulbert, 1973; Schardein, 1976; Robertson et al., 1979), our "positive" control, in this strain of rat. Dieldrin was included in these studies as an indifferent chemical, not previously associated with adverse reproductive effects (Dix et al., 1977; Chernoff et al., 1975). This expectation was confirmed when Dieldrin alone was given. The administration of Dieldrin in corn oil modified the anticipated effect of the dose of ethanol by an as yet unexplained interaction. While the use of Dieldrin as a pesticide is severely restricted, it is still being detected in products for human consumption (Rothschild, 1980), and this interaction is worthy of further study.

REFERENCES


*Received October 20, 1981
Accepted January 26, 1982*