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Fetotoxicity following Chronic Prenatal Treatment of Mice with Tobacco Smoke and Ethanol

K. L. Peterson, R. W. Heninger, and R. E. Seegmiller

Department of Zoology, Brigham Young University, Provo, UT 84602

In today's society pregnant women are exposed to a wide variety of chemicals, some of which produce harmful effects on their offspring. The effects of a single substance at low level exposure, however, may not be major but may be potentiated by interaction with other chemicals. Evidence in laboratory animals, for example, has shown that the teratogenic effects of aspirin are increased when pregnant animals are concurrently dosed with the food preservative benzoic acid (KIMMEL et al. 1971). Since the majority of birth defects in man have an unknown yet likely multifactorial etiology (FRASER 1977), much remains to be learned about the teratogenic effects of drugs acting in combination. Although it might prove beneficial to study a number of different compounds, we selected ethanol and tobacco smoke for reasons that (a) the chemicals cross the placenta, (b) their independent deleterious effects on prenatal development are well documented, (c) both substances are used by women with increasing frequency, and (d) they are commonly used "hand-in-hand."

Approximately a decade ago a pattern of defects among the offspring of chronically alcoholic women was observed (LEMOINE 1967; JONES et al. 1973). Included were prenatal and postnatal growth deficiencies, cardiac anomalies, microcephaly, maxillary hypoplasia, short palpebral fissures, epicanthic folds, delayed motor development, and mental deficiency. Animal models have helped to understand the mechanism for the ethanol induced fetotoxicity (TZE & LEE 1975; KRONICK 1976; CHERNOFF 1977, 1980; SCHWETZ et al. 1978; RANDALL & TAYLOR 1979; STREISSGUTH et al. 1980; WEST et al. 1981). For example, it has been shown that ethanol is teratogenic providing that blood alcohol levels remain high enough during critical periods of prenatal development. Furthermore, the extent of dysmorphology and growth retardation varies according to strain and nutritional status of the dam.

Studies on the fetotoxic effects of tobacco smoke have not been as conclusive as studies on alcohol. Nevertheless they have provided evidence that maternal smoking results in shortened gestational period, intrauterine growth retardation and increased perinatal mortality (BUTLER et al. 1972; COLE et al. 1972; LONGO 1976; MEYER 1978; PERSSON et al. 1978; LANDESMAN-DWYER & EMANUEL 1979). Since tobacco smoke contains several potentially fetotoxic substances, research in laboratory animals has been focused on one or more of these, particularly nicotine and carbon monoxide. In rats, following injection of nicotine into the maternal circulation, it was observed that fetal concentrations of nicotine

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were greater than maternal concentrations (MOSIER et al. 1974). ASTRUP et al. (1972) observed in rabbits that increased COHb concentrations were associated with lowered birth weight of the offspring. In a study of the effect of carbon monoxide on glucose metabolism in rat fetuses, ROBIN & COCKROFT (1978) reported that decreased oxygen and increased COHb caused a shift in glucose metabolism to anaerobic conditions, an effect which resulted in less available energy and decreased fetal growth.

The similar effects of ethanol and tobacco smoke on prenatal growth and perinatal viability suggest the possibility of chemical interaction in the production of fetotoxic effects. Studies addressing this issue have provided evidence both for and against this hypothesis (LITTLE 1977; MARTIN et al. 1977; ABEL et al. 1979). We have felt it important to address this issue in mice because the many interacting and confounding variables inherent in human studies can be controlled for in the laboratory. In the present study pregnant mice were chronically treated with ethanol injected intraperitoneally, with tobacco smoke via inhalation, or with ethanol plus tobacco smoke, and each group was compared with its sham-treated control.

MATERIALS AND METHODS

Animals

ARS(ICR)f outbred Swiss albino mice (Sprague Dawley Laboratories, Madison, Wisconsin) were fed Wayne Breeder Blox and water ad libitum. Mice were bred by placing virgin females with fertile males overnight and segregating them the next morning. Pregnancy (day 0) was determined by the presence of a vaginal plug.

Experimental Design

Females were randomly placed into three groups and treated from day 6 through 17 in the following manner: (1) one group received intraperitoneal injections of 0.03 ml per gram body weight of a 25 percent (v/v) solution of 95 percent ethanol and buffered saline, a dose which produced peak maternal blood alcohol levels of greater than 300 mg percent; (2) another group was treated with tobacco smoke by inhalation of one and a half cigarettes per day (2.35 percent nicotine/cigarette, Univeristy of Kentucky) via a smoke inhalation chamber (10 percent smoke in the chamber); (3) a third group was treated with tobacco smoke followed by ethanol injection four hours later. Ethanol treatment within the four-hour recovery period was found to be lethal to the pregnant females. Three other groups served as sham controls, viz., those receiving daily intraperitoneal injections of saline, those placed daily on the inhalation chamber with an unlit cigarette, and those receiving daily a combination of the above sham treatments.

The females were sacrificed on day 18 of gestation and the number of resorbed, dead and live fetuses were recorded. The extent of fetal mortality was determined by dividing the number of resorbed and dead fetuses by the total number of implantations.

The live fetuses were weighed, measured for crown-rump length, and examined for external malformations. The fetuses were then fixed in four percent buffered formalin, cut into 2-3 mm sections, and examined for internal malformations.

Statistical Evaluation

Differences in fetal weights and lengths for the different experimental groups were analyzed for statistical significance by use of the analysis of variance test. To determine if the effects of combined treatment were greater than the effects of ethanol or tobacco smoke alone, a computerized analysis of the linear model Y(i,j,k)=Treatment(i)+Mother(i)-j+E(i,j)k was used (GRAYBILL 1976; SCOTT et al. 1980).

RESULTS

Fetal Mortality

No resorbed nor stillborn fetuses were observed among either of the sham controls for the tobacco smoke and ethanol treatment groups (Table 1). A resorption frequency of seven percent was observed for the control group of the combined treatments. The group receiving tobacco smoke alone had a resorption frequency of five percent. The ethanol-treatment group had a significantly higher resorption frequency of 31 percent. A highly significant resorption frequency of 67 percent was observed in the group receiving both tobacco smoke and ethanol.

Fetal Weight

The mean weights for the controls ranged form 1.21 to 1.31 g. The mean weight of the controls for the combined treatment group did not differ from that of either of the other controls (Table 1). Furthermore, the weights of offspring from dams treated separately with tobacco smoke or ethanol were not significantly different from their respective controls. The mean birth weight of .90 g for the combined ethanol-tobacco smoke group was significantly lower than that of its control.

Fetal Length

The parameter crown-rump length was constant among all control groups and the groups receiving ethanol or tobacco smoke alone. These means ranged from 2.46 to 2.51 cm (Table 1). By comparison, fetuses treated with the combination of tobacco smoke and ethanol demonstrated a significantly reduced crown-rump length of 2.20 cm.

Malformations

The only malformations observed were cleft palate. None of the control groups nor the tobacco-smoke treatment group had any observable malformations. Eight percent of the fetuses in the ethanol treatment group and four percent of the fetuses in the combined treatment group had cleft palates (Table 1).

TABLE 1

EFFECTS OF TOBACCO SMOKE AND ETHANOL

ON FETAL WEIGHT, LENGTH, RESORPTIONS, AND MALFORMATIONS

Treatment	No. Litters	No. Live Births	Mean ± S.E.M. Fetal Weight (G)	Mean ± S.E.M. Fetal Length (CM)	Implants Total	Implants $\bar{X}$	Resorptions No.	Resorptions %	Malformations No.	Malformations %
Control	3	35	1.31 ± 0.01	2.51 ± 0.01	35	12.0	0	0	0	0
Tobacco Smoke	4	37	1.26 ± 0.02	2.46 ± 0.02	39	10.0	2	5	0	0
Control	2	18	1.27 ± 0.03	2.51 ± 0.01	18	9.0	0	0	0	0
Ethanol	5	38	1.21 ± 0.02	2.46 ± 0.02	55	14.5	17	31*	3[a]	8
Control	3	26	1.21 ± 0.03	2.50 ± 0.01	28	9.0	2	7	0	0
Tobacco Smoke Plus Ethanol	10	28	0.90 ± 0.02**	2.20 ± 0.02*	84	8.4	56	67**	1[a]	4

*P < 0.05 **P < 0.01 [a]Cleft Palate

DISCUSSION

From the results of this study it is suggested that, at the dose given, ethanol produces no effect on fetal weight and length in ARS(ICR)f Swiss albino mice treated by intraperitoneal injection on days 6-17 of pregnancy. The mean crown-rump length of the offspring treated with tobacco smoke during the same period of gestation similarly was not significantly different from that of its sham control. Fetuses prenatally exposed to both ethanol and tobacco smoke, however, had a highly significant reduction in weight and length. Treatment with ethanol resulted in 31 percent resorptions; tobacco smoke had no significant effect on mortality, with only five percent resorptions; and the combined treatment resulted in a highly significant resorption frequency of 67 percent.

From these data, it appears that the effects of ethanol on viability are potentiated by treatment with tobacco smoke. Furthermore, tobacco smoke plus ethanol treatment produced an effect on prenatal growth that was not produced by ethanol or tobacco smoke alone.

Although studies in humans and laboratory animals have shown that ethanol is teratogenic, results from the present study suggest that Swiss albino mice may not be as sensitive to ethanol induced teratogenesis. These findings are supported by a recent study on strain sensitivity to ethanol (GIKNIS & DAMJANOV 1980). Because the frequency of malformations (cleft palate) in the ethanol-tobacco smoke treatment group was not higher than that of the ethanol treatment group, it appears that tobacco smoke does not potentiate the teratogenic effects of ethanol. However, since the percentage of resorptions was so high for the combined treatment group, the possibility exists that in this group malformations were severe enough to frequently cause death.

The high resorption frequency and growth retardation observed among fetuses of the combined treatment group indicate that ethanol and tobacco smoke may interact to produce fetotoxicity. BROWN et al. (1979) suggest that ethanol retards fetal growth by inhibiting cell proliferation. Tobacco smoke lowers the amount of oxygen available for fetal glucose metabolism, thus decreasing the amount of available energy needed for embryonic growth and differentiation (ROBKIN & COCKROFT 1978). Thus through different but related mechanisms, it is possible that smoking potentiates the effects of ethanol on intrauterine growth and viability.

Nutrition is another factor that influences fetal growth. Malnourishment has been identified as a cause of low birth weight in laboratory animals and in humans. In the present study, animals treated with ethanol or smoke plus ethanol were comatose for nearly four hours. During this time while ethanol treated animals were not able to consume food and water, control animals were. Thus the mice exposed to ethanol may have been undernourished relative to their controls. SHOEMAKER et al. (1980) have shown that ethanol treated animals receiving adequate nourishment do not produce low birth weight fetuses but do have

high rates of fetal mortality. It is possible that in the present study, mice exposed to ethanol alone were not as severely malnourished compared to mice treated with smoke plus ethanol. When ethanol was administered in combination with tobacco smoke, the animals desire for food or ability to eat may have been suppressed. In connection with a nutritional effect on the fetus, another factor that merits consideration is the effect of fasting or undernourishment on blood alcohol levels. A study by WIENER (1980) indicates that blood alcohol levels are higher in fasted or undernourished animals than in adequately nourished animals. The growth retardation effects observed in the present study may be due to malnourishment, causing higher than usual blood alcohol levels in animals pretreated with tobacco smoke. Experiments are presently being conducted in which all treated and control mice are pair-fed to control for nutritional changes; furthermore, blood alcohol levels of pregnant mice receiving various doses of ethanol are being monitored to ascertain dose-response effects.

In conclusion, the present study provides evidence, albeit inconclusive, that ethanol and tobacco smoke interact to cause intrauterine growth retardation and fetal mortality. The teratogenic effects of ethanol in this strain of mice did not appear to be potentiated by tobacco smoke. This study provides an animal model in which other chemicals such as caffeine, diazepam and marijuana may be studied in combination with ethanol or tobacco smoke to test for possible synergistic effects on teratogenicity and general fetotoxicity.

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