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Aspirin Dose-Dependently Reduces Alcohol-Induced Birth Defects and Prostaglandin E Levels in Mice

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ABSTRACT The purpose of the present study was threefold. The first purpose was to determine if aspirin (ASA) decreases alcohol-induced birth defects in mice in a dose-dependent fashion. The second purpose was to see if the antagonism of alcohol-induced birth defects afforded by ASA pretreatment was related to dose-dependent decreases in prostaglandin E (PGE) levels in uterine/embryo tissue. The third purpose was to determine if ASA pretreatment altered maternal blood alcohol level. In experiments 1 and 2, pregnant C57BL/6J mice were administered ASA (0, 18.75, 37.5, 75, 150, or 300 mg/kg) on gestation day 10. One hour following the subcutaneous injection of ASA, mice received alcohol (5.8 g/kg) or an isocaloric sucrose solution intragastrically. In experiment 1 the incidence of birth defects was assessed in fetuses delivered by caesarean section on gestation day 19. In experiment 2 uterine/embryo tissue samples were collected on gestation day 10 1 hr following alcohol intubation for subsequent PGE analysis. In experiment 3 blood samples were taken at five time points following alcohol intubation from separate groups of alcohol-treated pregnant mice pretreated with 150 mg/kg ASA or vehicle. The results from the three experiments indicated that 1) ASA dose-dependently reduced the frequency of alcohol-induced birth defects in fetuses examined at gestation day 19, (2) ASA decreased the levels of PGE in gestation day 10 uterine/embryo tissue in a similar dose-dependent fashion, and 3) ASA pretreatment did not significantly influence maternal blood alcohol levels. These results provide additional support for the hypothesis that PGs may play an important role in mediating the teratogenic actions of alcohol.

During the last decade, mouse models have been used frequently to investigate issues related to the teratogenic actions of alcohol. Because of the rigorous experimental control that can be applied to the testing situation, it has been possible to conclude that alcohol is, in fact, a teratogen in mice (Chernoff, '77; Randall and Taylor, '79), that the effect is dose-related (Lochry et al., '82), and that acute alcohol administration on a single day of pregnancy is sufficient to produce offspring with birth defects (Webster et al., '80; Sulik et al., '81; Randall and Anton, '84). As with other classic teratogenic agents, the type of defect produced by acute alcohol administration depends upon the stage during pregnancy when exposure occurs (Webster et al., '83). The mechanism of action for the teratogenic actions of alcohol remains to be determined. Given that the mouse demonstrates both morphological and behavioral (Becker and Randall, '89) anomalies following maternal alcohol administration, it has the potential for being an excellent animal model to investigate the underlying mechanism(s) for many of the deleterious effects of prenatal alcohol exposure.

Our laboratory has chosen to focus initially on the etiology of alcohol-induced

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birth defects in C57BL/6J mice, since this strain has been shown to be sensitive to the teratogenic effects of alcohol (Randall and Taylor, '79; Sulik et al., '81). Although there are a host of possible mechanisms of action (e.g., Randall et al., '90; Schenker et al., '90), the mechanism of interest to our laboratory is possible alcohol-induced alterations of maternal and/or fetal prostaglandins (Randall and Anton, '84; Randall et al., '87a; Anton et al., '90). The reason for focusing on prostaglandins (PGs) is that PGs are critically involved in all stages of pregnancy from implantation through the initiation of labor (Keirse, '78). Some prostaglandins are vasoactive and regulate placental blood flow (Mäkilä et al., '86; Parisi and Walsh, '86) while others have been implicated in developmental processes (Wickremasinghe, '88). The particular prostaglandin of interest in this study is PGE. The reason for initially concentrating on PGE is that exogenous administration of PGE has been shown to cross the placenta (Persaud and Clancy, '74) and to be teratogenic in mice (Persaud, '78). Additionally, acute alcohol administration has been shown to elevate PGE levels in several tissues, including mouse reproductive tissue (Anton et al., '90). We hypothesize that elevated PGE levels may be an etiologic mechanism in the production of alcohol-induced birth defects. Hence, blockade of PG synthesis in general and PGE synthesis in particular, might protect against fetal insult that follows acute alcohol exposure.

The present study represents a follow-up to our previously published reports demonstrating a decrease in alcohol-induced birth defects in C57BL/6J mice given PG synthesis inhibitors (Randall and Anton, '84; Randall et al., '87a). Although the results of these studies led to the speculation that PGs may represent an underlying mechanism of alcohol-induced birth defects (Randall et al., '87b), these initial studies are limited in interpretation. Before any specific conclusions can be drawn from them for a role of PGs in mediating the teratogenic actions of alcohol, it is necessary to determine if the protective effect of ASA against alcohol-induced birth defects exists on a continuum from no-effect to complete blockade, to determine if there is a correlation between ASA protection and tissue PGE levels, and to rule out ASA-induced decreases in maternal blood alcohol level as an explanation for the protective effect of ASA. The purpose of the present three experiments in this study, therefore, was to extend our previously published results by addressing these important issues.

MATERIALS AND METHODS

Experiment 1

Animals and breeding

Female C57BL/6J mice 10–14 weeks of age weighing approximately 20 g at the time of breeding (Jackson Laboratories, Bar Harbor, ME) were maintained in temperature and humidity controlled rooms on a 12 hr light/dark cycle (lights on at 0600 hr) with free access to standard laboratory chow and water. The mice were housed prior to breeding in groups of five in polypropylene cages with stainless steel lids and beta-chip bedding. Females were time-bred from 0900–1100 hr and checked for vaginal plugs. Successfully mated females were weighed, individually housed, and randomly assigned to a treatment group. Each female was given a code number.

Procedure

On gestation day 10 (plug day referred to as gestation day 1) at 1300 hr, each mouse received a subcutaneous injection of aspirin (acetylsalicyclic acid, ASA) in one of six doses: 0, 18.75, 37.5, 75, 150, or 300 mg/kg. These injections were given in a constant volume of .02 ml/g. One hour following the s.c. injection of ASA (or the buffered vehicle), half of the mice in each ASA group received alcohol (5.8 g/kg, .03 ml/g) and the other half served as controls, receiving an equal volume of isocaloric sucrose solution intragastrically. Thus, the experimental design resulted in a total of 12 independent groups made up of 9–16 litters/group. Blood samples (30–50 μl) were drawn from the orbital sinus from all subjects 60 min following alcohol (or control) intubation for the purpose of blood alcohol analysis. Samples were taken from the controls, as well, in order to equate stress related to the blood collection procedure across groups. The mice were returned to their individual cages and then left undisturbed until gestation day 19.

Teratologic examination

On gestation day 19, pregnant females were weighed and then sacrificed. The uterine horns were exposed, and a count was made of viable, dead, and resorbed fetuses. Fetuses were removed and weighed to the

nearest .001 g before being stored individually in vials of Bouin's fixative for subsequent freehand teratologic evaluation using a modification of the method of Barrow and Taylor ('69). Group assignment was concealed from the examiner by assigning each fetus a code number. The code was not broken until all fetuses had been evaluated. Given that the primary types of soft tissue defects observed with acute alcohol exposure on gestation day 10 in C57BL/6J mice involve limbs and kidneys (Randall and Anton, '84), these were the only types of defects calculated. The dependent variables, thus, were prenatal mortality, litter weight, and frequency of limb/kidney anomalies.

Drug preparation

Aspirin (ASA) was purchased from Sigma Chemical Co. (St. Louis, MO). A solution of ASA was prepared daily by dissolving 375 mg ASA in 25 ml of 1% sodium bicarbonate buffer (300 mg/kg dose). Appropriate dilutions of this stock solution yielded the other doses. The buffer solution (pH 8.2) was prepared weekly and stored under refrigeration. The alcohol solution was prepared by diluting 6.25 ml of 95% ethanol in 25 ml of saline. The control intubation solution was prepared every other day by dissolving 8.5 g sucrose in 25 ml of saline and slightly warming the solution.

Experiment 2

Procedure

The procedure was similar to that reported for experiment 1 except that the pregnant mice were sacrificed 1 hr following alcohol (or control) intubation on gestation day 10 instead of being sacrificed near-term on gestation day 19. Immediately following maternal sacrifice, the uterine horns were exposed, and a section of uterus containing three embryos was removed, rinsed in cold saline, and weighed. The tissue sample was frozen in liquid nitrogen and stored in a −70°C freezer for subsequent radioimmunoassay (RIA) for PGE within 2 min from the time of maternal sacrifice. Only one tissue sample was taken from each pregnant female. This procedure is similar to that described previously (Anton et al., '90).

PGE radioimmunoassay measurement

Uterine/embryo tissue samples were removed from the −70°C freezer and placed in an ice bath. Phosphate-buffered saline (PBS, pH 7.4) was added using a tissue dilution factor of 2 μl PBS/mg tissue. The sample was sonicated in ice until homogeneity was apparent. A 50 μl aliquot of homogenate was placed in individual 12 × 75 polypropylene tubes in an ice bath. The tissue aliquot was vortexed with recovery counts (2,000 cpm of ^{3}H-PGE$_2$/50 μl, 200 Ci/mmol; New England Nuclear, DuPont) and buffer (120 μl) for a total volume of 220 μl. Samples were precipitated with 1 ml absolute alcohol and centrifuged at 900*g* (Sorvall RT6000B, DuPont, Wilmington, DE) for 15 min at 4°C. After centrifugation, 1 ml of ethanol supernatant was removed and placed in a fresh tube for dry down. The remaining pelleted tissue was saved and later assayed for protein content (Smith et al., '85). Ethanol was evaporated from the sample tubes by centrifugation under negative pressure. The samples were reconstituted in 1 ml PBS (pH 7.4), vortexed, and then acidified (pH 3.6) with 2.7% formic acid. To each sample tube, 3 ml of ethyl acetate was added, automatically shaken for 10 min, and centrifuged (700*g*) for 10 min at room temperature. The aqueous layer was frozen in liquid nitrogen. The ethyl acetate layer was decanted into a fresh tube and was evaporated under negative pressure. The tubes were then frozen under nitrogen at −70°C until RIA measurement of PGE.

The RIA for PGE was conducted as previously described (Anton et al., '88, '90). The antibody (Advanced Magnetics, Inc., Cambridge, MA) is highly specific for PGE, but it cross reacts with PGE$_1$ and PGE$_2$. Thus, the values are expressed as PGE. Sensitivity is 10 pg/tube, with intra- and inter-assay coefficients of variation of 4.8% and 12.7%, respectively. All sample PGE levels are the mean of duplicates. Procedure blanks were assayed with the samples. Extraction recoveries were determined for each sample (70%) and blank (75%). Sample PGE levels were corrected for recovery and blank levels were subtracted. PGE is expressed as pg/mg protein.

Experiment 3

Procedure

Twenty groups of mice derived from the factorial combination of 2 alcohol doses (5.8 g/kg alcohol vs. isocaloric control) × 2 ASA doses (150 mg/kg ASA vs. vehicle) × 5 time points (15, 30, 60, 120, and 240 min follow-

ing alcohol intubation) were used to assess whether ASA influences peak maternal blood alcohol level or alcohol disappearance from blood. The 150 mg/kg dose of ASA was chosen on the basis of previous work (Randall and Anton, '84) which indicated that this dose reliably reduced the incidence of alcohol-induced birth defects. Moreover, data (not presented) from experiment 1 revealed no significant effect of the different ASA doses on 60-min maternal blood alcohol level.

Blood alcohol analysis

On gestation day 10 maternal trunk blood was collected at the appropriate time following alcohol administration. Blood alcohol concentration was determined using an enzymatic assay that involves the conversion of ethanol to acetaldehyde. This reaction is catalyzed by the enzyme alcohol dehydrogenase (ADH). The cofactor, NAD is reduced stoichiometrically during the reaction to NADH, which is detectable by UV spectrophotometry.

Whole blood samples (10 μl) were diluted 1:50 in 3.4% perchloric acid, vortexed, and centrifuged (2,000 rpm) for 10 min. The supernatant (100 μl) was added to 2.6 ml Tris buffer (0.5 M, pH 8.9) containing NAD (1.083 mg/ml; Sigma Chemical Co.) and ADH (5.19 μl/ml; Sigma Chemical Co.). The samples were vortexed, incubated at room temperature for 40 min, and, then, spectrophotometrically assayed at 340 nm. Blood alcohol levels are expressed as mg/dl (mg%).

Data analysis

The effects of aspirin and/or alcohol treatment on pregnancy outcome variables (prenatal mortality, fetal weight, and incidence of birth defects), PGE levels in uterine/embryo tissue, and blood alcohol levels as a function of time following alcohol administration were analyzed by analysis of variance (ANOVA). Subsequent individual group comparisons were performed using Fisher's Least Significant Difference (LSD) test. Trend analyses were performed on ASA dose for the appropriate dependent variables in order to better describe the dose-related function. Data analyses on fetal weight and incidence of malformations were restricted to dams that produced at least four viable fetuses. In addition, fetal weight and malformation rate were expressed in terms of litter means. Thus, for these variables, the litter was used as the unit of analysis, rather than the individual pups (Abbey and Howard, '73). The confidence limit for all analyses was set at .05.

RESULTS

Experiment 1

Prenatal mortality

Prenatal mortality (expressed as a percent of the total number of implants) consisted primarily of resorption sites observed at the time of C-section. A total of four fetuses were dead. Two of these were in the 18.75 mg/kg ASA-alcohol group, one in the 300 mg/kg ASA-alcohol group, and one in the 37.5 ASA-isocaloric sucrose control group. Analysis of variance revealed that neither alcohol, nor ASA, nor the combination of the two drug treatments significantly influenced the incidence of prenatal mortality in comparison to control levels. The mean ± S.E. prenatal mortality rate for 0.0 mg/kg ASA-isocaloric sucrose control group was 12% ± 2%, while the 0.0 mg/kg ASA-alcohol group evidenced a prenatal mortality rate of 15% ± 6%. The number of implantation sites (i.e., viable and dead fetuses plus resorption sites) did not differ among groups. Implants averaged between 8 and 9 for each litter.

Litter weight

Litter weight as a function of alcohol treatment and ASA dose is illustrated in Figure 1. A two-way ANOVA revealed a significant main effect of alcohol ($F(1,96) = 223.54$, $P < .001$) and an alcohol × ASA dose interaction ($F(5,96) = 3.68$, $P < .004$). Decomposition of the latter term indicated that the 150 mg/kg ASA-alcohol and the 300 mg/kg ASA alcohol groups differed significantly from the 0.0, 18.75, and 37.5 mg/kg ASA-alcohol groups ($P < .05$). The 75 mg/kg ASA dose produced a marginal effect, not significantly differing from the vehicle condition or the 150 and 300 mg/kg ASA-alcohol groups. Thus, maternal alcohol treatment decreased litter weight. ASA pretreatment significantly antagonized the alcohol-induced reduction in fetal weight at the two highest doses employed.

Malformations

Malformation rate was expressed as a percentage of the number of viable fetuses in each litter with either a limb or kidney

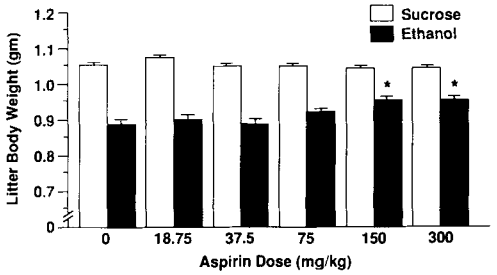

Fig. 1. Mean (±SE) litter body weight as a function of maternal alcohol treatment and aspirin dose. Values represent 9–16 litters/group and are averaged across litters for each group. *Differs from 0 mg/kg ASA-alcohol group ($P < .05$).

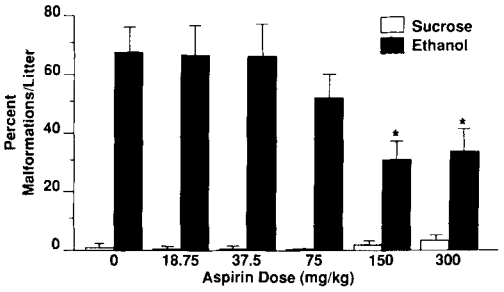

Fig. 2. Mean (±SE) percentage of fetuses/litter with either limb or kidney defects as a function of maternal alcohol treatment and aspirin dose (N = 9–16 litters/group). Those fetuses with *both* limb and kidney defects were only counted as a single malformed pup. *Differs from 0 mg/kg ASA-alcohol-treated group ($P < .05$).

defect. Fetuses with both types of defects were only counted once. As shown in Figure 2, litters treated with alcohol had significantly more malformed fetuses than control litters ($F(1,96) = 180.43, P < .0001$). Moreover, this effect of alcohol reliably varied as a function of ASA pretreatment ($F(5,96) = 3.77, P < .004$). That is, ASA significantly reduced the incidence of alcohol-induced birth defects in a dose-dependent fashion. The 75 mg/kg dose of ASA offered marginal protection while the 150 and 300 mg/kg ASA doses significantly reduced the teratogenic actions of alcohol. This was further supported by a trend analysis, which indicated that the data best fit a linear model ($F(1,48) = 14.97, P < .01$). Limb defects were limited primarily to the right forelimb and included missing digits (usually 4th or 5th), fused digits, or splayed digits. Renal defects observed were primarily hydronephrosis and hydroureter. Both right and left kidneys were equally affected. The relatively low incidence of malformations observed in the control groups (representing spontaneous anomalies) were due to renal anomalies (no limb defects were observed in the sucrose-treated groups).

Experiment 2

PGE levels

Figure 3 illustrates PGE levels in uterine/embryo tissue as a percent of baseline. As expected, ASA significantly reduced PG levels at all doses and in all groups ($F(5,72) = 122.73, P < .001$). Moreover, the percent reduction from baseline was dose-dependent. That is, the 75 mg/kg ($P < .05$) and the 150 and 300 mg/kg ($P < .001$) doses of ASA produced significantly greater reductions in PGE levels in comparison to the lowest dose of ASA (18.75 mg/kg). Alcohol treatment did not significantly influence the ASA-induced reduction in PGE levels.

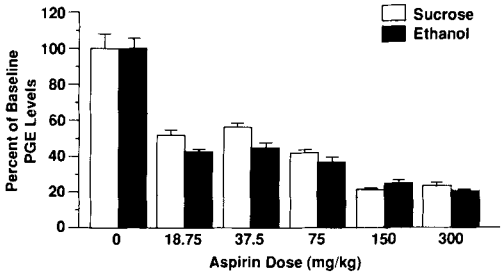

Fig. 3. Mean (±SE) PGE levels in uterine/embryo tissue as a percent of the baseline 0 mg/kg aspirin dose. Data is presented as a function of maternal alcohol treatment and aspirin dose (N = 7–11 litters represented/group). The 75, 150, and 300 mg/kg ASA groups differed significantly ($P < .05$) from the 0.0 and 18.75 mg/kg groups.

The dose-response function for ASA-induced inhibition of uterine/embryo PGE levels and ASA-induced reduction in the rate of birth defects was very similar (Fig. 4). That is, the greater the degree to which ASA inhibited PGE levels in uterine/embryo tissue, the greater its protective effects against alcohol-induced birth defects. As depicted in Figure 5, this positive correlation was highly significant ($r^2 = .96, P < .01$).

Experiment 3

Maternal blood alcohol level

Following an absorption phase, maternal blood alcohol concentration significantly de-

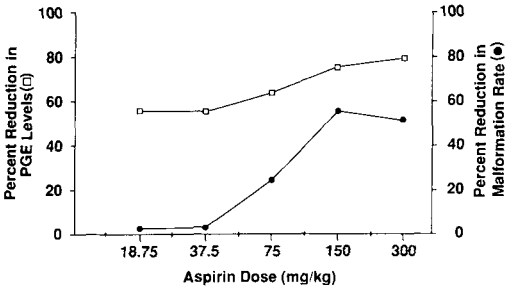

Fig. 4. Percent reduction from baseline (0 mg/kg aspirin dose) in uterine/embryo PGE levels and incidence of birth defects, as a function of ASA dose. All data are derived from alcohol-treated animals only.

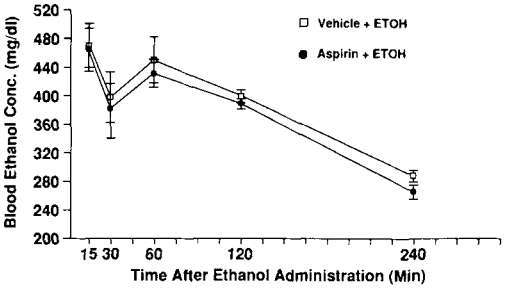

Fig. 6. Mean (±SE) blood ethanol concentrations as a function of aspirin treatment condition and time after alcohol intubation (N = 9–10 mothers/group).

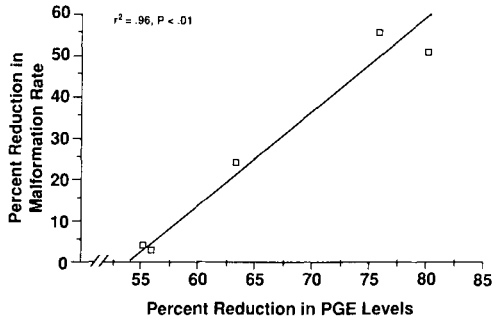

Fig. 5. Regression plot of aspirin-induced inhibition of PGE levels in uterine/embryo tissue against aspirin-induced reductions in the incidence of birth defects. All data are derived from alcohol-treated animals only.

creased in a linear fashion as a function of time ($F(4,79) = 18.99, P < .001$). Moreover, as can be seen in Figure 6, 150 mg/kg ASA did not significantly influence blood alcohol concentration at any time point in comparison to the vehicle controls. Therefore, these data suggest that the protective action against alcohol-induced birth defects afforded by pretreatment with ASA is not due to an alteration in the observed peak alcohol concentration or the elimination of alcohol from maternal blood.

DISCUSSION

As expected, acute alcohol administration (5.8 g/kg) on gestation day 10 produced deleterious effects on fetal growth and morphologic development in C57BL/6J mice (e.g., Randall and Anton, '84). That is, alcohol significantly reduced fetal body weight and increased the incidence of limb and kidney anomalies. Of particular significance is the fact that aspirin pretreatment reduced the incidence of alcohol-induced birth defects in a dose-related fashion. These data confirm and extend our previous report in which a single dose of 150 mg/kg ASA was found to reduce alcohol-induced birth defects by approximately 50% (Randall and Anton, '84). In addition, it is worth noting that the 300 mg/kg dose of ASA did not offer any further decrease in the teratogenic effect of alcohol in comparison to the 150 mg/kg dose of the drug. This suggests that while aspirin pretreatment markedly *reduces* the risk from prenatal alcohol exposure, it does not *prevent* alcohol-induced birth defects from occurring.

Aspirin pretreatment also significantly attenuated the alcohol-induced decrease in fetal body weight in a similar dose-dependent fashion. We have observed this significant attenuation previously (Randall et al., '87a). In our initial study (Randall and Anton, '84) the effect was in a similar direction but did not reach statistical significance. Taken together, it appears that ASA exerts ameliorating effects on alcohol-induced growth retardation, although the effect is not as reliable or robust as the antagonism of alcohol-induced dysmorphogenesis.

It is important to recognize that while high doses of ASA have been shown to be teratogenic in rodents in vivo (Larsson et al., '63) and in vitro (Greenaway et al., '82), none of the doses of ASA employed in the present study had a negative impact on pregnancy outcome. Thus, the protection against alcohol teratogenesis afforded by ASA pretreatment occurred at doses of the drug that did not, by themselves, produce deleterious effects on fetal growth and development.

Exactly how ASA reduces the incidence of

alcohol-induced birth defects is an important question. One mechanism by which ASA might attenuate the teratogenic actions of alcohol is by altering the circulating levels of alcohol in maternal blood. However, results from the present study demonstrated that ASA pretreatment did not significantly influence observed peak blood alcohol levels in the dams or the disappearance of alcohol from maternal blood. Thus, the prophylactic action of ASA against alcohol teratogenesis cannot be attributed simply to a pharmacokinetic interaction with alcohol on the maternal side.

An alternative mechanism by which ASA may reduce the embryotoxic effects of alcohol may be through its action on prostaglandins. As mentioned earlier, ASA inhibits PG synthesis by time-dependent irreversible inhibition of cyclooxygenase enzyme (Lands, '81). Given the demonstrated reduction of alcohol-induced birth defects by ASA, along with the fact that PGs are of crucial importance for normal fetal growth and development (Persaud, '78), it was hypothesized that alcohol-induced perturbation of the PG system may represent an important etiologic factor in the teratogenic actions of alcohol. Further evidence supportive of this hypothesis is the fact that another PG synthesis inhibitor, indomethacin, has been shown to similarly reduce the dysmorphogenic actions of alcohol in mice (Randall et al., '87b), chicks (Pennington et al., '85) and the suppression of fetal breathing movement in sheep (Smith et al., '90). Moreover, abnormally high levels of PGs have been shown to be teratogenic in rodents (Persaud, '75) and acute alcohol treatment has been demonstrated to elevate PG levels in mouse reproductive tissue (Anton et al., '90).

Additional and more direct supportive evidence for this PG hypothesis may be derived from the results of the present study in which ASA was shown to dose-dependently inhibit PGE production in gestation day 10 uterine/embryo tissue. Of particular significance is the fact that this dose-related inhibition of PGE levels in uterine/embryo tissue mirrored the dose-response function for the protective effects of ASA against alcohol-induced birth defects. This is most clearly depicted by the highly significant positive correlation between the magnitude of ASA-induced inhibition of PGE levels in uterine/embryo tissue and the extent to which ASA is effective in reducing the incidence of alcohol-induced birth defects (Fig. 5). Interestingly, the data suggest that ASA was ineffective in attenuating alcohol-induced dysmorphogenesis at doses that produced as much as a 60% reduction in PGE levels. Thus, it would appear that an additional 20% reduction in uterine/embryo PGE levels (produced by the 150 and 300 mg/kg ASA doses) is necessary for a protective effect of ASA to be realized. Why this is true is not clear at this time. Future studies examining other cyclooxygenase inhibitors will be needed to further elucidate this relationship between degree of PG inhibition in uterine/embryo tissue and protection against alcohol teratogenesis. In addition, other cyclooxygenase end products, such as thromboxane and prostacyclin, will need to be measured to ascertain any differential sensitivity of their production to ASA and alcohol exposure.

The site at which aspirin interacts with alcohol to produce its ameliorating effects is unclear at present. The fact that ASA was found to be much more efficacious than indomethacin in reducing alcohol-induced birth defects (Randall et al., '87a), along with the fact that ASA crosses the placenta much more readily than indomethacin during the early stages of gestation (Klein et al., '81; Randall et al., '87a), suggests that PG synthesis inhibition must occur on the fetal side of the placenta in order to observe significant protective effects against alcohol teratogenesis. On the other hand, we have previously reported that "naked" C57BL/6J day 10 embryos (divested of all surrounding membranes) were *in*capable of producing or metabolizing PGs (Anton et al., '88). Therefore, it is possible that ASA produces its effects at the level of the placental and extra-embryonic tissues including embryonic membranes, and placental vessels, all of which have a high capacity for PG production (Mitchell, '86). Clearly, additional research is required to more specifically identify the site of action.

Finally, it is possible that aspirin's prophylactic action against alcohol-induced dysmorphogenesis is mediated through prostanoids other than prostaglandins (e.g., thromboxane, prostacyclin). These prostanoids are particularly vasoactive and have an important role in controlling blood flow in intrauterine tissues (cf. Ylikorkala and Mäkilä, '85), since umbilical and placental

vessels are not under neural control (Reilly and Russe, '77). The fact that low concentrations of alcohol have been shown to constrict and produce spasms in umbilical vessels (Altura et al., '83; Savoy-Moore et al., '89) suggests that alcohol's effects on these vasoactive prostaglandins may contribute to its fetotoxic effects. Aspirin pretreatment then, might act to mitigate this action of alcohol. Support for this contention may be derived from studies that have demonstrated that inhibition of cyclooxygenase activity provides relief from constriction of umbilical vessels (MacLennan et al., '88). The role of thromboxane and prostacyclin in mediating the teratogenic actions of alcohol as well as the protective effects of aspirin in our model system remains to be determined, but certainly warrants further investigation.

In summary, results from this study have demonstrated that 1) aspirin pretreatment dose-dependently reduces the incidence of alcohol-induced birth defects, 2) the protective effects of ASA against alcohol teratogenesis is not related to an effect on observed peak blood alcohol levels or disappearance of alcohol from maternal blood, 3) ASA dose-dependently inhibited PGE levels in uterine/embryo tissue, and 4) the magnitude of PGE inhibition in uterine/embryo tissue by ASA is positively correlated with the extent to which ASA reduced the incidence of alcohol-induced birth defects. Taken together, these results provide additional support for the hypothesis that alcohol-induced alterations in the prostaglandin system may represent an important etiologic factor in the teratogenic actions of alcohol.

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