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Effects of Acetylsalicylic Acid on Ethanol – Induced Intrauterine Fetal Growth Restriction in Rats

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ABSTRACT

The effects of acetylsalicylic acid (ASA) on intrauterine growth restriction induced by maternal ethanol consumption were studied. A single intraperitoneal dose of ethanol (2.96 g/kg body weight) injected in the 10th day of pregnancy (PD10), significantly reduced maternal weight gain, fetal weight, amniotic fluid volume and umbilical cord length. Meanwhile, ethanol did not affect placental weight or litter size. A single intraperioneal dose of ASA (200 mg/g body weight). Injected on the PD10, did not modify maternal body weight gain, litter size, fetal and placental weight and amniotic fluid volume, but significantly diminished umbilical cord length. Intraperitoneal injections of ASA (200 mg/g body weight) and ethanol (2.96 g/kg length weight) on PD10. demonstrated that ASA antagonized in part at least, the effects of ethanol on maternal weight gain, fetal weight and amniotic fluid volume. Contrarily, ASA potentiated the effect of ethanol on umbilical cord length.

تأثيرات الاسبرين (Acetylsalicylic acid) على تحديد النمو الجنيني المحدث داخل رحم الجرذان الحوامل بواسطة الايثانول

الملخص

تم دراسة تأثيرات الاسبرين (اسيتايل ساليسيلك اسد) على محددات النمو المحدثة بواسـطة تجريـع الفئران الحوامل بمادة الايثانول. جرعة واحدة من الايثانول (2.96 غم/كغم وزن الجسم) اعطيـت مـن خلال زرقها تحت غشاء الجنب في اليوم العاشر من الحمل، ادى ذلك الى نقصان واضـح فـي الـوزن المكتسب ووزن الجنين، حجم السائل الامنيوتي وطول الحبل السري. وبنفس الوقت الايثانول لم يؤثر على وزن المشيمة ولاعلى حجم الولادات. جرعة واحدة من الاسبرين في اليوم العاشر من الحمل لم تغير في الوزن المضاف للامهات الحوامل ولا في حجم الولادات وكذلك لم تغير في اوزان الاجنة والمـشيمة ولا في حجم السائل الامنيوتي، ولكن هذه الجرعة تمحي بصورة واضحة النقصان في طول الحبل السري.

ان اعطاء جرع تحت غشاء الجنب لكل من الاسبرين (200 ملغم/غم من وزن الجسم) والايثـانول (2.96 غم/كغم من وزن الجسم) في اليوم العاشر من الحمل يوضح ان الاسبرين ضاد جزئيا على الاقـل من تأثيرات الايثانول على الوزن المضاف للجرذان الحوامل ووزن الاجنة وحجـم الـسائل الامنيـوتي. وعلى العكس تماما نجد ان الاسبرين يمحو بقوة تأثير الايثانول على طول الحبل السري.

INTRODUCTION

It is well known that high doses of ethanol administered during pregnancy results in adverse pregnancy outcome. The manifestations of prenatal ethanol exposure range from obvious marked growth deficiency and organ malformations to more subtle effects on central nervous system (CNS) development and abnormal function. These developmental alterations, appear at birth, characterized by growth retardation and severely compromised mental capabilities, collectively have been labeled fetal alcohol syndrome (FAS) (Jones and Smith, 1973).

Jollie (1990) sustained that prostaglandins (PG) may be involved in the etiology of FAS, due to the stimulating action of ethanol on PG levels. Accordingly, drugs which inhibit PG synthesis, as acetylsalicylic acid (ASA), could have a protecting action on ethanol effects (Randall et al., 1991a).

The purpose of this study is to assess the possible protective effect of ASA on the harmful action of ethanol on intrauterine growth.

MATERIALS AND METHODS

Animals, chemicals and treatment: Virgin female rats (*Rattus norvegicus*), weighting between 200 and 250g, were used. After a period of two weeks of acclimatation, the animals were mated overnight with fertile males, and the day on which sperm was detected in the vaginal smear was designated pregnancy day 1 (PD1). Pregnant rats were kept individually in polypropylene cages throughout pregnancy under controlled conditions of light (12 h – light, 12 h – dark cycle), humidity (50 ± 10%) and temperature (22 ± 2C) and fed with a commercial pelleted rodent diet and tap water.

After 10 days, twenty pregnant rats were randomly distributed in the following experimental groups:

Group I : Five rats injected with a single intraperitoneal dose of 2.96g/kg weight of ethanol between the 9:00 and 10:00h.

Group II : Five rats injected with a single intraperitoneal dose of 200mg/kg body weight of Aspirin (ASA solution of 200mg/ml), between the 9:00 and 10:00h.

Group III : Five rats receiving a single intraperitoneal dose of 200mg/kg body weight of Aspirin between the 9:00 and 10:00h., and, after one hour, an intraperitoneal injection of 2.96 g/kg body weight of ethanol.

Group IV: Control group, constituted by five animals injected on the PD10 with an equivalent volume of sterile saline, between the 9:00 and 10:00h.

Morphmetrical determination: On the morning of the PD20, rats were killed by anesthetic ether inhalation. The uterine horns were examined for resorption sites, live and dead fetuses. Each fetus was scrutinized for external anomalies and then fixed in a solution of 85 ml 80 % alcohol, 10 ml formalin and 5 ml glacial acetic acid. After 24h of fixation, fetuses and placentas were blotted dry and weighed in precision scale and the umbilical cords were measured.

Statistical analysis: statistical analysis was performed using one – way analysis of variance (ANOVA). Where supported by significant treatment effects, comparisons among groups were performed using the Tukey's test (Sahai and Ageel, 2000).

RESULTS

The maternal body weight gain from the PD10 to the PD20 showed significant differences among the groups (Table 1). The intraperitoneal inection of ethanol (group I and III) significantly reduced the maternal weight gain in pregnant rats in comparison either to the control group or to those injected only with ASA (group II). Animals injected only with ethanol, on the other hand, showed smaller body weight gain than those animals treated with ASA plus ethanol. The litter size also showed differences among different groups (Table 1).

The fetal body weight showed significant differences among the groups (Table 1). fetuses belonging to the two groups injected with ethanol weighed less than those of the control group. Fetuses from pregnant rats injected only with ethanol were lighter than those from animals previously injected with ASA. On the other hand, fetal weight showed no differences between group II (ASA) and either group III (ASA plus ethanol) or IV (control).

The placental weight showed no significant differences among the four group (Table I).

The amniotic fluid showed significant differences among different groups (Table 1). Fetuses from both ethanol – injected groups (group I and II) had significantly smaller amniotic fluid volume than either the control fetuses or the ASA injected group fetuses. On the other hand, the amniotic fluid volume was significantly higher in the ASA plus ethanol – injected group than in that only injected with ethanol.

The length of the umbilical cord showed significant differences among different groups (Table 1). Fetuses from all the treated groups had significantly shorter umbilical cords than the control fetuses (Table 1). Similarity, umbilical cords were shorter in the ASA plus ethanol – injected group than in the ethanol – injected group. On the otherhand, no significant differences were found between umbilical cord length of fetuses from the group II (ASA) and those from both groups I (ethanol) and III (ASA plus ethanol).

DISCUSSION

Pregnant rats injected with ethanol showed a significantly smaller weight gain during the last 10 days of pregnancy than either the control animals or those injected only with ASA. However, no significant difference of maternal weight gain was observed between animals injected with ASA and the control ones. Beside, body weight gain was higher in the animals injected with ASA plus ethanol than in those injected only with ethanol. In this way, ASA demonstrated a partial protecting effect on the action of ethanol in the weight gain during pregnancy.

It is well known that malnutrition and undernourishment are associated with chronic consumption of ethanol. In theses cases, intrauterine growth retardation was attributed to a secondary effect of the nutritional deficit due to ethanol consumption. Abel (1978) claimed that the effect of chronic treatment with ethanol (1 or 2 g/kg body weight) throughout pregnancy, appeared to be related to the reduction of the maternal intake of calories rather than to a direct effect of ethanol on the fetus. In the present study, however, ethanol was administrated in a single dose, on the PD10, being harmed the interpretation that decreased weight would be owed to reduced ingestion of nutrients.

Warkany and Takacs (1959) demonstrated a loss between 15 and 25g in the body weight of pregnant rats injected with methyl slaicylate, but the animals recovered their

weight after 3 or 4 days. Esperidião et al. (1998) demonstrated that maternal weight gain was not affected in rats receiving up to 100 mg/kg ASA, via gavage, during the whole pregnancy.

Table 1: Mean (± S.D.) of the morphometric parameters of fetuses and adnexae of the ethanol, ASA, ASA plus ethanol and control group, ANOVA and Tukey's post–hoc test.

Parameter	Group I (ethanol)	Group II (ASA)	Group III (ASA plus ethanol)	Group IV (control)	F	P
Maternal weight gain (g)	41.2±4.8**	64.3±5.4**	45.2±3.3**+§§	63.0±2.6	4.52	<0.01
Litter (n)	11±1.3	14±1.3	13±1.5	13±1.0	2.30	ns
Fetal weight (g)	2.15±0.20**	2.40±0.15**	2.30±0.15**	2.42±0.28	4.45	<0.01
Palcenta weight (mg)	460±50	500±45	470±46	541±43	2.08	Ns
Amniotic fluid (ml)	0.8±0.1**	1.0±0.1**	0.9±0.1*+§	1.0±0.1	3.95	<0.05
Umbilical cord length (mm)	30±3**	29±3**	27±3**	37±4	3.58	<0.05

* $p < 0.05$ (comparison with group IV – ethanol) $p < 0.05$ (comparison with group I – ethanol) § $p < 0.05$ (comparison with group II – ASA)

** $p < 0.01$ (comparison with group IV – control) $p < 0.01$ (comparison I – ethanol) §§ $p < 0.01$ (comparison with group II – ASA).

In the present study, the litter size did not show any difference among groups, as previously reported in mice (Pilström and Kiessling, 1967; Randall et al., 1991a, b). Tze and Lee (1975), on the otherhand, litter size reduction was observed after in utero exposure to ethanol.

Lubawy and Garratt (1977), demonstrated an increased amount of resorptions after administration of ASA (125 or 250 mg/kg/day), between the PD8 and the PD20. Esperidião et al., after daily administration of ASA (100 to 400 mg/kg), during all the pregnancy, via gavages, found smaller litter size.

Analysis of the fetal weight data indicated that both experimental groups of pregnant rats injected with ethanol showed lighter fetuses. Meanwhile, fetuses from rats injected with ASA plus ethanol were heavier than those from rats injected only with ethanol. It is well known that prenatal exposure to ethanol, it directly acts on the embryogenesis, causing growth alterations, characterized mainly by retarded intrauterine growth (Randall et al., 1991a, b). After Wunderlich et al. (1979), ethanol inhibits the synthesis of the choronic somatomammotropin. ASA (150 or 300 mg/kg), administrated on the PD10, 1 h before ethanol (5.8 g/kg), showed a protective action on the reduction of the fetal weight caused by ethanol in mice (Randall et al., 1991a, b). Our results

demonstrated only a partial protective effect of ASA on fetal weight reduction caused by ethanol in rats.

According to Pennington et al. (1985), intrauterine growth retardation caused by ethanol is mediated by the increase of prostaglandin levels. Thus, the simultaneous administration of inhibitors of PG synthesis would protect against the ethanol – induced hypoplasia. Anton et al. (1990) and Cook and Randall (1997) demonstration that ethanol (6 g/kg body weight, on the PD17) induced premature labor in mice and that this action is mediated by PGE and PGF2. Pretreatment with ASA on the PD17, inhibited PG synthesis and blocked premature labor. The authors concluded that premature labor, could have important implications for the concept, as well as for the normal fetal growth and development.

After oral administration of 125 or 250 g/kg/day of ASA to pregnant rats between the PD8 and the PD20, Lubawy and Garrett demonstrated decrease of both weight and length. Similarly, daily doses of 100 or 400 mg/kg/day of ASA to pregnant rats significantly decreased the fetal weight (Esperidião et al.).

In the present work no significant effects on the placental weight was demonstrated, after ethanol, ASA or ASA plus ethanol administration. Wunderlich et al. did not observe difference in the placental weight of fetuses treated with ethanol. Even without affecting the placental weight, it is possible that ethanol acts on the fetal weight by decreasing the placental transfer capacity, inhibiting enzymes or hormones, as chorionic somatomammotropin.

There are no references about any toxic action of ASA on the placenta. Lubawy and Garrett, after oral ASA administration (125 or 250 g/kg/day), between the PD8 and the PD20, and Esperidião et al. with daily doses of 400 mg/kg ASA, during all the pregnancy, demonstrated decreased placental weight. On the otherhand, Hamed et al. (1994) claimed that ASA did not affect the placental weight, both in well fed fetuses and in not well nurtured fetuses, wherease Wallenburg and Rotmans (1987), administering low doses of ASA, prevented the idiopathic uteroplacental insufficiency. In the present study, it was verified that ethanol, administered only or together with ASA, caused a significant reduction of the amniotic fluid volume, in comparison to both the control group and the ASA injected group. Administration of ASA during pregnancy caused no significant alteration of the amniotic fluid volume. There were significant difference between the group injected only with ethanol and that injected with ASA plus ethanol, this shows that ASA possesses a partial protecting action against the effects of ethanol on the volume of amniotic fluid.

Randall et al. (1991b) demonstrated renal malformations in 45 % of the mouse fetus treated with ethanol on the PD10, while in animals receiving ASA 1h before ethanol, the incidence of renal malformations dropped to 29 %. Gage and Sulik (1991) demonstrated that IP administration of two ethanol doses (2.9 g/kg) on the PD9 caused anomalies on the mouse urinary tract, mainly hydronephrosis and hudroureter, in 40.7 % of the fetuses. Randall et al. (1991a) showed a high incidence of urinary malformations in fetuses of mice injected with ethanol on the PD10, and demonstrated a protective action of 150 or 300 mg/kg ASA, administered 1h before ethanol. The authors claimed that ASA pretreatment dramatically reduces the risks of the prenatal exposure to ethanol, even though it does not prevent the ethanol – induced congenital defects. On the otherhand, treatment with ASA or with ASA plus ethanol significantly reduces umbilical cord length

when compared with the control group. Similarly, fetuses from the group injected with ASA plus ethanol have shorter umbilical cords than fetuses from the group injected only with ethanol. Thus ASA acts synergically with ethanol in shortening the umbilical cord length.

In conclusion, ASA seems to reduce, in the pregnant rat, the deleterious effects of ethanol on some of the morphometric fetal parameters, confirming the results obtained by Randall et al. (1991a, b) in mice.

REFERENCES

Abel, E.L., 1978. Effects of Ethanol on Pregnant Rats and Their Offspring. Psychopharmacology, Vol. 57, pp.5-11.

Anton, R.F., Becker, H.C. and Randall, C.L., 1990. Ethanol Increases PGE and Thromboxane Production in Mouse Pregnant Uterine Tissue. Life Sci., Vol. 46, pp.1145-53.

Cook, J.L. and Randall, C.L., 1997. Early Onset of Parturition Induced by Acute Alcohol Exposure in C57BL/6J Mice: Role of Uterine PGE and PGF2 alpha. Repr. Fertile. Dev., Vol. 9, pp.815-23.

Esperidião, S., Ajeje, R., Kopelman, B.I., Simões, M.J., Evêncio – Neto, J. and Kulay, Jr.L., 1998. Acãorônica do ácido acetulsalicìlico na prenhez da rata. Rev. Bras. Ginec. Obstet., Vol. 20, pp.245-9.

Gage, J.C. and Sulik, K.K., 1991. Pathogenesis of Ethanol – Induced Hydronephorsis and Hydrourtere as Demonstrated Following in Vivo Exposure of Mouse Embryos. Teratology, Vol. 44, pp.299-312.

Hamed, M.R., Al – Assay, Y.S. and Ezzeldin, E., 1994. Influence of Protein Malnutrition on Tetratogenicity of Acetylsalicylic Acid in Rats. Hum. Exper. Toxicol., Vol. 13, pp.83-8.

Jollie, W.P., 1990. Effect of Sustained Dietary Ethanol on the Ultrastructure of the Visceral Yolk – Sac Placenta of the Rat. Tetraology, Vol. 42, pp.541-2.

Jones, K.L. and Smith, D.W., 1973. Recognition of the Fetal Alcohol Syndrome in Early Infancy. Lancet, Vol. 2, pp.999-1001.

Lubawy, W.C. and Garrett, R.J., 1977. Effects of Aspirin and Acetaminophen on Fetal and Placental Growth in Rats. J. Pharm. Sci., Vol. 66, pp.111-3.

Miller, M.W., Higginbottom, M.C. and Smith, D.W., 1981. Short Umbilical Cord: Its Origin and Relevance. Pediatrics, Vol. 67, pp.618-21.

Pennington, S., Allen, Z., Tunion, H., Farmer, P., Rowland, L. and Kalmus, G., 1985. Prostaglandin Synthesis Inhibitors Blocks Alcohol – Induced Fetal Hypoplasia. Alc. Clin. Exper. Res., Vol. 9, pp.433-7.

Pileström, L. and Keissling, K.H., 1967. Effects of Ethanol on the Growth and on the Liver and Brain Mitochondrial Functions of the Offspring of Rats. Acta. Pharmacol. Toxicol., Vol. 25, pp.255-32.

Randall, C.L., Anton, R.F., Becker, H.C., Hale, R.L. and Ekblad, U., 1991a. Aspirin Dose – Dependently Reduces Alcohol – Induced Birth Defects and Prostaglandin E Levels in Mice. Teratology, Vol. 44, pp.521-9.

Randall, C.L., Becker, H.C. and Anton, R.F., 1991b. Effects of Ibuprofern on Alcohol – Induced Teratogenesis in Mice. Alc. Clin. Res., Vol. 15, pp.673-7.

Sahai, H. and Ageel, M.I., 2000. The Analysis of Variance. Fixed, Random and Mixed Models. Boston, Birkhäuser.

Tze, W.J. and Lee, M., 1975. Adverse Effects of Maternal Alcohol Consumption on Pregnancy and Feotal Growth in Rats. Nature, Vol. 257, pp.479-80.

Wallenburg, H.C. and Rotmans, N., 1987. Prevention of Recurrent Fetal Growth Retardation by Low–Dose Aspirin and Dipyridamole. Amer. J. Obstet. Gyncol., Vol. 157, pp.1230-5.

Warkany, J. and Takacs, E., 1959. Experimental Production of Congenital Malformations in Rats by Salicylate Poisoning. Amer. J. Pathol., Vol. 35, pp.315-31.

Wunderlich, S.M. Surendrabaligga, B. and Munro, H.N., 1979. Rat Placental Protein Synthesis and Peptide Hormone Secretion in Relation to Malnutrition from Protein Deficiency on Alcohol Administration. J. Nutr. Vol. 109, pp.1534-42.