Bull. Environm. Contam. Toxicol. 22,175-181 (1979)

Toxic Effects of Cadmium and Amaranth on the Developing Hamster Embryo

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Until recently FD and C Red No. 2 or amaranth, the trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3, 6-disulphonic acid, was a widely used red coloring agent in foods and drugs. In 1976 the Food and Drug Administration barred its future use in foods, drugs and cosmetics based on the suspected carcinogenicity of this dye in rats (BOFFEY 1976). Data on the embryolethal and teratogenic effects of amaranth in rats, rabbits, mice and hamsters is equivocal. DRAKE (1975) summarized the results of the eleven different embryotoxicity studies in these animals. Amaranth was considered a teratogen in rats in only one of these reports (SHTENBERG and GRURILENTO 1970). In four of the studies amaranth produced an increased frequency of resorptions while no significant embryolethal or teratogenic effects were observed in the seven other studies (DRAKE 1975).

The administration of a water soluble salt of the environmental contaminant, cadmium, is known to damage the developing hamster embryo. The major areas affected include the head, ribs and limbs; the area damaged is determined by the time of maternal exposure to this metal (FERM and CARPENTER 1968, MULVIHILL et al. 1970, FERM 1971, GALE and FERM 1973).

The embryotoxic response produced by a single teratogen is often modified by the administration of combinations of two teratogens or a teratogen and a non-teratogen. For example, cadmium-induced cranial abnormalities are prevented by the simultaneous treatment with lead (FERM 1969). Also, treatment with the non-teratogen, zinc helps protect the embryo from the detrimental effects of mercury administration (GALE 1973). The purpose of the present study was to determine whether the maternal exposure to both amaranth and cadmium would alter the known embryotoxic effects of cadmium on the developing embryo.

MATERIALS AND METHODS

On the 8th gestation day, timed pregnant golden hamsters (Charles River Lakeview) were anesthetized with sodium pento-

0007-4861/79/0022-0175 $01.40

barbital. At that time ten females were injected with amaranth (IV, 100 mg/kg, KEK Laboratories, Inc.) and ten others received cadmium sulfate (IV, 2 mg/kg, Fisher Scientific Co.). Another ten females were administered a combination of amaranth (IV, 100 mg/kg) and cadmium sulfate (IV, 2 mg/kg) as two separate injections. Control animals received demineralized-distilled water alone (IV, 5 ml/kg). On the 15th gestation day all pregnant hamsters were killed by an overdose of ether. At that time the number of resorption sites in each uterus was recorded and all fetuses were examined for externally detectable congenital malformations. The fetuses from half of the females in each experimental group and the controls were fixed in Bouin's solution. Subsequently each fetus was dissected in order to study the incidence and types of internal malformations. The dissection protocol involved the removal of the mandible and tongue in order to observe the stage of palate development. The rest of the head was sectioned coronally with a razor blade and the resulting 1 mm sections studied with the stereomicroscope to detect abnormalities within the brain, eyes and nasal cavities. Next, removal of the ventral body wall allowed for the detection of malformations of the viscera of the thoracic and abdominal cavities. The remaining fetuses were fixed in 95% alcohol and subsequently stained by an alizarin red S technique which enabled a systematic search for defects in the developing skeletal system. Tables of binomial confidence limits (MAINLAND et al. 1965) were used to determine the statistical significance of the frequency of the different malformations. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The data on the embryocidal and teratogenic response resulting from exposure of pregnant hamsters to amaranth and cadmium alone and in combination is summarized in the two Tables. Table 1 includes the incidence of resorptions and externally detectable malformations. A resorption is an implantation site which contains a non-viable fetus. While the table indicates that amaranth alone is neither embryolethal nor teratogenic, all of the cadmium-induced embryotoxic effects are significantly greater than controls. For each externally detectable malformation a comparison was made between the cadmium-treated group and the cadmium and amaranth-exposed group in order to determine whether amaranth alters cadmium-induced damage. The brain malformation category was the only one in which a statistically significant difference was observed between the two treatment groups. The brain damage category included exencephaly and encephalocele while eye defects consisted of microphthalmia and a few cases of anophthalmia. Only a few internal malformations (cleft palate and hydrocephalus) were detected in this study and their incidence was not significantly greater than that of controls.

TABLE 1

EXTERNALLY DETECTABLE ABNORMALITIES

Treatment	Dose mg/kg	No. Females	No. Implantation Sites	No. Live Fetuses	Percent Resorption Sites[a]	Percent Abnormal Fetuses[b]	External Defects[b] Eye	Brain	Miscellaneous
Amaranth Alone	100	10	133	130	2	0	0	0	0
Cadmium Alone	2	10	127	83	<u>35</u>	<u>52</u>	<u>33</u>	<u>29</u>	<u>27[c]</u>
Cadmium + Amaranth	2 + 100	10	117	67	<u>43</u>	<u>55</u>	<u>15</u>	3*	<u>15[d]</u>
Water (control)	5 ml/kg	6	75	75	0	0	0	0	0

a % = No. ÷ No. implantation sites X 100
b % = No. ÷ No. live fetuses X 100
c abnormal tail (3); edema (4); cleft lip (6); limb defects (7); micrognathis (2)
d cleft lip (2); limb defects (8)
<u>Underlined %</u> = significantly different statistically from water controls
* % = significantly different statistically when compared with cadmium alone

TABLE 2

MALFORMATIONS WITHIN THE DEVELOPING SKELETAL SYSTEM

Treatment Dose(mg/kg)	No. of Fetuses	Percent of Fetuses with Abnormal Ossification of: Skull Bones	Hyoid Bone	Vertebral Column	Forelimb	Hindlimb	Sternum	Ribs	Extra Ribs
Amaranth (100)	73	0	0	7	0	47	26	1	41
Cadmium (2)	42	60	26	69	31	79	81	67	48
Cadmium (2) and Amaranth (100)	23	13*	4	35	17	39*	70	87	83
Water Controls (5ml/kg)	37	0	0	0	0	32	35	0	32

Underlined % = significantly different statistically from controls
* % = significantly different statistically when compared with cadmium alone

As indicated by Table 2 considerable skeletal system damage was detected in this study. In general the most severe and extensive damage occurred following exposure to cadmium alone while treatment with amaranth alone disrupted ossification to a minimal degree. When compared with the cadmium-treatment group, combined treatment with both cadmium and amaranth decreased the incidence of skeletal malformations to a statistically significant extent only in the skull and hindlimb bones.

The assessment of the degree of ossification of the skeletal system of the normal 15th gestaion day hamster fetus is based on previous studies (BEATTY and HILLEMANN 1950, GALE and FERM 1973, GALE 1975). The following ossification centers of the skull, all of which are well established by the 15th gestation day, were studied in this experiment; mandible, maxilla, premaxilla, nasal, frontal, parietal, squamosal, zygomatic, basioccipital, supraoccipital, exoccipital and interparietal. The most prevalent defect observed in the cadmium-treated group involved a poorly ossified interparietal bone alone or together with poor ossification of the parietal bones. The squamosal bones were also poorly developed in many fetuses. Each of the other ossification centers in the skull, except the occipital sites, were damaged in a few fetuses in the cadmium-treated group. Only three fetuses in the amaranth + cadmium group (13%) exhibited abnormal skull bones; one had a hole in the basioccipital, another had shortened premaxillae and maxillae and another exhibited poorly ossified premaxillae, maxillae, squamosals and the interparietal bone.

The hyoid bone is normally well ossified by the 15th gestation day. One fetus in the combined amaranth and cadmium treatment group had a poorly ossified hyoid while in 26% of the fetuses from the cadmium-treated animals, the hyoid was either absent or poorly developed.

The vertebral column of the hamster fetus contains 7 cervical, 13 thoracic, 6 lumbar, 4 sacral and caudal vertebrae. By the 15th gestation day most of these vertebrae contain 3 distinct ossification centers, i.e. one in the centrum and 2 in the developing vertebral arch. However, in the cervical region most of the centra are still cartilagenous and ossification in the caudal region is limited to the more cranial one or two vertebrae. In this study abnormal vertebral column ossification consisted of absent, distorted or poorly ossified centra and arches. The damage observed after amaranth treatment alone was minimal; only a few fetuses exhibited damaged thoracic vertebrae and sacral bodies. The most extensive damage was observed in the cadmium-treated fetuses i.e. the 3 ossification sites of the cervical, thoracic, lumbar, and sacral vertebrae were involved. In the combined treatment group vertebral malformations were observed only in the thoracic and sacral regions.

The sternum of the normal 15th gestation day hamster fetus contains established ossification sites within the manubrium and sternebrae 1, 2, and 5 while sternebrae 3 and 4 remain unossified (BEATTY and HILLEMANN 1950). While most of the control and amaranth-exposed fetuses conformed to this sternal ossification pattern, 35% and 26% of each group, respectively, also exhibited an unossified second sternebra. Sternal ossification was most severly disrupted in the cadmium-treated group, since in most cases the sterna were completely unossified (81%). Sternal damage in the amaranth and cadmium-treated group was characterized by the absence of the manubrium and sternebrae 2 and 5. Thus the different treatments altered sternal ossification quantitatively and qualitatively.

While the normal hamster rib cage contains 13 pairs of thoracic ribs one or more cervical or lumbar ribs are common. Such supernumerary ribs were detected in the controls and all experimental groups. Both the cadmium-treated group and the combined group also presented a high frequency of fused ribs i.e., 67% and 87% respectively.

Most of the larger bones of both the forelimbs and hindlimbs of the 15th gestation day hamster contain detectable ossification centers except the carpals, tarsals, metacarpals and metatarsals of digits 1 and 5, and most of the phalanges. In this study abnormal ossification in the forelimbs was minimal since it was characterized by the absence of one or more of the three metacarpal ossification sites. Hindlimb bone formation was more severely affected. While most of the hindlimbs of the water controls conformed to the 15th day ossification pattern described by BEATTY and HILLEMAN (1950), 32% exhibited less than the three expected metatarsal ossification sites. In both the amaranth and the combined amaranth and cadmium experimental groups abnormal hindlimb bone formation was also limited to an absence of metatarsals. While the cadmium-treated group also contained this defect it also exhibited delayed ossification in some of the larger more proximal bones i.e., ischium, pubis, femur, tibia and fibula.

Several conclusions can be drawn from this study. 1) The IV administration of amaranth (100 mg/kg) to pregnant hamsters on the 8th gestation day is not embryotoxic i.e., it does not kill fetuses or produce any significant external, internal or skeletal system malformations. Thus this data supports earlier work that indicates that orally administered amaranth is not embryotoxic (DRAKE 1975). 2) This study also supports published reports on the nature of the known embryolethal and teratogenic response induced by cadmium in hamsters (FERM and CARPENTER 1968, MULVIHILL et al. 1970, FERM 1971, GALE nad FERM 1973). 3) Based on a comparison of the frequency of malformations in the cadmium-treated versus the cadmium and amaranth-treated groups in this study,

amaranth only protects the hamster fetus from cadmium-induced damage of the brain and bones of the skull and hindlimb.

ACKNOWLEDGEMENTS

The technical assistance of Mrs. Martha Hill is greatly appreciated. This study was supported by NIH grant ES00697.

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