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Arsenic as a Teratogenic Agent

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Sodium arsenate induces developmental malformations in a variety of experimental animals. In the golden hamster, the intravenous (or intraperitoneal) administration of 20 mg/kg of sodium arsenate during day 8 to 9 of gestation induces a rather specific spectrum of congenital malformations. This period corresponds to the period of very rapid differentiation and major organogenesis in this animal. The spectrum of defects produced by arsenate in the hamster includes exencephaly, encephaloceles, skeletal defects, and malformations of the genito-urinary system. This teratogenic effect can be significantly reduced by the simultaneous administration of selenium. Recent studies in this laboratory have demonstrated the permeability of the placenta to ^{74}As during the early critical stages of embryogenesis and the distribution of this isotope in maternal, placental and embryonic tissues. We have also recently demonstrated the marked potentiation of the teratogenic effect of sodium arsenate by subjecting the mothers to short periods of hyperthermia immediately following the administration of subteratogenic or minimal teratogenic levels of arsenate.

Introduction

Arsenic has been known for some time to have a deleterious effect on the developing vertebrate embryo. Ancel (*1*) first demonstrated that disodium methylarsenate interfered with tailbud development of the chick embryo. Subsequently, Ridgway and Karnofsky (*2*) showed that sodium orthoarsenate caused mild micromelia and abdominal edema when injected into the yolk sac of 4-day chick embryos. We first demonstrated the clear-cut mammalian teratogenic effect of sodium arsenate in the hamster in 1968 (*3*). These findings have been confirmed in the mouse (*4*) and rat (*5*).

Animal Model

We began to study the effects of sodium arsenate on the developing hamster embryo, utilizing the teratogenic screening model employed in this laboratory for a number of years. Our model consists of breeding the animals under laboratory conditions to obtain accurately timed matings and then injecting the animals either intravenously or intraperitoneally on day 8 of gestation. At this time of development the embryo has implanted and is beginning to undergo axial orientation. Differentiation immediately thereafter is extremely rapid and progresses through the stages of somite differentiation, neural tube closure, visceral arch formation, limb bud formation, and embryonic heartbeat, all within a 24-hr period. Our first experiments with sodium arsenate showed that the toxic level (the amount which would kill all of the embroys *in utero*) was somewhere over 30 mg/kg of this salt. Dosage levels of 10 mg/kg induced minimal teratogenic effects. The optimal teratogenic dose (the one causing approximately 50% fetal resorption and a large percentage of malformation in the surviving embryos) was determined to be 20 mg/kg of sodium arsenate.

Because we had determined that certain other metals (lead, cadmium, and indium), induced rather specific teratogenic effects in this same animal system (*6*) it was of interest to find that sodium arsenate, when injected on day 8 of gestation, induced a high percentage of exencephaly in fetuses as well as a number of fetuses with varying degrees of encephaloceles. When the time of injection is varied, that is when the injections are spread out over the 24-hr period between days 8 and 9 of gestation, the spectrum of malformations changes. This probably means that the organ systems undergoing differentation are also changing at this time and are apparently sensitive to the amount of arsenic to which they are exposed.

We discovered quite early that one of the peculiar teratogenic effects of sodium arsenate was its effect on the developing urogenital system. Careful dissections of fetuses recovered from mothers treated

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with this agent revealed a surprisingly high number of urogenital anomalies. These were not restricted to one sex and consisted of malformations of the uterus, ovaries, and testes, together with a rather high incidence of unilateral and even bilateral renal agenesis. We as yet have no explanation for this and have not noted this in animals treated with other metals.

Protective Effect of Selenium

Early in our experimental work with arsenic in combination with various metals, we noted that selenium had a marked protective effect against the teratogenic effect of arsenic. Selenium in itself is not a significant teratogen in our animal model. However, when injected into pregnant animals which have received arsenic shortly before, the selenium significantly reduces the teratogenic response (*7*). It might be added here that selenium has this same protective effect on cadmium-induced developmental malformations.

Synteratogenic Effect of Hyperthermia and Sodium Arsenate

The remarkable consistency of malformations of the brain induced by arsenic are quite striking. Recently, we noted that similar malformations could be induced in hamster embryos when the mothers were exposed to a hyperthermic environment on day 8 of gestation (*8*). Essentially these animals were kept in a darkened incubator for a period of 1 to 1.25 hr and the temperature raised to approximately 41°C. Pretreatment and post-treatment rectal temperatures were recorded on these animals and then the embryos recovered some 4 to 5 days later. Those embryos recovered from treated mothers showed a high incidence of encephaloceles and exencephaly. Reducing the time of exposure to the hyperthermic treatment reduced the number of malformations. Times above this caused almost complete fetal absorption. Therefore the exposure time of 1.25 hr seemed to be fairly critical.

Utilizing the minimal teratogenic dose of 10 mg/kg of sodium arsenate we then exposed the animals to the subteratogenic dose (1 hr) of hyperthermia. Whereas neither of these treatments alone would cause many malformations in the offspring, the additive effect of these two stimuli was clearly demonstrated by the increased incidence of malformations in those embryos exposed to the synergistic treatment. Several explanations may account for this. One is that the increased heat stress causes an increase in the permeability of the placenta so that more arsenic ions are available to the developing embryo; another is that the fever induced by this treatment may accelerate chemical reactions in general and thus potentiate the arsenic effect; and finally, because actively dividing cells are known to be more sensitive to both heat and arsenic, the additive effect may be simply one involving a suppression of mitotic activity. Limarzi (*9*) has shown that arsenic causes abnormal mitotic figures and chromosome scattering in human bone marrow cells, and Edwards (*10*) has shown that exposure of pregnant guinea pigs to hyperthermia caused inhibition of mitotic activity and subsequent cell death in fetuses.

Permeability of the Placenta to Arsenic

The permeability of the placenta to arsenic has always been of some interest and certainly so when one tries to explain the teratogenic effect of this agent on developing tissues. The transmission of organic arsenicals from pregnant rabbits and cats to their fetuses apparently is negligible as demonstrated by the studies of Underhill and Armatruda (*11*), although the stages of embryonic development were not correlated with the times of injection. Snyder and Speert (*12*), however, concluded that the rate of transmission of neoarsphenamine across the rabbit placenta increased as pregnancy progressed. Term human placentas from patients receiving the therapeutic doses of prenatal arsphenamine showed a greater retention of arsenic in the fetal portion of the placenta as compared to the maternal portion (*13*). Lugo et al. (*14*) have documented a human case of arsenic trioxide poisoning in human pregnancy, and has shown the ease with which inorganic arsenic crosses the human placenta at term with extremely high levels in the fetal liver, brain and kidneys. James et al. (*15*) showed that potassium arsenate given to pregnant ewes apparently did not accumulate in detectable amounts in the lambs of ewes fed potassium arsenate during pregnancy, but only four animals were checked following dietary treatment.

We recently have studied the placental transmission of ^{74}As in the teratogenic model we use in this laboratory (Hanlon and Ferm, unpublished data). Pregnant hamsters on day 8 of gestation were injected intravenously with a teratogenic dose of sodium arsenate (20 mg/kg) to which had been added a known amount of radioactive arsenic. Maternal and embryonic tissues were collected either 24 or 96 hr later. The tissues were then counted for radioactivity and it was clear that arsenic traversed the placenta during the critical stage of embryogenesis and appeared in the embryonic tissues.

Specimens taken 4 days after injection showed a rather rapid clearance of this isotope from the tissues. It is significant to note, however, that a fair amount of arsenic did cross the placenta and is available to the embryonic tissues during the critical states of embryogenesis if this is indeed the manner of its teratogenic activity.

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