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Transplacental Transfer of Asbestos in Pregnant Mice

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Occupational exposure to asbestos has been on decline in the US over the last 2-3 decades. There is, however, concern that environmental asbestos exposure is increasing through the use of asbestos-containing products (Churg 1988; Edelman 1988; Jarvholm et al. 1988; Sebastein et al. 1989). Most of the previous studies on asbestos exposure have included adult subjects only. However, recent studies have demonstrated asbestos fibers in the tissues and/or placenta digests of a series of autopsied stillborn infants (Haque et al. 1992; 1995; 1996). One of these studies found a highly significant difference ($p = 0.001$) between the mean asbestos counts of tissues from 92 autopsied stillborn infants (52,894 fibers/g) and 45 control placentas of healthy liveborn infants (19 fibers/g) (Haque et al. 1996). A possible relationship between stillbirths and asbestos fiber presence was also suggested by this study. Additionally, significant association was found between a maternal history of previous abortions and asbestos fiber presence in the stillborn tissues ($p = 0.007$) (Haque et al. 1996).

Although asbestos is a known carcinogen and a mutagen, its effects on the developing fetus are not known. In fact, it was unknown and unsuspected that asbestos fibers could be present in human placenta and fetal tissues until the recent studies on the stillborn infants were reported (Haque et al. 1996). Asbestos fibers have been shown to enter the circulation via the gastrointestinal and/or respiratory tract in both human and animals (Carter and Taylor 1980; Cook 1983; Hallenbeck 1983). There is also evidence that circulating maternal cells can enter placental circulation and subsequently enter the fetus (Zarou et al. 1964). There is only one previous experimental study that demonstrated transplacental transfer of asbestos fibers in rats (Cunningham and Pontefract, 1974). Another study in mice failed to demonstrate transplacental transfer of asbestos fibers (Krowke et al. 1983); however, it is possible that the methodology used to detect the fibers may not have been sensitive. In the same abstract, Krowke et al, (1983) also reported a dose-dependent interference with limb development when organ cultures were exposed to crocidolite and chrysotile asbestos. Based on the previous knowledge, and the results of our human stillborn studies, we hypothesized that the asbestos fibers found in the stillborn fetal tissues and placentas were transferred from the pregnant mothers, who were exposed either through inhalation or ingestion of asbestos fibers. These fibers subsequently entered the maternal circulation and

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the placenta. The long duration of human pregnancy would allow the fibers to enter the fetal circulation and to be deposited in fetal tissues.

The interaction between asbestos fibers and the developing fetal and placental cells may produce DNA damage, and/or induce cytokines, the mediators of inflammation. Both these events are known to adversely affect pregnancy (Kenne et al. 1986; Lemaire et al. 1986; Libbus et al. 1989; Perkins et al. 1993; Zhang et al. 1993). Fetal tissues are highly susceptible to chemicals and drugs such as ethyl carbonate, nitrosoureas, nitrosamines, and benzo(a)pyrene (Bulay and Wattenberg 1970; Rice 1973; 1979). Thus, while prenatal exposure to chemical carcinogens is known to result in permanent mutagenic or neoplastic changes in fetal tissue (Rice 1979), there are no known studies on the effects of asbestos exposure on the developing fetus. Experiments in rodents indicate that their fetuses are highly sensitive to chemical carcinogens in the prenatal period (Rice 1973; 1979). In order to examine the effects of asbestos on developing fetus, one has to first establish the occurrence of transplacental transfer of asbestos fibers. To our knowledge, there is no previous documentation of transplacental transfer of asbestos fibers in mice. Thus, the objective of our study was to investigate whether transplacental transfer of asbestos occurs in pregnant mice. We report the results of our light and electron microscopic examination of fetal and placental tissues following intravenous injection of asbestos fibers in pregnant mice.

MATERIAL AND METHODS

Pregnant ICR mice were received from Harlan Laboratories on the tenth day of pregnancy and allowed to acclimatize for two days. Since there is no previous data on the dose or optimum treatment days in pregnant mice, we chose gestational day (GD)12 and 16 as the early and mid-gestational days for asbestos injection. On the 12^{th} GD, two mice were given 0.2 ml, and one mouse was given 0.45 ml of crocidolite asbestos suspension via tail vein injection. On GD16, an additional mouse received 0.2 ml of crocidolite suspension; one control mouse received 0.2 ml injection of normal saline on GD15. The asbestos suspensions contained approximately 345,000 fibers/ml. Since we were not certain of the time when the transfer of fibers may occur, the mice were sacrificed at three different times at 1, 48 and 72 hours following treatments. Further details of treatment are shown in Table 1. At the end of exposure times, the mice were sacrificed by cervical dislocation, the fetuses and placentas were removed by Caesarean section, and numbered starting from the left upper end of the uterus. The amniotic sacs were opened and each fetus euthanized by severance of the umbilical cord. The fetus and placenta were weighed separately. Sagittal halves of each fetus and placenta were frozen at -70 °C, and the other halves were fixed in 10% buffered formaldehyde solution for asbestos counts and histological examination. For the light and electron microscopic asbestos counts, the formalin-fixed fetuses and placentas were processed using a digestion-extraction method that has been used in our laboratory for the past 10-11 years (Dodson et al. 1985;

Williams et al. 1982). Briefly, 110 mg to 200 mg of formalin-fixed tissue was digested in 9.2% sodium hypochlorite solution (bleach). The digested tissue was filtered through a 0.2 µm Nuclepore filter, washed with water, and treated sequentially with 4% potassium permanganate, 8% oxalic acid, 9.2% sodium hypochlorite, 8% oxalic acid, and water. The filter was divided into four quarters; one quarter of the filter was placed face down on a glass slide, and the filter dissolved with chloroform and mounted with a coverslip. Using a Nikon Labophot microscope, fibers on the entire slide were counted at x400 magnification, and the number of fibers/g calculated using a standard formula.

Tissue for light microscopic evaluation of pathologic changes was processed using standard histologic laboratory technique. Briefly, following the paraffin embedding of the tissue, sections were cut at 4 µm, deparaffinized, stained with hematoxylin and eosin, dehydrated, cleared and mounted on glass slides. All sections were examined for the presence of alterations such as inflammation, edema, hemorrhage, necrosis, and for asbestos fibers. Tissue for ultrastructural examination was also processed using the standard electron microscopic technique. Briefly, the tissue was fixed in a solution of 2% paraformaldehyde and 2% gluteraldehyde, post-fixed in osmium tetraoxide and stained with 1% uranyl acetate, followed by dehydration in ethanol. After embedding in epoxy, sections were cut on an ultramicrotome and examined in a Phillips CM100 electron microscope using x2000 to x 20,000 magnification to detect, localize and measure the asbestos fibers. Positive identification of fibers was accomplished by using energy dispersive x-ray analysis (EDXA) attached to the electron microscope.

RESULTS AND DISCUSSION

A total of 36 fetuses and corresponding placentas from the 5 mice were examined for asbestos fibers (Table 1). Average asbestos fiber count of fetal digests from the one hour treated mouse was 4,288 fibers/g (range: 116 to 30,342 fibers/g), and the average placental digest count was 10,857 fibers/g (range: 1,335 to 48,337 fibers/g). Average fiber count of the fetal digests from the 48 hour mouse was 254 fibers/g, (range: 0 to 706 fibers/g), and the average placental digest count was 23, 529 fibers/g (range: 6,026 to 104,481 fibers/g). Fetal digests of the 72 hours mouse (0.2 ml dose) had an average fiber count of 1,010 fibers/g (range: 378 to 2,225 fibers/g), and the corresponding placental digests had an average of 5,222 fibers/g (range: 1,171 to 11,800 fibers/g). The fetal digests from the 72 hour mouse with a higher asbestos dose of 0.45 ml had an average count of 855 fibers/g (range: 139 to 1,440 fibers/g), and the placental digests had an average of 11,683 fibers/g (range: 2,781 to 17,483 fibers/g). The fetal and placental digests from the control mouse had 0 fibers/g.

Histologic examination of sections from the fetuses and placentas showed focal areas of coagulative necrosis with a mild neutrophil infiltration in 29% of the placentas from the treated mice. No other pathologic changes were seen in sections from the fetuses or placentas. Using light microscopy at high

magnification (X 1,000), no fibers were visible in the areas of placental necrosis. However, electron microscopy of the placental tissue taken from the areas of necrosis showed very small (100 nm to 700 nm) asbestos fibers. Some of the asbestos fibers were found within the cytoplasm of the placental sinusoidal cells, but interestingly, a few small fibers were actually present within the cell nucleus (Fig. 1).

These experiments demonstrate transfer of asbestos fibers to the fetus and placenta in pregnant mice within an hour of intravenous injection. The study also shows that asbestos fibers continue to be present in both the placenta and the fetus at 48 and 72 hours following a single intravenous injection. There was some variation in the individual fetal and placental fiber counts from the same litter. This may be explainable by the uterine anatomy in mice. The uterus in mice is U-shaped, and derives its blood supply from the aorta. Each fetus has its own placenta and amniotic sac, and receives blood through an individual branch from the uterine artery. It is therefore possible that, the fetuses closer to the origin of the uterine artery may receive a higher dose of asbestos. The differences in fiber counts of the 1 hour, 48 hour and 72 hours may be due to individual variation in circulation and retention of fibers. On the other hand, there may be a recirculation of the fibers from the fetus to the placenta with redistribution of fibers in the fetus and placenta. Additionally, one fetus in the one hour mouse, and one placenta in the 48-hour mouse had high fiber counts; an unusually high count may occur when a clump of fibers lodges in the placental vessels and breaks through the vascular spaces to enter the fetal circulation (Cunningham and Pontefact, 1974). These observations, however, are based on small number of animals and need further exploration.

Table 1. Asbestos fiber counts of fetal and placental tissues

Exposure times	Treat Day	Sac Day	Dose in ml	# of Fetus	Avg Fet. Wt. (g)	Avg Plac. Wt. (g)	Avg # F/g in Fetus	Avg # F/g in Placenta
1 hr	16	16	0.20	11	0.705	0.134	4,288	10,857
48 hr	12	14	0.20	7	0.257	0.120	254	23,529
72 hr	12	15	0.20	7	0.331	0.141	1,010	5,222
72 hr	12	15	0.45	5	0.512	0.162	855	11,683
control	15	15	0.20 saline	6	0.592	0.149	0	0

Exposure time, time between injection and sacrifice; Treat Day, gestational day of treatment; Sac Day, gestational day of sacrifice; Avg. Fet. Wt. (g), average fetal weight in grams; Avg. Plac. Wt. (g), average placental weight in grams; Avg #F/g, average number of fibers per gram.

Our study confirms a transplacental transfer of asbestos in mice following a single intravenous injection, and also demonstrates that the fibers continue to be present in both the fetus and placenta for up to 72 hours after injection. To our knowledge, in addition to our human studies (Haque et al 1992, 1995, 1996), there is only one experimental study demonstrating transplacental transfer of asbestos, (Cunningham and Pontefract 1974). In this study, pregnant wistar rats received three intravenous injections of 1 to 3 mg of chrysotile asbestos at 2 day intervals between the 10^{th} to 14^{th} day of gestation; asbestos fibers were found in the fetal livers and lungs of >50% of the tissue digests examined. Additionally, our study demonstrated small asbestos fibers within the cytoplasm and nuclei of placental cells, a finding that may help in understanding the mechanisms of fiber toxicity.

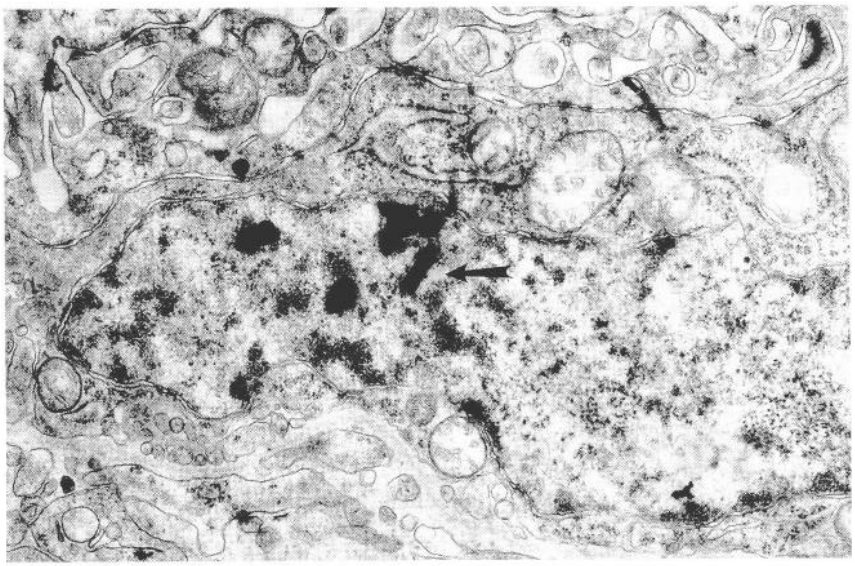

Fig. 1. Electron micrograph of a placenta from a mouse injected with 0.2 ml of crocidolite asbestos suspension. Small asbestos fibers (arrow) are seen within the nucleus (X 17,250).

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