Short Communication

A Preliminary Report on the Tumorigenic Effect of Long-Term Exposure to n-Hexane in the Rat Testis

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Recent studies suggest that exposure of rats to n-hexane and n-hexane's metabolite 2,5-hexanedione (2,5-HD) is toxic to male rat genitalia). In the course of our experiments, histological examination of various organs in addition to the testes uncovered Leydig cell hyperplasia and Leydig cell tumors after treatment with n-hexane.

Materials and Methods

Animals: F344/Jcl male rats (Clea Japan, INC., Japan) were obtained at 72 days of age and randomly divided into two groups: the n-hexane-exposed group and the unexposed control group. The rats were housed in stainless steel wire cages, one rat per cage, at a temperature of 23 ± 2°C, a humidity of 55 ± 5%, and a 12 hr dark/light cycle. The cages were kept a semi-barrier system at the experimental animal center of the Toho Univ. Sch. of Med. The rats were fed a laboratory diet (CE-7 diet blocks, Clea Japan, Inc., Japan) and given tap water ad libitum.

Chemical substance and Exposure: Special grade n-hexane (Wako Chemical, INC., Japan) was vaporized by the diffusion tube method, with a permeacal permeater (PD-1 B, Gastec, INC., Japan). An air flow of 439 ml/min from a compressed air bottle was regulated by a flowmeter. N-hexane was conveyed through a diffusion tube immersed in a thermoregulated water bath (at 50°C). The rats were exposed to either ambient air or to air containing 1000 ppm n-hexane, one rat per glass metabolic chamber (Sugiyama-gen, Inc., Japan), for 4 h per day, 6 days per week, and 415 days altogether. The concentration of n-hexane in the chambers was monitored with a Gastec gas detector (Gastec, INC., Japan). The measured n-hexane concentrations were 1100 ppm to 900 ppm (n=156, 983 ± 32 ppm). The control group was kept in chambers ventilated with fresh air. The temperature in the glass chambers was maintained at 24°C. Only water was given during the hours of exposure.

During the period of the experiment, the animals were observed daily and any clinical indications of neuropathy-ataxia, everted flat foot placement, or hind limb weakness were noted. The rats' weight and food intake were recorded daily.

Histology: All rats were decapitated without anesthesia, 24 h after the experiment was completed. Samples of the testes were fixed in 10% neutral buffered formalin, embedded in paraffin, and cross-sectioned in 3 µm slices. Sections were stained with Mayer's hematoxylin and eosin (H&E) or periodic acid-Schiff's reagent and Mayer's hematoxylin (PAS/H). The 14 typical cellular associations in the seminiferous epithelium, according to the standard criteria of Leblond and Perey.

Results

Body weight, testes weight, food intake, the frequency of 14 cellular associations in the seminiferous epithelium and light microscopical histological findings in single testes of the exposed group did not significantly differ from the controls, but the incidences of Leydig cell hyperplasia and Leydig cell tumors in the 6 rats exposed to n-hexane were 100% (6/6), and 33.3% (2/6), respectively. In contrast, the incidences of Leydig cell hyperplasia (Fig. 1 B) and Leydig cell tumor in the control group of 6 rats were only 16.7% (1/6) and 0%, respectively. There are no morphologic differences between Leydig cell hyperplasia and Leydig cell tumors, according to the criteria established by the National Toxicology Program. Leydig cell hyperplasia and Leydig cell tumors were significantly more frequent (p<0.05) than in the control group (Table 1).

Discussion

In the present study, the incidences of Leydig cell hyperplasia and Leydig cell tumors in the 6 rats exposed to n-hexane in the present study were 100% and 33.3%, respectively. That benign Leydig cell tumors spontaneously develop in male F-344 rats with increasing age is well known. It has been reported that the incidence of these tumors is 60-80% by the age of 18 months and reaches 98% by the age of 24 months. Hamada et al. examined spontaneous development of Leydig cell tumors in aged male F-344 rats by comparing various parameters in rats without Leydig cell tumors at age 6-12 months and those with Leydig cell tumors at age 18-24 months. Aged rats with Leydig cell tumors had lower plasma testosterone and higher progesterone levels but comparable plasma luteinizing hormone, follicle stimulating hormone and prolactin levels, but the mechanism has not yet been elucidated.

Chapin et al. compared the plasma hormone levels in 2,5-HD treated rats to those in control rats, and the plasma testosterone levels did not significantly differ at 1, 3 and
Fig. 1. Light micrographs of testis sections stained with PAS/H.
(A) n-hexane exposed group: Leydig cell tumors (*) are evident.
(B) control group: Leydig cell hyperplasia (→) is evident. Bar equals 100 µm in A and B. (x 20)

Table 1. Histological determination of the number of Leydig cell hyperplasia and Leydig cell tumors in n-hexane exposed rats and controls in cross section. Significant difference evinced between the n-hexane exposed group and the control group (*p<0.05 by Student’s t-test)

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Control group</th>
<th>n-hexane exposure group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Leydig cell hyperplasia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leydig cell Tumors</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
</tr>
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6 weeks. Luteinizing hormone levels also did not differ at 1 and 3 weeks, but were significantly higher in the 2,5-HD exposed rats at 6 weeks.

Leydig cell tumors in male F-344 rats exposed to n-hexane apparently differ from those in aged male F-344 rats.

In our study, premature Leydig cell hyperplasia and Leydig cell tumors were observed in the n-hexane group, suggesting that the rat testes were damaged by n-hexane.

Our results suggest that long-term exposure to n-hexane is potentially tumorigenic in male F-344 rat testes.

References
4) Boekelheide, K. Rat testis during 2,5-hexanedione intoxication and recovery. II. Dynamic of pyrrole reactivity tubulin content, and microtubule assembly. Toxicol Appl Pharmacol 1988b; 92: 28–33.