Indenopyridine Hydrochloride Induced Testicular Spermatogenesis Failure with High Seminal Alkaline Phosphatase Levels in Male Dog

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Indenopyridine hydrochloride (IH), an antispermatogenic agent, was tested to determine the testicular pathological changes, seminal spermatozoa concentrations and seminal plasma alkaline phosphatase levels in male dogs. A single oral dosage of 30 mg IH/kg BW induced the dissociation and premature release of germ cells into the lumens of seminiferous tubules. Ring-shaped spermatid nuclei, nuclear pykonosis of spermatocytes and multinucleated cell associations were also observed. Thereafter, the spermatogenic index (SI) significantly decreased one day after IH administration. Moreover, seminal spermatozoa concentrations decreased two weeks after drug treatment; and there was a statistically significant difference in spermatozoa production inhibited by IH compared to the control. Reversible spermatogenesis was noted 7 weeks after IH treatment in male dogs. Meanwhile, seminal plasma alkaline phosphatase levels also significantly increased two weeks after IH treatment. These data confirm that IH might induce a two-month inhibition of spermatogenesis in male dogs.

Key words indenopyridine hydrochloride; spermatogenesis; alkaline phosphatase; male dog

Materials and Methods

Animals Thirty adult mongrel male dogs, weighing 6—15 kg, were used in the present study. All male dogs were acquainted with the laboratory environment, researchers and procedures such as the ordinary restraint and collection of semen. They stayed in individual cages with sufficient space and were provided with commercial dog food (Altromin standard diet), tap water and normal exercise. They were isolated in quarantine for at least two weeks for observation and acclimation prior to this study.

Drug Indenopyridine hydrochloride (IH) (C_{22}H_{28} · HCl) (Fig. 1) was purchased from National Products & Pharmaceutical Laboratory, Natural Pharmacia International, Inc., U.S.A. The purity of IH was greater than 99.5%.

Histological Survey An experiment to determine the effects of IH on antispermatogenesis in male dogs was performed as follows. Twenty-one adult male dogs received a single oral treatment of 30 mg/kg of IH.5) Subgroups of 3 male dogs were castrated per day serially within days 1—7 after IH treatment. Another three male dogs accepted an empty capsule as a control, and their testes were also collected by castration. The testes were fixed in Bouin’s solution and embedded in paraffin. The 5 μm sections were observed with hematoxylin/eosin stain by light microscopy. A testis was rated for its modified spermatogenic index (SI) on a scale of 0 to 6. The indices were as follows: 0, no spermatogenic cells; 1, only spermatogonia present; 2, spermatogonia and spermatocytes present; 3, spermatogonia, spermatocytes and round (early) spermatids present with <5 late spermatids per tubule; 4, spermatogonia, spermatocytes, and round spermatids present, and up to 25 late spermatids per tubule; 5, all cell types present and 50—75 late spermatids per tubule; 6, all cell types present and >100 late spermatids per tubule. One hundred seminiferous tubules per testis were observed by microscopy.

Seminal Spermatozoa Counting and Seminal Plasma Alkaline Phosphatase Assay Six adult male dogs received a single oral treatment of 30 mg/kg of IH, or an empty capsule as a control before drug administration. The ejaculates were obtained from all male dogs one week before IH treat-

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Fig. 2. (A) Seminiferous Tubules of Adult Dogs 24 h after a Single Oral Dosage of 30 mg IH/kg BW Administration
Pyknotic spermatocytes (P) were observed. Original magnifications: 80×.

(B) Seminiferous Tubules of Adult Dogs 2 d after IH Treatment
Dissociation and premature release of germ cells with multinucleated cell associations (M) into the tubular lumens induced disorganization. Some spermatozoa (S) migrated to the basal layer of tubules. Original magnifications: 400×.

(C) Epididymal Lumen of Adult Dog 3 d after IH Treatment
Degenerative germ cell (Dg) mixed with many spermatozoa in the epididymis was observed. Original magnifications: 400×.

(D) Seminiferous Tubules of Adult Dog 5 d after IH Treatment
Shrinkage of tubules and increased interval between tubules were noted. Cell debris and multinucleated giant cells were observed in these lumens. Original magnifications: 40×. (Hematoxylin-eosin stain.)

(E) Quantitative Analysis of Spermatogenic Index (SI) by Microscopic Observation in Testis Cross-sections of Control and IH-Treated Male Dogs
The lesions per seminiferous tubule were grouped into three categories of SI: 0—2, 3—4 and 5—6. A total of 200 randomly selected tubule cross sections were analyzed at each time point with data expressed as a percentage per total. Each bar represents the mean ± S.D. * indicates statistically significant difference (p<0.05) compared with the control. (n=3)
ment and 1, 2, 3, 4, 5, 7 and 12 weeks after IH treatment. The number of spermatozoa was reported as the total sperm per ejaculate and was counted with a hemocytometer. Meanwhile, the semen samples were centrifuged as for serum (e.g., 3000 rpm for 10 min), and the supernatant seminal plasma were harvested for alkaline phosphatase detection. The values of seminal alkaline phosphatase were analyzed using an automatic analyzer (Express Plus, CIBA-Corning Diagnostics Corp., U.S.A.).

Statistics All of the quantitative data, calculated as a percentage of total or values were expressed as the mean±S.D. Significant differences between groups (p<0.05) were determined using Duncan’s multiple range after single factor analysis of variance.

Ethics of Experiment The Experimental Animal Committee on Management of National Chung Hsing University approved the experiments in this study.

RESULTS

Histological Observation of Seminiferous Tubules As early as 24 h after treatment with 30 mg/kg of IH, ring-shaped spermatid nuclei and pyknotic cells were observed in some tubules (Fig. 2A). Two days after treatment there was dissociation and premature release of germ cells with multinucleated cell associations into the tubular lumens. Thereafter, spermatozoa migrated to the neighboring area of the basal layer of seminiferous tubules (Fig. 2B). Meanwhile, many degenerative germ cells appeared in the epididymis (Fig. 2C). Some of these tubules consisted of Sertoli cells with spermatogonia only, especially 5—7 d after IH treatment. Lumens of these tubules were filled with cell debris or intercellular stroma. In addition, a shrinkage of tubule diameter with an increased interval between tubules was noted (Fig. 2D). Moreover, IH significantly induced spermatocytic death. Thereafter, the SI shifted to a level from 5—6 to 0—2 during the duration of IH treatment (Fig. 2E).

Seminal Spermatozoa Counting and Seminal Plasma Alkaline Phosphatase Assay The spermatozoa number per ejaculate was significantly decreased two weeks after IH treatment. Moreover, there was reversible spermatogenesis with elevated spermatozoa numbers from 7 weeks after IH administration in male dogs (Fig. 3A). Significantly increased seminal plasma alkaline phosphatase levels (>20000 U/l) were also noted from 2 to 12 weeks, except in the fifth week, after IH administration (Fig. 3B).

DISCUSSION

The results of the present experiment indicate that a single oral dose of 30 mg IH/kg BW inhibited spermatogenesis within 24 h in male dogs. The main pathological lesions of degenerative changes in the male dogs were similar those in the spermatogenic tissue of mice and rats after IH treatment.2—5) These lesions after IH treatment were characterized in the germinal epithelium that resulted in one or more of the following: a disruption of the original orderly cellular arrangement within the germinal epithelium, or the presence of a degenerative nuclear change with ring-shaped spermatid nuclei and nuclear pyknosis (Figs. 2A, B). Meanwhile, multinucleated giant cells and part or complete depletion of the tubules (Figs. 2B, D) were regarded as the result of a prior degenerative insult.2,4) Therefore, this premature release of degenerative germinal cells was transferred into the epididymis (Fig. 2C). Indenopyridine derivatives inhibited the meiotic and post-meiotic phases of spermatogenesis,10) and these induced the germinal epithelium to disappear rapidly except for spermatogonia and Sertoli cells, which apparently remained intact. Thereafter, IH indirectly led to the disorganization of tubules with the dissociation of germ cells and the migration of spermatozoa (Fig. 2B). Thus, structure-activity relationship (SAR) studies based upon such compounds can lead to clinically useful medicines and new areas of investigation.

The SI represents the spermatogenic potency of the testes.1,9) The SI scores for the control dogs ranged mainly from 5 to 6. Those for the IH-treated dogs ranged from 0 to 2 (Fig. 2E). The significantly decreased SIs corresponded to a previous histological survey. Therefore, the SI measure was a sensitive indicator of tubular dysfunction. In general, infertility resulted when the SI rating was 3.5 or lower.11) Because the process of sperm production takes 60—62 d (average 54.4 d),8,11) the spermatozoa values per ejaculate returned to normal range in the seventh week and twelfth week after IH
treatment (Fig. 3A). In addition, libido, ability of erection and ejaculation of male dogs were not influenced by IH. Thus, IH might become an ideal contraceptive in male dogs.

The major portion of alkaline phosphatase in a dog’s seminal plasma does not come from the prostate but from the epididymis. Furthermore, a dog’s seminal plasma alkaline phosphatase is presented at relatively high levels. The values of spermatozoa per ejaculate (≥600×10⁶) and seminal plasma alkaline phosphatase (≥1000 U/l) measured in samples of semen from the male dogs of the control group were normal in the present experiment (Figs. 3A, B). It meant there were no problems of bilateral obstruction to outflow in these male dogs. Moreover, the correlation between the decreased numbers of spermatozoa per ejaculate and increased seminal plasma alkaline phosphatase in the first month after IH treatment was also noted (Figs. 3A, B). These results confirmed that IH induced testicular spermatogenesis in male dogs. In addition, the values of seminal plasma alkaline phosphatase are a good indicator for observation of the antispermatic effect of IH in male dogs.

REFERENCES