REPRODUCTIVE EFFECTS OF PYRIDOXAL ISONICOTINOYL HYDRAZONE IN RATS : EFFECTS ON ESTRUS CYCLE AND IMPLANTATION

N. Sookvanichsilp, W. Panintrarux, S. Suksathan and S. Rattanajarasroj

Department of Pharmacology, Faculty of Pharmacy Mahidol University, Bangkok 10400, Thailand

Pyridoxal isonicotinoyl hydrazone (PIH) is a highly effective compound in the management of iron overload in animals. The reproductive effects of this compound were studied in rats. PIH doses of 100, 200 and 400 mg/kg body weight given orally for 20 consecutive days produced no effects on rat's estrus cycle. When these rats were caged with males and the PIH doses mentioned were continued feeding till day 7 of pregnancy, it was found that PIH could interfere with implantation in some rats. Male-mediated effect of PIH on implantation was also studied. Male rats were fed with PIH at doses of 100 and 200 mg/kg body weight for one month before they were housed with proestrus females. All mated rats were pregnant and no significant differences (at $p = 0.05$) in the number of implantation sites were found in these pregnant rats as compared to those mated with the control males.

INTRODUCTION

Pyridoxal isonicotinoyl hydrazone (PIH) is a complex of pyridoxal hydrochloride and isonicotinic acid hydrazide (INH) in equimolar amounts. It is a highly effective compound in the management of iron overload in rats when given orally[1-4]. Toxicological data of this compounds are limited. There was a recent report of PIH on acute toxicity in mice and rats and subchronic toxicity in rats[5]. In the present study, the effects of PIH on estrus cycle and implantation in rats were performed. In addition, male-mediated effect of this compound on implantation was also studied.

MATERIALS AND METHODS

Effect of PIH on Estrus Cycle in Rats

Forty female Wistar rats (145-150 g), from the National Laboratory Animal Center-Salaya, with regular estrus cycles were divided into 4 groups of 10 animals. PIH (prepared by oxidation of pyridoxine HCl to pyridoxal and reacted with INH - described elsewhere[6]) was dispersed in distilled water and doses of 100, 200 and 400 mg/kg body weight were given to each group by gavage with a feeding needle for 20 consecutive days. Water was given to the control group. Vaginal smears were performed every morning. The smears were stained with methylene blue (0.5% solution) and examined microscopically at 200X.

Effect of PIH on Implantation in Rats

All female rats mentioned above were caged with males. PIH doses of 100, 200 and 400 mg/kg body weight were kept on feeding the females. The day when sperm were present in the vaginal smears was designated as day 1 of pregnancy. The females, mated at the first or second estrus stage were then used. PIH and water were still given consecutively from day 1 till day 7 of pregnancy. Laparotomy was performed under ether anesthesia on day 9 of pregnancy. After laparotomy, the females were allowed to complete gestation.

Effect of PIH on Male-Mediated Implantation in Rats

Twenty-five male Wistar rats (250-280 g) were divided into 3 groups of 8-9 animals. PIH doses of 100 and 200 mg/kg body weight were given by gavage with a feeding needle. Water was given to the control group. The animals were fed for 1 month before they were individually housed with the proestrus females (150-170 g) with regular estrus cycles (one male per one female). Vaginal smears were taken and examined daily. Unmated female rats were replaced by the new proestrus females. PIH or water was withdrawn when sperm were found in the vaginal smears.

Statistical Analysis

Results were analysed by Student's t-test. P-value of less than 0.05 was regarded as statistical significance.

RESULTS

Effect of PIH on Estrus Cycle in Rats

PIH doses of 100, 200 and 400 mg/kg body weight given orally to female rats for 4-5 consecutive estrus cycles produced no significant changes in the period of estrus cycle. The average cycle length was in the range of 4-5 days. The epithelial cytology found in vaginal smears was normal, i.e. proestrus, estrus, metestrus and diestrus.

Effect of PIH on Implantation in Rats

PIH doses of 100, 200 and 40 mg/kg body weight given to rats for 4-5 consecutive estrus cycles and continued feeding till day 7 of pregnancy could interfere with implantation in some rats as indicated in Table 1. Resorbing sites were detected in certain gravid and non-gravid uteri from the treated groups. However, no significant differences (at $p = 0.05$) were found in the number of implantation sites in pregnant animals from the treated groups as compared to those from the control group. All pregnant rats exhibited normal gestation periods (22-24 days). The litters were examined externally and no abnormalities could be observed.

Effect of PIH on Male-Mediated Implantation in Rats

All male rats from PIH treated and control groups mated with the females at the first, second or third night. A lot of sperm were detected in the vaginal smears taken from female rats mated with the males from any groups. All mated rats were pregnant and the number of implantation sites were indicated in Table 2. No significant differences (at $p = 0.05$) were found in the number of implantation sites in pregnant rats mated with the treated males as compared to those mated with the control males. Serving behavior was occasionally observed and no differences were found between treated and control males. No external abnormalities could be detected when their litters were examined externally.

Table 1. Effect of PIH on implantation sites in rats

Dose (mg/kg/day)	No. of pregnant rats No. of rats mated	No. of implantation sites (mean ± SEM)	No. of litter sizes (mean ± SEM)
water	8/8	8.13 ± 0.95 (10, 5, 12, 5, 8, 6, 11, 8)	7.13 ± 0.69 (10, 5, 9, 5, 7, 6, 9, 6)
100	6/8	8.17 ± 0.98 (12, 8, 10, 7, 6, 6)	7.33 ± 0.71 (10, 8, 8, 7, 5, 6)
200	4/9	7.25 ± 1.31 (9, 5, 5, 10)	6.50 ± 0.96 (7, 5, 5, 9)
400	4/9	8.75 ± 1.88 (10, 8, 4, 13)	7.50 ± 1.26 (8, 8, 4, 10)

Table 2. Number of pregnant rats and implantation sites found in the females mated with the PIH treated males

Dose of PIH (mg/kg/day)	No. of pregnant rats No. of rats mated	No. of implantation sites (mean ± SEM)
water	9/9	8.33 ± 0.88 (11, 4, 7, 8, 5, 9, 9, 12, 10)
100	8/8	7.88 ± 0.86 (7, 10, 5, 5, 12, 9, 8, 7,)
200	8/8	8.13 ± 0.91 (6, 8, 11, 4, 6, 10, 11, 9)

DISCUSSION

Effect of PIH on Estrus Cycle in Rats

The rat's estrus cycle can be divided into four stages and can be recognized by changes in vaginal cytology : proestrus, estrus, metestrus, and diestrus[7]. An estrus cycle length is 4-5 days. Ovulation occured during estrus stage can be readily detected by cornification of vaginal epitheleum. The estrus cycle is regulated by hormones from the hypothalamus (LHRH), pituitary (FSH and LH) and ovary (estrogens and progesterone). In this study, oral PIH doses of 100, 200 and 400 mg/kg body weight given to female rats for 20 days showed no effects on estrus cycle. This may lead to a suggestion that PIH at doses given has no critical effects on those hormonal levels.

Effect of PIH on Implantation in Rats

The timing of blastocyst formation and implantation in rats requires 3-4 days and 5-6 days from ovulation, respectively[8] The prepared endometrium, which is under the influence of hormones and other factors, is necessary for implantation. Fertilized ova need various kinds of nutrients for further development. Iron is an important component of heme and also involves in various enzymatic reactions. Iron deficiency causes hematologic disorders of pregnancy[9]. In the present study, oral PIH doses of 100, 200 and 400 mg/kg/day given to female rats for 20 days and continued feeding till day 7 of pregnancy could inhibit normal implantation in some rats. This leads to a suggestion that PIH probable interferes with the available iron for blastocysts.

Effect of PIH on Male-Mediated Implantation in Rats

The potential hazard of a chemical to reproduction is difficult to assess because of the complexity of the reproductive process. The gametogenic and the secretory functions of testes are dependent on the secretion of the adenohypophyseal gonadotropins, FSH and LH (ICSH). Sexual behavior is under the influence of sex hormones, and the physiologic processes of erection and ejaculation are controlled by the central nervous system (CNS) but are modulated by the autonomic nervous system (ANS)[7, 10]. Various drugs act on the CNS and the ANS and cause impotence[11]. In this study, as there were no observable effects of PIH on serving behavior in male rats and on implantation in female rats mated with the PIH treated males, this may indicated that PIH at given regimens has no influence on total reproductive capacity in male rats. However, since the process of spermatogenesis in rats requires 3.5 cycles of the germinal epithelium (about 45 days)[10], administration of PIH for 1 month could not indicate the effect of the compound to spermatogenesis.

In conclusion, PIH doses of 100, 200 and 400 mg/kg body weight given orally to female

rats for 20 consecutive days before the animals were caged with the males, and continued feeding till day 7 of pregnance could interfere with implantation in some rats. PIH at doses of 100 and 200 mg/kg body weight had no effects on pregnancy when these PIH doses were given to male rats for one month before they were housed with the females.

REFERENCES

1. Hoy T, Humphrys J, Jacobs A, Williams A and Ponka P. Effective iron chelation following oral administration of an isoniazid - pyridoxal hydrazone. Br J Haematol 1979; 43: 443.
2. Cikrt M, Ponka P, Necas E and Neuwirt J. Biliary iron excretion in rats following pyridoxal isonicotinoyl hydrazone. Br J Haematol 1980; 45: 275-83.
3. Hershko C, Avramovici-Grisaru S, Link G, Gelfano L and Sarel S. Mechanism of in vivo iron chelation by pyridoxal isonicotinoyl hydrazone and other imino derivatives of pyridoxal. J Lab Clin Med 1981; 98: 99-108.
4. Williams A, Hoy T, Pugh A and Jacobs A. Pyridoxal complexes as potential chelating agents for oral therapy in transfusional iron overload. J Pharm Pharmacol 1982; 34: 730-2.
5. Sookvanichsilp N, Nakornchai S and Weerapradist W. Toxicological study of pyridoxal isonicotinoyl hydrazone : acute and subchronic toxicity. Drug Chem Toxicol 1991; 14: 395-403.
6. Sookvanichsilp N. Pyridoxal isonicotinoyl hydrazone : Preparation, physical properties and acute toxicity in mice. Mahidol University J Pharm Sci 1992; 19: 1-3.
7. Thomas JA. Toxic responses of the reproductive system, in "Casarett and Doull's Toxicology, the Basic Science of Poisons", 4th ed. Amdur MO, Doull J and Klaassen CD, eds. New York : Pergamon, 1991: 484-520.
8. Manson JM and Kang YJ. Test methods for assessing femals reproductive and developmental toxicology, in "Principles and Methods of Toxicology", 2 nd ed. Hayes AW, ed. New York : Raven, 1989: 311-59.
9. Kelton JG and Cruickshank M. Hematologic disorders of pregnancy, in "Medical Complications During Pregnancy". Burrow GN and Ferris TF, eds. Philadelphia : WB Saunders, 1988: 66-94.
10. Zenick H and Clegg ED. Assessment of male reproductive toxicity : a risk assessment approach, in "Principles and Methods of Toxicology", 2 nd ed. Hayes AW, ed. New York : Raven, 1989: 275-309.
11. Millar JGB. Drug-induced impotence. Practitioner 1979; 223: 634-9.