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Effect of Cocaine Administration on the Development of Mouse Embryos

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Morphological normal of unfertilized oocytes, which was collected 12-14 hours after human Chorionic Gonadotropin(hCG) injection, was not influenced by chronically administration of cocaine for 2 weeks in mice. Proportion of normal unfertilized oocytes in non-cocaine treated group (control), and 10 mg/kg and 20 mg/kg cocaine treated group based on body weight with subcutaneous(s.c) daily injection of cocaine for 2 weeks were 92.9%, 85.6% and 90.9%, respectively. There is no significant difference between control and cocaine treated groups. Two to 8 cell stage embryos collected 24-48 hours post hCG in control group were 66.7%, whereas, 10 mg/kg and 20 mg/kg groups treated with cocaine was 12.5% and 27.3%, respectively. Although control and treated groups are significantly different ($p<0.05$) the developmental score of 2 to 8 cell stage embryos collected at 24-48 hours post HCG, there is no difference between 10 mg/kg and 20 mg/kg treated with cocaine groups. These results indicated that the normal embryos of the groups of cocaine administration were significantly arrested when compared with that of control group. The proportion of 2 to 8 cell stage embryo reaching the blastocyst stage, which were cultured 48-52 hours with 5% CO_2 in air at 37°C, were 93.9% in control group and, 70.4% and 71.9% in each 10 mg/kg and 20 mg/kg cocaine treated group. These results were shown that developmental capacity to blastocyst *in vitro* culture was significantly limited embryos obtained from cocanized mice compared with those of control mice. These results suggest that episode of cocaine intoxication can cause impairment of early embryogenesis in the mouse.

Key words: Superovulation, unfertilized egg, 2- and 8 cell embryos, blastocyst, episode of cocaine intoxication

INTRODUCTION

Drug abuse has so permeated our society that the use of alcohol, marihuana, methamphetamine, cocaine and other mood-altering substances has become commonplace among juvenile delinquent (Jhoo *et al.*, 1990 a,b).

It is clear that a number of central nervous system (CNS) agents, including drugs of abuse, can inhibit reproductive function. This is especially true since young men and women of reproductive age are the segment of the population most heavily involved in drug abuse (Smith and Ricardo, 1987). There is convincing evidence that the drugs of abuse can disrupt neuroendocrine and gonadal function with sufficient magnitude to infertility and sexual dysfunction (Conrad and Ross, 1985). More recently, the dramatic increase of cocaine abuse during pregnancy has led to the growing concern about its effect on the exposed fetuses (Plessinger and Wood, 1991). Women using the cocaine during pregnancy had a higher incidence of spontaneous abortions and abruption placentae (Acker *et al.*, 1983). Related reports from animal studies (Mahalik *et al.*, 1980; Fantel and Macphail, 1982) explained toxic effects of cocaine used during pregnancy and teratogenic effects on the fetus and newborn. According to most teratologist, the preimplantation period is thought to be of little relevance for the study of embryotoxic mechanism. One reason may be that organogenesis has not begun, and insults to the late preimplantation embryo are either lethal or completely harmless (Austin, 1973; Wilson, 1977). Recent studies indicated that treatment of preimplantation embryo may not result in the alternatives of death or normal development, but rather in abnormal development (Kajiwara and

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Inouye, 1986; Olsen and Storeng, 1986; Tam, 1988; Yu and Chan, 1988). Up to the present, though the effects of cocaine on fetuses have been well documented, little is known about its effect on preimplantation embryos.

The purpose of this study was to examine the susceptibility of preimplantation embryo for the influence of cocaine treatment *in vivo*.

EXPERIMENTAL METHODS

Materials

Cocaine hydrochloride was obtained from Merck Co. HCG and pregnant mare serum gonadotropin (PMSG) were purchased from Intervet Co. (Holland). All other reagents used were special grade.

Animal Treatments

ICR female mice of 6 to 8 weeks old were obtained from laboratory animal breeding center in Kangwon National University or Seoul National University. They were placed in quartine room and they were housed in plastic cages for 2 weeks before handling. Samyang Laboratory Rodent chow and tap water were provided *ad libitum*. The animal room was maintained at approximately 20℃-25℃ with a relative humidity of 50%-55 % and 12 hours of light-dark cycle.

The control sroup received saline solution by s.c. The testing groups received cocaine (10 mg/kg or 20 mg/kg in saline) daily s.c. injection for 2 weeks. Oocytes and fertilized eggs were collected after 2 weeks of cocaine injection.

Superovulation and Mating

After last cocaine injection, mice were superovulated with a intraperitoneal injections (i.p.) of 5 I.U. PMSG followed 44-45 hrs later with an injection of 5 I.U. hCG. They were placed with males and mating was confirmed the following morning by the presence of a vaginal plug. Noon on the day of the vaginal plug was considered by 0.5 of pregnancy.

Collection of Fertilized or Non-fertilized Eggs

Oocytes from non-mated female mice and 2 to 8 cell embryos were collected to examine the cocaine effect on morphological changes of oocytes. Mice were killed 12-14 hrs or 24-48 hrs after hCG injection by cervical dislocation. Oviducts were cut out from reproductive organs. The connective tissues and blood clots were removed from the oviducts. Fine oviducts were transfered into watch glass containing M16 medium, and then eggs were collected and examined (Whittingham, 1971; Mcgaughey, 1978; Gates, 1965).

The developmental and morphological changes of eggs were examined by light microscope (40-100x).

In vitro Culture of Fertilized Eggs

The drops (100 μl) of M16 medium supplimented with bovine serum albumine (BSA) was covered with sterilized paraffin oil in small culture dish (Fallcon, USA) and was equilibrated for 2 hours before culture with 5% CO_2 in air at 37℃. All of 2- and 8-cell embryos were examined for development to the blastocyst at 4-5 days (Smithberg and Ricardo, 1982; Chen and Hsu, 1982). Medium was changed every 2 days.

Statistical analysis

The differences in response between control and cocaine-treated embryos were evaluated by the Chi-square test.

RESULTS

Effects of Cocaine on Morphological Integrity of Oocytes After Superovulation

As shown in Table I, 92.9% of the oocytes in the control was intact in oocytes cytoplasm. The groups treated with 10 mg/kg and 20 mg/kg of cocaine had no significant effect on the integrity of oocytes.

Effect of Cocaine on Developmental Capacity of the Embryos After Mating

As Table II and Fig. 1 indicated, 66.7% of the oocytes in control group was developed into 2-8 cell sta-

Table I. Effect of cocaine on morphology of ooctytes collected at 12-14 hours after hCG injection

Cocaine Dose (mg/kg)	No. of mouse	No. of oocytes	No. of oocytes (%)	
			Normal cytoplasm	Degenerated cytoplasm
0	7	85	79 (92.9)[a]	6 (7.1)[a]
10	8	90	77 (85.6)	13 (14.4)[a]
20	8	88	80 (90.9)[a]	8 (9.1)[a]

Same superscripts within coloum are not different, $p<0.05$

Table II. Effect of cocaine on early development *in vivo* of mouse embryos collected at 24-48 hours after mating

Cocaine (mg/kg)	No. of mouse	No. of oocytes	No. of oocytes (%)6	
			2-8 cell	degenerated
0	7	84	56 (66.7)[a]	28 (33.3)[a]
10	8	72	9 (12.5)[a]	63 (87.5)[b]
20	8	88	24 (27.3)[b]	64 (72.7)[b]

Different superscripts within column are diferent, $p<0.05$

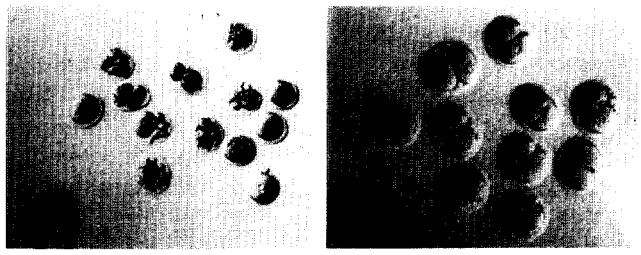

Fig. 1. Early development *in vivo* of mouse oocytes 24 hrs after natural mating. 1) Noncleaved and degenerated oocytes in cocaine 10 mg/kg group 2) Normal cleaved oocyte.

Table III. EFfect of cocaine on blastocyst development of 2- to 8-cell *embryos in vitro*

Cocaine (mg/kg)	No. of mouse	No. of[1)] embryos	No. of blastocyst (%)
0	6	49	46 (93.9)[a]
10	5	54	38 (70.4)[a]
20	5	64	46 (71.9)[a]

[1)] 2 to 8 cell embryos collected at 24-48 hours after mating. Different supersripts within column are different, $p<0.05$.

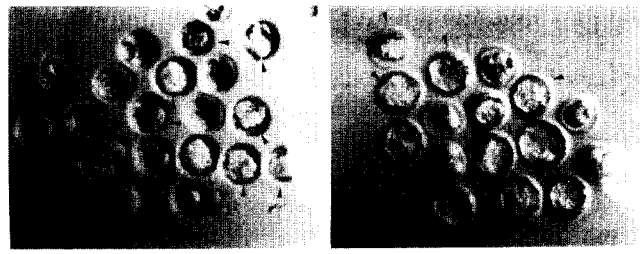

Fig. 2. Blastocysts (arrowheads) developed from 2 cell and 8 cell embryos after 96 hrs culture *in vitro*. The formation of blastocysts in the presence of cocaine (20 mg/kg)[1)] was significanlty inhibited compared with that in the absence of cocaine[2)].

ges. Developments from oocytes to 2-8 cell stages were significantly inhibited by exposured cocaine insults (10 mg/kg and 20 mg/kg) ($p<0.05$).

Effects of Cocaine on Blastocyst development *In vitro*

In control group, 93.9% of fertilized eggs was developed to the blastocyst stage, whereas both cocaine groups (10 mg/kg and 20 mg/kg) were significantly interfered with the development of blastocyst during 3-4 days culture (Table III and Fig. 2) ($p<0.05$).

DISCUSSION

This study indicated the influence on early embryo by episode of cocaine intoxication before implantation. We found that preimplantation mouse embryos cultured *in vivo* and *in vitro* were highly sensitive to cocaine exposure. As shown in Fig. 1, cocaine lost the capacity to cleave at a normal rate although nonferitilized eggs in the presence of cocaine maintained their morphologic architecture. However, the etiologies for cocaine's effects under the present experimental system are not ready to reply. According to Kajiwara and Inouye (1986), abnormal embryos in this experiment were defined as underdeveloped embryos (not reaching blastocyst stage). However, in our study necrotic preembryos were not found as shown by cadmium treatemtn (Yu *et al.*, 1985; Yu and Chan, 1986). It is well known that the presence of a sufficient number of trophoblast and inner cell mass (ICM) cells in the blastocyst is a prerequite for successful implantation and embryogenesis (Tam, 1988). In particular, the ICM is often preferentially affected by a variety of chemicals. The deleterious effect are usually manifested by the loss of ICM cells and a suppression of differentiation of ICM (Yu *et al.*, 1985; Eibs and Spielman, 1977; Spielman and Jacob-Muller, 1981). It has been suggested that embryogeneses would not occur if cellular loss (caused by either cell death or arrested cellular proliferation) exceeds a certain proportion of the ICM population. The significant impairment of blastocyst formation *in vitro* in this study raised the possibility that may relatively reduce ICM population in the cocaine group. Therefore, our results indicated that blastocyst in the presence of cocaine would have a reduced embryonic capacity and a poor ability of implantation in the uterus. It is imperative in future experiments to access the viability of ICM by vital staining (Hoppe and Bavister, 1984) for better evaluation of developmental potential of embryos. Our results showed that the embryopathic effects of cocaine were not dose dependent manner. Therefore, more intensive studies are needed to achieve better understanding on dose-dependency of cocaine exposure.

Finally, our study proposed the possibility that heavy intoxication of cocaine in female mice can induce deleterious effect on the preimplantation, whilst many other studies (Plessinger and Wood, 1991; Mahalik *et al.*, 1980; Fantel and Macphail, 1982; Tam, 1988; Eibs and Spielman, 1977; Sherif and Yehia, 1991; Nesrin, 1986) primarily have examined the cocaine effects on the post-implantation.

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