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Testicular Degeneration and Sperm Loss Induced by Chronic Administration of Cocaine in Mice

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The effect of cocaine on reproductive system were studied in male ICR mice. Daily injection of cocaine HCl (10, and 40 mg/kg, s.c for 15 days) disrupted epididymal sperm levels and testicular morphology. These disruptions were manifested in a decreased number of sperm, an increased number of dead sperm as compared with control vehicle, and were evidenced by vacuolations, multinucleated giant cell formations and coagulation necrosis in the testicular seminiferous tubules. Responses in the current study were trended in dose dependent pattern. These results suggest that chronic cocaine intoxication can perturb on male reproductive organ functioning.

Key words: Chronic cocaine intoxication, epididymal sperm level, testicular morphology, coagulation necrosis, reproductive organ functioning

INTRODUCTION

The escalating incidence of drug seeking behavior among juvenile delinquent in our society has become a major concern (Jhoo *et al.*, 1990a,b). Since adolescence is a critical development period of life, heavy drug use and involvement in juveniles have a greater negative impact than they do in adults.

The physiological events associated with normal adolescent development rely on the maturation of hypothalamic centers that controls the release of gonadotropin releasing hormone (GnRH). Most study indicated that drug abuse can interfere with the secretion of GnRH (Smith and Ricardo, 1987). Thus, drug abuse by adolescents may result in delayed or arrested repoductive development. Recently, cocaine abuse is significantly increasing (Madden *et al.*, 1986; Chavez *et al.*, 1989). Nevertheless, the impact of cocaine use on male reproductive system development has not been clearly answered. Since the major drug action of cocaine involves blocking reuptake of norepinephrine at nerve terminal, norepinephrine remains at the point of release at the nerve terminal and/or release into the blood stream. Norepinephrine binds to vascular α_1 and α_2 receptors, which produce smooth muscle constriction and vasoconstriction (Chavez *et al.*, 1989). Many of damaging effects of cocaine, therefore, may be due to vasoconstriction. Cocaine will increase luteinizing hormone (LH) at lower doses, whereas LH release is inhibited at higher doses. This should lead, respectively, to an increase in testosterone at low doses and a decrease at higher doses (Burul and Harclerode, 1989). Testosterone is necessary for spermatogenesis and cocaine or its metabolites may directly affect early sperm development (Johnson *et al.*, 1984). Testicular function also could be detrimentally influenced by reduced blood flow. Therefore, the potential for harmful effects of cocaine on the male reproductive system should not be overlooked.

The objective of this study is to investigate the association of cocaine use with male reproduction measured by epididymal sperm level and testicular morphology.

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EXPERIMENTAL METHODS

Materials and Animal Treatment

ICR male mice of 4 to 8 weeks old weighing 20± 1 grams were housed at 22°C in a 12h light: 12h dark photoperiod. Laboratory Rodent chow and water were ad libitum. Three concentrations (0, 10, and 40 mg/kg) of cocaine HCl (Merk Co.) were dilluted, using sterile saline as the solvent. The dosages were calculated for the free amine from the drug. However, the animals

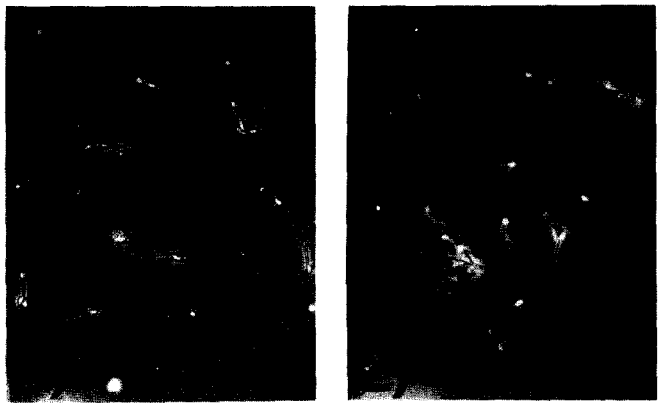

Fig. 1. Identification of sperm using by supravital staining method.
(1) ☆ means live sperm; (2) ✡ indicates dead sperm (100×).

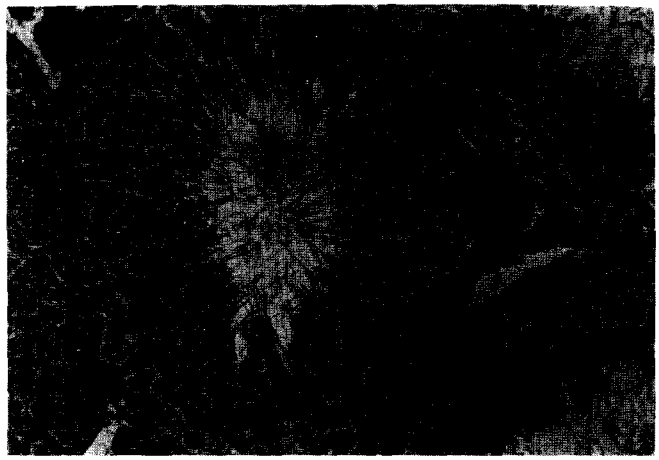

Fig. 2. Section of a testis from a control mouse. The seminiferous tubules show full spermatogenesis. (400×)

were subcutaneously injected with the hydrochloride salt or vehicle alone. The animals received 15 daily injections and each animal was sacrificed by time schedule after last injection.

Determination of Sperm Number

For the sperm count, the suspensions were prepared as follows: epididymis were minced with fine scissors in 3% NaCl solution and following vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80 μm stainless steel mesh, the sperm number per ml was determined with hemocytometer count (Bruce *et al.*, 1974).

Dead Sperm Assessment

The percentage of stained spermatozoa was determined according to the supravital staining method using eosin and nigrosin, as described by Eliasson (Eliasson, 1977). As shown in Fig. 1, dead sperm heads were stained blue, while live ones were not. The nigrosin only provided a background in the stain.

Fig. 3. Tubular degeneration evidenced by multinuclear giant cell at days after final injection in cocaine 10 mg/kg group. (400×) Note the tubules with spermatogenesis.

Histopathology

Testes used for histocytology were fixed in Bouin's solution, embedded in paraffin wax, and sectioned at 5 μm. After hematoxyline and eosin staining, the sections were examined by light microscopy.

Statistical Analysis

The data were analyzed using PROC GLM (SAS, 1990). To examine the changes of total and dead sperm numbers over time, regression analysis were employed. The graphic presentation was illustrated in Fig. 6. The equations used were as:

$$y = b_0 + b_1 x + b_2 x^2 + e \quad (1)$$

where: y = response variable (No. of total, or dead sperm)
x = Time (hr)
b_0 = intercept
b_1, b_2 = regression coefficients
e = random residual

To compare the differences among the treatment groups for a given time interval, the following model was used:

$$y_{ij} = \theta + \tau_i + e_{ij} \quad (2)$$

where: y_{ij} = response variable (No. of total, or dead sperm)
θ = overall mean
τ_i = i^{th} effect of treatment (control, 10 mg/kg, 40 mg/kg)
e_{ij} = random residual

Fig. 4. Section showing marked tubular vacuolation 2 days after final injection in cocaine 10 mg/kg group. Note the abscence of spermatozoa. (400×)

Fig. 5. Representative section of a testis 7 days after final injection in cocaine 40 mg/kg group showing coagulation necrosis with vacuolation and complete absence of spermatozoa. (100×)

Comparisons between control and treatment groups were made for each time interval. Least Significant Difference (LSD) test was applied for the multiple mean comparisons among the groups. The results were presented in Fig. 6.

RESULTS

The Effects of Cocaine on Total Sperm Number

In control group, the sperm number did not change as time progressed. However, the sperm number of the groups treated with 10 mg/kg and 40 mg/kg of cocaine highly significantly ($p<0.01$) decreased over time compared with that of control group. No significant differences, however, were observed between the two treatment groups of 10 mg/kg and 40 mg/kg.

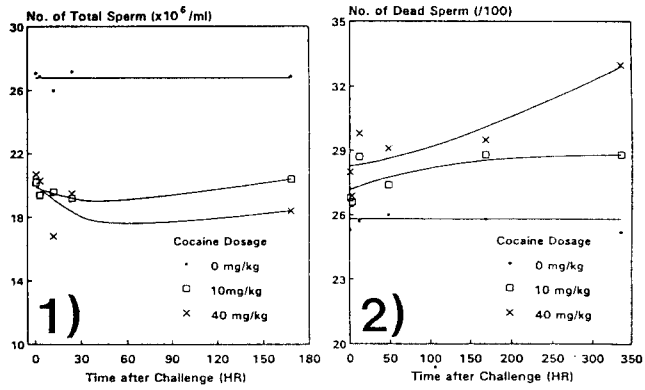

Fig. 6. Changes of total and dead sperm number in the epididymis over time for different cocaine doses. The estimated regression equations are (1) $y=19.9-0.0349x+0.000226x^2$ ($R^2=0.75$) of cocaine 10 mg/kg and $y=20.06-0.083x+0.000434x^2$ ($R^2=0.24$) of cocaine 40 mg/kg, and (2) $y=27.2+0.0142x-0.00003x^2$ ($R^2=0.53$) of cocaine 10 mg/kg and $y=28.3+0.0056x+0.000025x^2$ ($R^2=0.79$) of cocaine 40 mg/kg.

The Effects of Cocaine on Number of Dead Sperm

The number of dead sperm in control group also did not change as time progressed. The number of dead sperm in treatment group of 10 mg/kg were dramatically increased ($p<0.01$) at 12 hr and 336 hr in comparison with that of control group. And also more significant trend was found in the treatment group of 40 mg/kg. Especially, the number of dead sperm in the treatment group of 40 mg/kg was highly significantly increased ($p<0.01$) compared with that of 10 mg/kg at 336 hr after completion of cocaine injection.

The Effects of Cocaine on Testicular Morphology

Testes of control group did not show any pathologic degeneration. The distribution of microscopic findings occured in a haphazard pattern: some tubules had severe degenerative changes, while in nearly tubules the changes were minimal. Early changes followed by final injection of cocaine consisted of irregular vacuolated cytoplasmic swelling of the spermatids. In late stages after last cocaine injection, coagulation necrosis in tubules was observed (Fig. 2-5).

DISCUSSION

The important finding of this study was that prolonged cocaine use had marked effects on the density of sperm in the epididymis as well as considerable cytotoxic effects within the testes itself. The mammalian epididymis is important for the normal maturation of sperm (Soufir *et al.*, 1989). This process include strucutral stabilization of the sperm, changes in sperm

motility patterns and energy metabolism, and the acquisition of fertilizing capacity (Calvin and Bedford, 1971; Hoskins *et al.*, 1978). Any significant interference in the normal maturation process and/or storage which the sperm undergo in the epididymis could interfere with the fertilizing potential (Working *et al.*, 1985). Therefore, it was examined epididymal sperm concentration to achieve better understanding of fertilizing potential in this study. As shown in Fig. 6 of regression relationship, the trend of changes of total sperm numbers and dead sperm numbers was closely coincided. Since time dependent response from the group of 40 mg/kg was low ($R^2=0.24$), further research is needed such as defining the ultrastructure of sperm itself due to frequent observations of abnormal sperm morphology caused by a typical reprodotoxicant of tetrahydro cannabinol (THC) (Zimmerman *et al.*, 1979; Dixit *et al.*, 1974; Dixit *et al.*, 1977).

Apparent reduction in testosterone production may possibly be involved in the lowering of production (Johnson *et al.*, 1984). Testosterone is required for the completion of meiosis during spermatogenesis and sperm production rates are closely related to the percentage of cell loss during postprophase of meiosis (Johnson *et al.*, 1981). A reduced production of testosterone could lower sperm production rates by incerasing cell loss during meiosis. Epididymal sperm maturation also requires the secretion of testosterone within the epididymis (Johnson *et al.*, 1984). Thus, it is possible that lowered concentration of epididymal testosterone could have interfered with the normal maturation of sperm. The present results indirectly confirm the findings of Berul and Harclerode (1989) and Gordon and Mostofsky (1980) as reflected by hormonal study. Therefore, cocaine could act by inhibiting gonadotropin secretion, by suppressing spermatogenesis, or by interfering with sperm maturation and storage (Wong *et al.*, 1987).

The absolute weights of testes and sex accessary organs in this study were found in severe variation, although it was known that the toxic testicular weight is less than that of a normal animal (Ribelin and Calif, 1963). The present study is shown that confirms the pathologic changes within testicular seminiferous tubule by chronic cocaine insults. Cocaine administration in this study produced considerably lasting histopathologic effects, which was evidenced by giant cell formation, vacuolation usually associated with fatty change in other organs, and coagulation necrosis in mice testes, may leads to a prolonged period of oligospermia. It is proposed that the toxic effects of cocaine may in part be mediated through a direct effect on epididymal stored spermatozoa. Especially, in the high dose of cocaine (40 mg/kg), epididymal sperm loss and testicular damage were clearly exampled. Additionally, it must be reflected that endocrine component of the testis exhibits rhythm fluctuations according to season and environmental conditions (Johnson, 1991; Fentes *et al.*, 1991). Although this study did not permit an evaluation of precise mechanism, it was concluded that cocaine could ultimately affect male reproductive function.

The possibility of an adverse effect of cocaine intoxication and/or cocaine abuse on male reproductive function in man is alarming. Equally importantly, cocaine induced vasoactive effect (Chasnoff *et al.*, 1988; King and Prescott, 1978; Moerman *et al.*, 1984) on genitourinary tract explained by prostatic hypoplasia, urethral obstruction and cryptoorchidism, must be considered for better understanding of the pathogenesis.

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