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Research article

SPERM MATURATIONAL DEFECT AFTER CYCLOPHOSPHAMIDE TREATMENT

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ABSTRACT: During normal spermatogenesis, most of the round spermatid's cytoplasm was phagocytosed as 'residual bodies' by the Sertoli cell at spermiogenesis, and only a small cytoplasmic residue i.e. 'cytoplasmic droplet' remains applied to the elongated spermatid after release from the germinal epithelium. A characteristic morphological change on spermatozoa during epididymal transit was the caudal migration of the cytoplasmic droplet away from the neck via the principal piece, however, while studying the Cyclophosphamide (CPA) induced sperm morphological changes from the cauda epididymis in male Wistar rat, *Rattus norvegicus* using phase contrast microscope it was noticed that the sub-chronic and acute doses of CPA caused retention of cytoplasmic droplet on the mid-piece. Thus from the foregoing it was concluded that beside CPA being an inhibitor of spermatogenesis, it also interferes with the maturation of spermatozoa by the retention of cytoplasmic droplet perhaps due to alteration in epididymal secretory and absorptive functions thus leading to infertility.

Keywords: Cyclophosphamide, cytoplasmic droplet, epididymis, sperm maturation, infertility.

INTRODUCTION

The cytoplasmic droplet of epididymal spermatozoa was a small localized outpunching of cytoplasm on the tail of unknown significance. Generally a greater proportion of spermatozoa looses their cytoplasmic droplet during its transit from corpus to cauda epididymis. This indicates that spermatozoa maturation takes place between the corpus and cauda epididymis since loss of cytoplasmic droplet was considered as an index of spermatozoa maturation in mammals (Bedford, 1975). Further, the location of cytoplasmic droplet changes from proximal to the distal end of the mid-piece in an increasing percentage of spermatozoa during their epididymal transit (Cortadellas and Durfort, 1994; Cooper and Yeung, 2003). While studying the effect of an anticancer drug Cyclophosphamide on the male reproductive organs in Wistar rat *Rattus norvegicus* the authors observed retention of cytoplasmic droplet and their progressive decline and shift in position from the middle piece to principal and to the distal end of the tail, and these changes are dose dependent.

MATERIALS AND METHODS

Drug

The anticancer drug Cyclophosphamide (Endoxan-N, CAS no. 50-18-0), with the chemical formula $C_7H_{15}Cl_2N_2O_2P$ and molecular weight, 261.086 g/mol. manufactured by Candila Healthcare Limited, Goa was used for the present experiments.

Animals

Male Wistar rat *Rattus norvegicus* weighing between 250-300g were obtained from Department of Biochemistry, RTM Nagpur University, Nagpur. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended procedures by fulfilling all the necessary ethical standards. They were fed *ad libitum* with standard pellet diet and had free access to water and kept on a 12hrs light-dark cycle.

Treatments

Animals were allowed to acclimatized for a period of week before being treated. They were selected randomly and divided into three groups with six animals in each group. Vehicle-treated control (Group-I), 5mg and 15mg/KgBW/day for 15days as a sub-chronic dose (Group-II) and 70mg and 100mg/KgBW once 7 days (Group-III). The drug was administered intraperitoneally.

Epididymal Sperm Analysis

The animals were sacrificed using chloroform 24 hours after the last day of each experiment. The spermatozoa present in the right cauda epididymidis were collected after mincing/slicing the tissue in a cavity block containing 1ml of physiological saline centrifuged at 600rpm for one minute (WHO, 2010) and observed under phase contrast microscope. Sperm count was done by using Neubauer's haemocytometer. The sperms from the minced epididymis were counted in five thoma ruled chambers after charging the haemocytometer with solution and calculated by using the formula 50,000nd where 'n' was the number of sperm and 'd' was the dilution which was 1ml. The cytoplasmic droplet were counted and its percentage was calculated.

Statistical Analysis

Statistical analysis were reported in terms of mean ±SEM. Difference between the groups was statistically determined by Student 't' test (Delgaard, 2008). The average data generated for the group of rats treated with Cyclophosphamide were compared with data on vehicle-treated control group of rats. A significant level of $P<0.05$ was accepted.

RESULTS

Table-1 depicts the vehicle-treated control sperms from the right cauda epididymis, either with no or few cytoplasmic droplets but an increase in their percentage of retention on the mid-piece, end of mid-piece and on the distal end of the tail after CPA treatment (Figs.1 and 2).

Table-1: The percentage of cytoplasmic droplets and sperm count after CPA treatment. (Figures in parentheses are number of animals used).

Group	Treatment	Sperm condition	Percentage of sperm with cytoplasmic droplet	Change in position of cytoplasmic droplet on the sperm
I	Vehicle-treated control (6)	Normospermic	5.06%	Few cytoplasmic droplet either on mid-piece or distal end.
II	5mg/KgBW CPA for 15days (6)	Oligozoospermic	47.73%	On the mid-piece and at the end of mid-piece
	15mg/KgBW CPA for 15days (6)	Oligozoospermic /azoospermic	58.16%	On the mid-piece, at the end of mid-piece and on the principal piece
III	70mg/KgBW CPA once 7days (6)	Oligospermic	20.99%	On the distal end of the tail
	100mg/KgBW CPA once 7days (6)	Oligospermic	23.60%	On the distal end of the tail

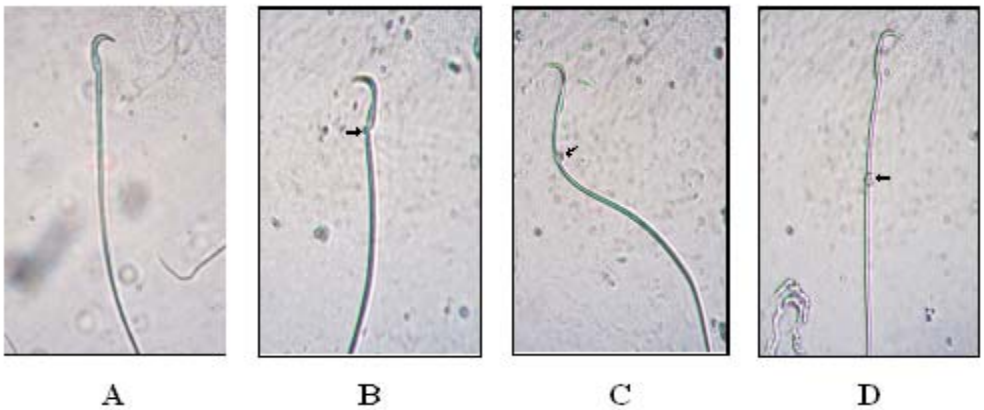

Figure 1: Phase contrast photograph of A) Vehicle-treated control sperm and CPA treated sperm showing, retention of cytoplasmic droplet B) at mid-piece, C) on the principle piece D) at mid-piece.

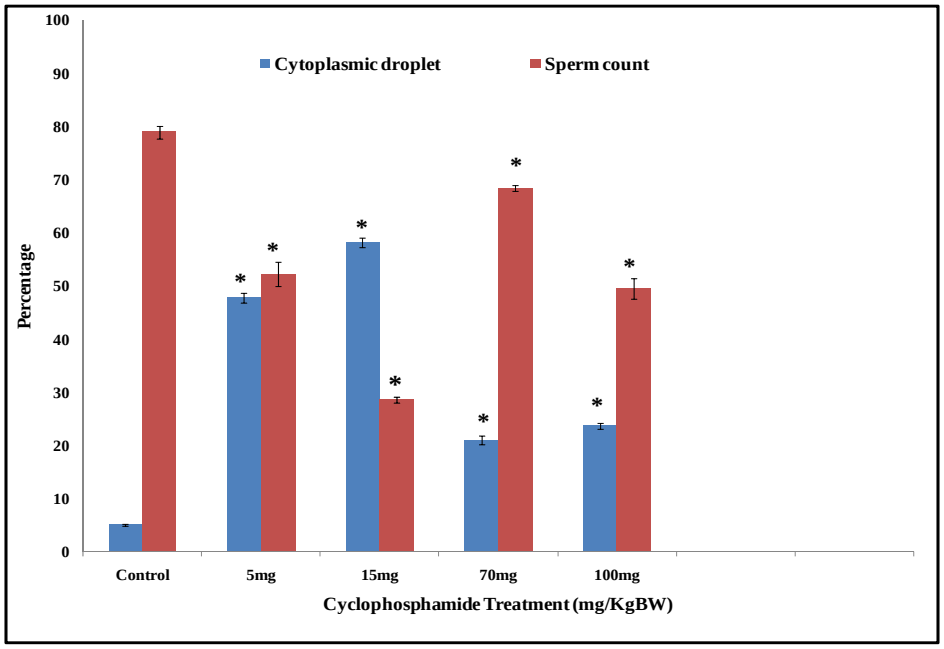

Figure 2: **Percentage of cytoplasmic droplet and sperm count after various doses of CPA. Values are expressed as ± SEM. *P < 0.05.**

DISCUSSION

During spermiogenesis the spermatozoa eliminate most of their cytoplasm, and the organelles become closely packed. Attachments between structural components must be significant to maintain cell integrity in the uniformly designed and vigorously motile spermatozoa (Cortadellas and Durfort, 1994; Yu et al. 2005). In most species studied such as in Guinea pig, in Ericsson, in rabbits, in ram, in bull and in lambs there was convincing evidence that cytoplasmic droplet migrates along the mid-piece from neck to annulus during proximal epididymal transport and remains on the majority of cells in the epididymis (Cooper and Yeung, 2003) but no report was available on the percentage of retention of cytoplasmic droplets.

In the present piece of work the location of the cytoplasmic droplets changes from the middle piece to principal and to the distal end of the tail, and these changes are dose dependent. Our observations are supported by following workers who studied retention of cytoplasmic droplets after anticancer drug treatment (Kaur et al. 1997; Cooper, 2006; Suzuki-Toyota et al. 2004 and Suzuki-Toyota et al. 2007). Thus the results show that CPA which was an inhibitor of spermatogenesis also interferes with the maturation of spermatozoa perhaps due to alterations in epididymal secretory and absorptive functions and due to decline in the level of serum testosterone.

The droplet lost at ejaculation was necessary for fertility (Cooper et al. 2004; Cooper and Barfield, 2006; Hinton and Cooper, 2010) due to poor adherence to the zona pellucida (Thundathil et al. 2001) and oviduct epithelium (Khalil et al. 2006). Most reports relate to the retention of proximal droplets (at the neck), indicative of a failure of normal epididymal maturation. This occurs in young bulls where success rates of IVF are below normal, despite the selection of spermatozoa, and the situation improves as the male ages (Amann et al. 2000). Further analysis reveals that the reduced cleavage and fertility rates reflect poor passage through hyaluronate swim-up medium and a failure to bind to the zona pellucida (Thundathil et al. 2001). In the mouse, too, infertility in the male was associated with retention of the droplet on sperm in the uterus of females mated to *c-ros* knockout males (Yeung et al. 2000).

There are also many reports indicating that the presence of excess residual cytoplasm on spermatozoa was associated with poor sperm function. For example, the presence of a 'cytoplasmic droplet' on human spermatozoa was associated with infertility in men who smoke (Mak et al. 2000) or who suffer varicocoele (Zini et al. 2000) and the retention of such a droplet was related to a shorter axoneme (Gergely et al.1999), poor sperm motility (Zini et al. 1998), abnormal head and mid-piece morphology (Huszar and Vigue, 1993; Gomez et al. 1996; Gergely et al. 1999), lower fertilizing capacity (Keating et al.1997), reduced binding to the zona pellucida (Huszar and Vigue, 1994; Ergur et al. 2002), greater extents of chromatin breaks and DNA damage (Fischer et al. 2003) and increased chromosomal disomies (Kovanci et al. 2001). We have also observed shorter tails, poor motility, abnormal head and mid-piece morphology, nucleus defects with CPA treatment.

CONCLUSION

Thus from the foregoing it was concluded that CPA which was an inhibitor of spermatogenesis also interferes with the maturation of spermatozoa perhaps due to alterations in epididymal secretory and absorptive functions thus leading to infertility.

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