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# 5-FLUOROURACIL (5-FU) INDUCES THE FORMATION OF GIANT CELLS AND SLOUGHING OF SEMINIFEROUS EPITHELIUM IN THE RAT TESTIS

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**Abstract :** The chemotherapeutic agent, 5-fluorouracil (5-FU) has been widely used in the treatment of a variety of cancers. Its effect on the testis has not been substantially studied. Present study was conducted to evaluate the gonadotoxicity of 5-FU in male albino rats. Animals were injected with single dose of 5-FU (10, 50 and 100 mg/kg, i.p.) and sampled on 1, 3, 15 and 30 day post exposure. Animals were anaesthetised, testes were perfusion fixed by Bouin's fluid. Five micron thick paraffin sections were stained with haematoxylin and eosin. Slides were screened for the incidence of partially and extensively sloughed tubules. Data were analysed by Mann Whitney 'U' test. Only 100 mg/kg induced multinucleated cells on 3rd day. All doses of 5-FU induced sloughing of the seminiferous epithelium. Maximum number of partially sloughed tubules were seen on third day. Partial sloughing was not dose dependent except on 15th day. The extensive sloughing was dose dependent except on 30th day. The result indicates that all the doses of 5-FU tested in this study cause sloughing of epithelium and only 100 mg/kg induces the formation of giant cells on third day.

**Key words :** 5-fluorouracil sloughing seminiferous epithelium testis

## INTRODUCTION

The chemotherapeutic agent 5-FU and its metabolities inhibit enzyme thymidylate synthetase and get incorporated into DNA or RNA (1). This results in spontaneous lesions in nucleic acids, leading to single strand breaks and cell death (2). 5-FU has been found to be clastogenic in paternal germ cells, but not in the maternal germ cell lines (3, 4). Russell and Russell (5) reported that 5-FU has no effect on rat testis

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earlier than 21 days post exposure during which it produced spermatid arrest, malorientation of spermatid and abnormally positioned manchette in cells. It is not known, whether 5-FU has any early effect on synchronisation of seminiferous epithelium. The early effects such as sloughing of germ cells would lead to atrophy of seminiferous tubules. This finding will be of importance as there is increasingly more cancer survival rates and accompanied infertility (5). The aim of the present study was to elucidate the effects of 5-FU on the seminiferous epithelium and we report that it causes sloughing and formation of multinucleated giant cells in the epithelium of rat testis.

## METHODS

Male Wistar rats (90–100 days old), were maintained under standard laboratory conditions with free access to food and water ad libitum. On the day of experiment, the animals were segregated into 13 groups with 5 animals in each group. A single dose of 0.1 mL distilled water was injected (i.p.) to one group which served as control. Remaining 12 groups were given the single injection of various doses of 5-FU (Fluracil; Biochem). Four groups recieved 10 mg/kg, another four groups 50 mg/kg and remaining four groups were injected 100 mg/kg intraperitoneally. Four sample periods were selected arbitrarily for 1, 3, 15 and 30 d post exposure. On the day of biopsy the animals were anaesthetised (Pentobarbital sodium, 40 mg/kg; Sigma chemicals) and the mediastinum was opened. Perfusion fixation was started by injecting heparinised (1 mL/L) normal saline through the left ventricle to wash out the blood from the vessels. This was followed by the injection of Bouin's fluid until the testes turned yellowish (6). Laparotomy was conducted and testes along with the epididymes were removed. After the removal of tunica vaginalis from the testis the organs were immersed in the Bouin's fluid for 10 h. They were removed and cut into 3-4 mm thick blocks and placed in 70% alcohol for 5 days with the daily change of alcohol. Further, the tissue was processed for paraffin embedding (7). Five micron thick sections were obtained and stained with heamatoxylin and eosin according to the standard procedure (8).

Slides were coded and analysed for qualitative changes in the tubules. The preliminary study revealed that 5-FU caused sloughing of epithelial cells into the lumina of tubules. Hence, the incidence of partially and completely sloughed tubules was studied. Partially sloughed tubules were the one in which the surface cells were separated, or the epithelium was separated in the middle partially. Extensively sloughed tubules were showing complete or more than 50% of epithelial cells exfoliated into the lumen. From each animal, 5 sections cut at 10 micron apart were screened according to the method of Allard et al (9). One hundred seminiferous tubules were counted/section and sloughed tubules were classified into two types as mentioned above. Average of 5 readings provided the percentage of partially and extensively sloughed tubules for that animal.

Statistical analysis was done by Mann-Whitney 'U' test and $P<0.05$ was considered as the level of significance.

## RESULTS

The results obtained in the present study revealed that 5-FU induced disintegration of seminiferous epithelium. Only 100 mg/kg induced formation of giant cells on third day (Fig. 1). Giant cells were usually multinucleated ones however, mononucleated cells were also present. Another effect of 5-FU was distortion of seminiferous epithelium in terms of sloughing. In the control rats number of seminiferous tubules showing partial sloughing was only $3.40 \pm 0.51$. There were no extensively sloughed tubules in the control. The animals which have recieved lowest dose (10 mg/kg) showed partial sloughing on day 1 itself, and

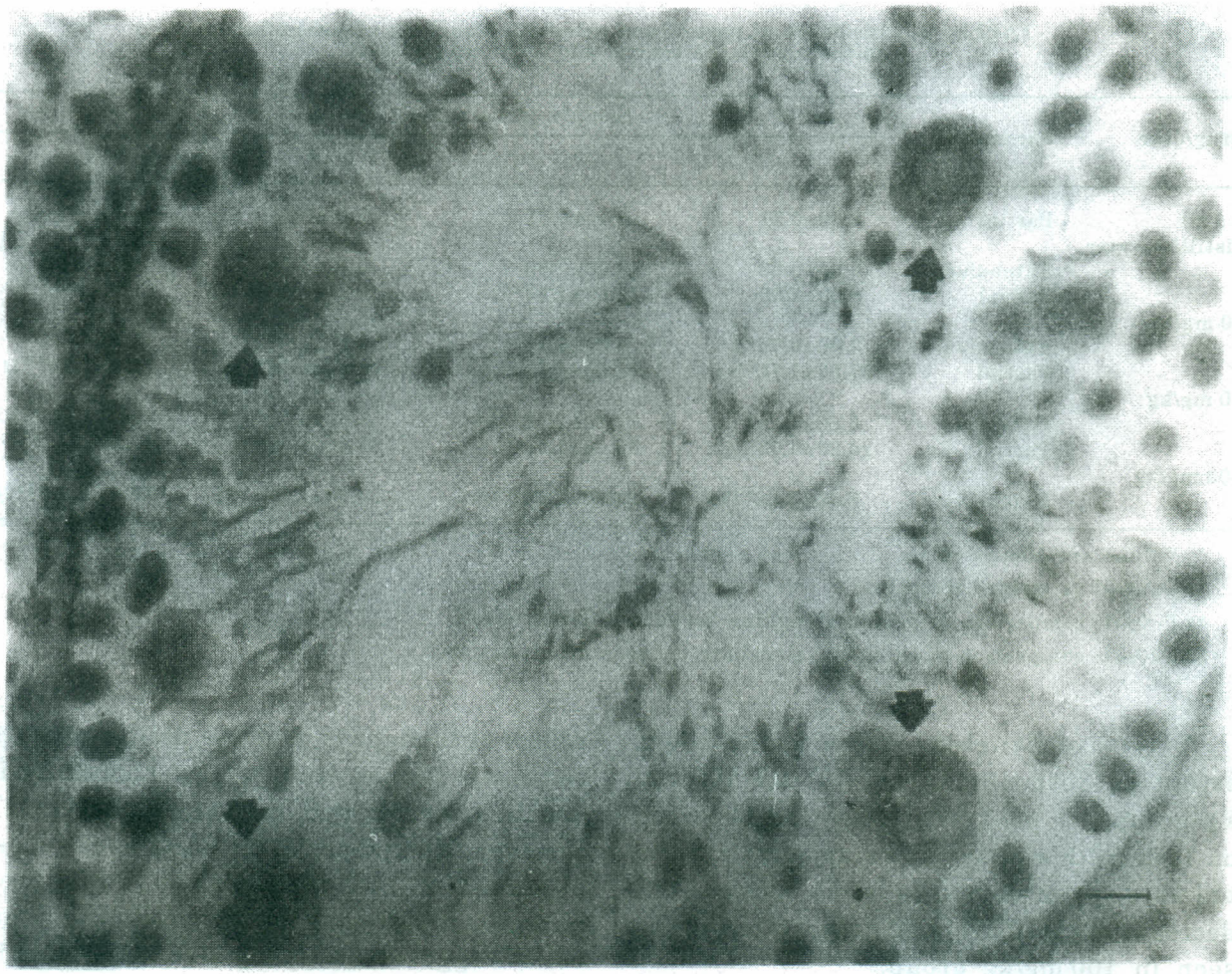

Fig. 1: Shows the multinucleated giant cells (arrows) in a seminiferous tubule of rat testis treated with 100 mg/kg 5-FU and sampled on third day. Note that the tubule is showing extensive sloughing with a few germ cells seen in the tubule. H&E, scale bar = 14u.

on day 3 more tubules exhibited partial sloughing (P<0.01). There was a peak in the incidence of partially sloughed tubules on third day. In 50 mg/kg group, there was another rise on 30th day. Third day onwards, the partial sloughing was reduced in 100 mg/kg group. However, lower doses caused partial sloughing variably from third day onwards (Table I).

TABLE I: Incidence of partial and extensive sloughing of seminiferous epithelium in the control and 5-FU treated rats.

| Drug/dose | Parameter | Sample period (days) | | | |
|---|---|---|---|---|---|
| | | 1 | 3 | 15 | 30 |
| 0.1 ml distilled water | Partial | 3.40±0.51 | 3.40±0.51 | 3.40±0.51 | 3.40±0.51 |
| | Extensive | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 10 mg/kg | Partial | 5.00±1.00 | 13.60±1.44** | 9.60±1.29** | 11.00±0.89** |
| | Extensive | 3.00±0.71** | 3.00±0.71** | 6.20±1.59** | 2.40±0.51** |
| 50 mg/kg | Partial | 27.80±1.74**†† | 38.60±4.76**†† | 18.80±0.86**†† | 41.80±1.28**†† |
| | Extensive | 5.60±1.29** | 17.80±2.78**$$ | 7.60±1.21** | 30.80±2.22**$$ |
| 100 mg/kg | Partial | 20.20±1.02**†† | 38.60±1.89**†† | 28.20±1.69**†† | 25.00±0.58**†† |
| | Extensive | 8.40±0.68**$$ | 24.80±2.40**$$ | 20.40±1.60**$$ | 16.20±1.43**## |

Values are Mean ± SEM

** Control vs treated P<0.01

†† P<0.01, comparison of partial sloughing between different doses.

$$ P<0.01, comparison of extensive sloughing between different doses.

Extensive sloughing was dose dependent except on 30th day. In 10, 50 and 100 mg/kg groups, a peak response was observed on 15th, 30th and 3rd day respectively. From third day onwards, a time dependent decrease of extensively sloughed tubules was seen in 100 mg/kg group.

However, this pattern was not observed in the animals treated with the other two lower doses.

## DISCUSSION

Appearance of giant cells is known to have association with the final common pathway of degeneration of germinal cells in animals treated with variety of chemicals (10, 11). The mechanism of formation of giant cells is uncertain, but, some authors believe that cell fusion of damaged spermatids is the reason (12). Another school of thought is that the multinucleated cells are formed due to the karyokinesis which is not followed by the cytokinesis (11). Further, the absence of mitotic figures, observation of membranous residues and residual plasma membrane in the giant cells suggest that they are formed due to the cell fusion (13). Giant cells with single nucleus are mostly degenerating cells and found in the lumen of the tubule. Hence, multinucleated cells are young and

mononucleated cells are the older in the process of degeneration. In the present study, only 100 mg/kg of 5-FU induced the giant cells on 3rd day, however, disappearing by 15th day. We presume that this disappearance is largely due to the exfoliation and drainage of giant cells away from the testis. As a support for this hypothesis, the abnormal cells have been found in the epididymes (D'Souza et al unpublished data). Nevertheless, this finding is in contrast to the previous reports on 5-FU (5), where even higher dose of the drug did not induce multinucleated cells.

The present study showed that the extent of partial sloughing was dose dependent only upto 50 mg/kg. Beyond this dosage the same response was not observed. This study also indicated that 5-FU or its metabolites have maximum effect on days 3 and 30 as reflected in the higher incidence of partially sloughed tubules on these two days. Further, the partial sloughing was more in 50 mg than in the animals treated with 100 mg. The reason for such discrepancy between these two doses is not known. Period response was discordant for both partial and extensive sloughing indicating that the extent of response was independent of doses at different time intervals. However, if not linear a time dependent decrease was seen in the percentage of sloughed tubules in 100 mg group from third day onwards. Dose response was seen in extensive sloughing upto 15th day, but by 30th day dose did not matter; however, the duration of exposure did.

In the present study, even low dose (10 mg/kg) of 5-FU damaged seminiferous epithelial harmony. This indicates that 5-FU or its metabolites affect either Sertoli cells or intercellular bridges between germ and Sertoli cells which results in the destruction of cellular association. Nakai and Hess (14) reported that epithelial sloughing was induced by chemicals which affect the microtubules in the Sertoli cells. Further, the destruction of vimentin filaments which are required as an anchoring device for the germ cells also initiates exfoliation of the epithelium (15). Hence, epithelial sloughing induced by 5-FU indicates that this drug affects the microtubules and anchoring device in the cells of seminiferous epithelium. The deformation of Sertoli cell nucleus was found in the tubules showing sloughing in rats treated with carbendazim (16). Whether 5-FU has similar effect on Sertoli cell nucleus is not known. The cellular anomalies and delayed release of spermatids in 5-FU treated rats (5), result in the increased incidence of abnormal spermatozoa and reduced sperm count. However, it is not possible to predict the effect of 5-FU on these two parameters based on the findings of present study. Hence, there is a need to conduct sperm morphology assay and sperm count in 5-FU treated rats to confirm our hypothesis.

The sloughed germ cells flow away from the testis towards the efferent ductules and epididymis. If this occurs in large scale, sloughed cells would block the efferent ductules inducing a back flow of cells towards the seminiferous tubules (17). This results in further destruction of

seminiferous epithelium and consequently increases tubular diameter finally causing tubular atrophy (18). In this regard, the extent of sloughing is more important than the number of tubules sloughed. We believe that, 5-FU induced sloughing does not initiate blocking of post testicular ductal system, therefore question of backflow of exfoliated cells does not arise. Favouring this opinion, the seminiferous tubular diameter was not found to be increased in 5-FU treated rats after a month. This indicates that such phenomenon is not involved with the toxicity of 5-FU (D'Souza et al, unpublished data). We conclude that even small dose of 5-FU is gonadotoxic in male rats. The toxicity is found in terms of epithelial sloughing and the induction of multinucleated giant cells by higher dose.

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