LIST OF PROFESSIONAL PUBLICATIONS


15. S. Banik, C. Deb and B. Paul: Possible Involvement of Pituitary in Mitomycin C Induced Inhibition of Testicular Steroidogenesis. Communicated to *Endokrinologie.*
Profound influence of mitomycin C (MC) on the alteration of testicular histology has been reported by many authors (Ehling - 1974; Bempong and Trower - 1973/75; Mohanta and Parida - 1976). According to Mohanta and Parida (1976), MC induced chromosomal aberration in spermatocytes due to the interaction of MC with the nucleoprotein complex of the chromosome. Kersten and Rauen (1961) reported the selective effects of MC on DNA in a bacterial system. In vitro studies suggest that MC blocked DNA synthesis by cross-linking with complementary DNA strands (Matsumoto and Lark - 1963). It is generally accepted that treatment with LH increases the average DNA content per nucleus of Leydig cells (Lee - 1960) and promotes increased production and secretion of testosterone (Brady - 1951). Recently, on the basis of histochemical studies, Banik et al. (1979) suggested that MC causes a reduction in testicular steroidogenesis in mature rats. The present paper is concerned to confirm the effect of MC specifically on testicular steroidogenesis by quantitative measurement of serum testosterone and simultaneously, an in vitro experiment was performed to evaluate the possible mode of action of MC regarding testicular steroidogenesis.

Materials and Methods

20 closed colony bred sexually mature male rats of 90 days age, weighing 140 - 150 gm were selected for the present investigation. They were housed at a constant lighting schedule of 12 hrs light per day and fed on a standard rat diet. The rats were divided equally into two groups. One group of rats received MC (intraperitoneally, 500 µg/kg) in distilled water, every alternate day for 20 days, and the other group of animals received only distilled water, in the same amount and in the same manner and treated as controls. Prior to sacrifice blood was drawn by cardiac puncture under light ether anesthesia and it was collected in the centrifuge tube. Blood was centrifuged and serum was separated and kept at -20°C until assayed. Serum testosterone was measured by radioimmunoassay following a method of Bardin and Peterson (1967). All the animals were sacrificed 24 hrs. after the last injection and the testes and sex accessory were immediately dissected out and their wet weights were recorded.

20 sexually mature male rats were used in an in vitro experiment for histochemical demonstration of 
\[ \Delta^\alpha 3\beta\text{-hydroxysteroid dehydrogenase (}\Delta^\alpha 3\beta\text{-HSD)} \] and glucose 6 phosphate dehydrogenase (G-6-PDH). The testes were incubated in Kreb-Ringer-Bicarbonate Solution (PH 7.6) at a temp of 37°C with a gas phase of 95% O₂ - 5% CO₂ (Brady - 1951).

Key words: Mitomycin C - steroidogenesis
The testis (both sides) of all the rats were equally divided into 4 groups (1, 2, 3, 4) and incubated in different containers. Group 1 serves as the control and added 0.9% sodium chloride solution whereas in group 2, 3 and 4, MC was added in different concentrations (50 μg, 100 μg, 200 μg/ml) in the incubation medium. The duration of incubation was continued for various lengths of time period viz., 0, 2, 4, 6 hours respectively. At the end of the respective incubation period, both control and MC treated testes were cut at 20 μm on a cryostat at -20°C. The frozen sections placed on coverslips were incubated for 30 minutes at 37°C for the histochemical demonstration of Δ5 3β-HSD activity using dehydroepiandrosterone as substrate and G-6-PDH activity using glucose 6 Phosphate as substrate (Deane et al. 1962) Parallel sections were incubated in corresponding substrate - free media and served as control. After incubation the sections were fixed in 10% neutral formalin and mounted in glycerol jelly for microscopic observation.

Results and Discussion

After treatment with MC serum testosterone level reduced significantly (Table 1) along with remarkably fall in the weights of testis and sex accessories (Table 1). The

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean testicular wt (mg ± SE)</th>
<th>Mean seminal Vesicle wt. (mg ± SE)</th>
<th>Mean Prostate wt (mg ± SE)</th>
<th>Testosterone ng/ml of serum (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: distilled water (10)</td>
<td>2563 ± 12 949</td>
<td>201 ± 16 499</td>
<td>221.95 ± 8.94</td>
<td>8.105 ± 0.06</td>
</tr>
<tr>
<td>Mitomycin C every alternate day for 20 days (10)</td>
<td>2143 ± 18 006</td>
<td>161 ± 4.22 266</td>
<td>164 ± 2.40 44</td>
<td>4.56 ± 0.16</td>
</tr>
</tbody>
</table>

Level of significance (P Value)*

- Control: Mitomycin C
  - P < 0.001
  - P < 0.001
  - P < 0.001
  - P < 0.001

Numbers within parenthesis indicate number of animals used

* Calculated by student t-test

The results of the present investigation are consistent with the previous findings of Banuk et al. (1979). In which paper, a histochemical observation was made on a key steroidogenic enzyme Δ5 3β-HSD activity after treatment with MC for a various lengths of period and suggested that MC suppressed testicular steroidogenesis. Recently Deb et al. (1979) have also been observed that MC caused a reduction in ovarian steroidogenesis. The consideration of the above findings corroborated our reports of the present investigation. Moreover quantitative measurement of serum testosterone following administration of MC lead to conclude firmly that MC reduces testicular steroidogenesis.
So far literature data are available it is observed that till now no attention was paid to delineate the probable mode of action of me in the alteration of testicular steroidogenesis whether directly or via the alteration in pituitary gonadotropin secretion. The in vitro studies on histochemical demonstration of testicular steroidogenic enzymes activity of the present paper showed (Table 2) that the addition of me (50, 100, 200 μg/ml) in the incubation medium resulted a diminution in the Δ⁵ 3β-HSD and G-6-PDH activity in the interstitial cells of the testes in comparison to control groups. The direct involvement of Δ⁵ 3β-HSD in the production of steroid hormones (Knorr et al. - 1976; Inano and Tamaoki - 1966) and the rate limiting role of G-6-PDH through the supply of NADPH for steroid hydroxylation have been reported earlier by a number of workers (Koritz and Peron - 1958; Savard et al. - 1963). Therefore, here again, it is suggested that me may directly reduced the testicular steroidogenesis through the suppression of steroidogenic enzyme activity.

The existence of hypothalamo-pituitary-testicular axis in the regulation of testicular steroidogenesis through the liberation of gonadotropin from the pituitary has generally been accepted now. A number of observations have been published regarding the mode of action of LH on testicular steroidogenesis and the review of the papers suggested that LH stimulation on testicular steroidogenesis possibly via the increased synthesis of DNA in the nucleus of the Leydig cells (Liu - 1960). On the other hand several authors reported that me has got a potent inhibitory action on DNA synthesis (Mohanata and Parida - 1976; Matsumato and Lark - 1963). Bamk et al. (1979) also reported that me reduced the Leydig cells nuclear area. So, on the basis of above discussion in connection with the present findings it is suggested that me suppressed testicular steroidogenesis possibly by its direct action but the involvement of pituitary cannot be excluded without further studies.

**Table 2: in vitro studies on histochemical activity of Δ⁵ 3β-HSD and G-6-PDH in the interstitium of testis after incubation with Mitomycin C**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 hrs</th>
<th>2 hrs</th>
<th>4 hrs</th>
<th>6 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ⁵ 3β-HSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>*+ + +</td>
<td>+ + +</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>+ + +</td>
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<tr>
<td>3</td>
<td></td>
<td>+ + +</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G-6-PDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>*+ + +</td>
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<td>2</td>
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<tr>
<td>4</td>
<td></td>
<td>+</td>
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</tr>
</tbody>
</table>

* denotes the intensity of formazen deposition in the interstitial spaces gr-1 control, added-0 9% sodium chloride, gr-2,3,4, me was added (50 μg/ml, 100 μg/ml, 200 μg/ml) in the incubation medium.

Incubation medium Krebs-Ringer Bicarbonate buffer with a gas phase of 95% O₂ 5% CO₂ mixture (Brady - 1951).

Summary

Testicular steroidogenesis was determined by radioimmuno assay of serum testosterone and histochemical demonstration of the key steroidogenic enzyme (Δ⁵ 3β hydroxy-
steroid dehydrogenase) activity in the Leydig cells after treatment with mitomycin C (mc), (500 µg/kg, Bwt). In vivo experiments showed mc treatment causes a remarkable fall of serum testosterone level along with significant reduction on the weights of testis and sex accessories. In vitro studies resulted in a diminution in the Δ3,5 hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activity in the section of the testis after addition of mc (100, 200 µg/ml) in the incubation medium. The consideration of the above findings corroborated to the suggestion that mc reduced testicular steroidogenesis.

Einfuß von Mitomycin C auf die Steroidgenese im Hoden und ihr möglicher Modus bei Albino-Ratten

Zusammenfassung


References


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andrologia 12 (1980)


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**Announcements**

June 16-20, 1980, Jerusalem:
3rd International Congress on Twin Studies. Prof. P. Parisi, Piazza Galeno 5, 00161 Rome, Italy

June 18-20, 1980, Washington:
62nd Annual Meeting of the Endocrine Society. Endocrine Society, 428 East Preston Street, Baltimore, MD 21202, US.

July 7-11, 1980, London:

July 14-17, 1980, Oxford:
Annual Meeting of the Society for the Study of Fertility. SSF Business Office, 141 Newmarket Road, Cambridge CB5 8HA, UK.

July 14-18, 1980, London:
Meeting of Society for Drug Research: Drugs and the Foetus. Dr J D. Flack, Beecham Pharmaceuticals, Medicinals Research Centre, The Pinnacles, Harlow, Essex.

August 12-14, 1980, Ann Arbor:
13th Annual Meeting of the Society for the Study of Reproduction. Business Manager, Society for the Study of Reproduction, 309 W Clark St, Champaign, IL 61820, US.

September 29 – October 1, 1980, Oxford:
International Congress on Endocrinology of Human Infertility: New Aspects. C. Ferrari, PO Box 995, Milan, Italy.

December 11-12, 1980, London:
Winter Meeting of Society for the Study of Fertility. SSF Business Office, 141 Newmarket Road, Cambridge CB5 8HA, UK.
Role of Testosterone on Mitomycin C Induced Changes in Testicular Histology in Rats

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Received 19 June 1980, revised 30 January 1981

Histological degeneration of the testis and reduction in the testicular weights due to Mitomycin C treatment were improved to a large extent when Mitomycin C was administered together with testosterone propionate (1 mg/animal) in immature rats.

Mitomycin C (MC) treatment causes various deleterious effects on different tissues. The drug (MC) also brought about a rapid degeneration of testicular epithelium. The direct action of MC on spermatogonial cells causes testicular involution. Recently a remarkable fall of testicular steroidogenic enzyme activity was noted after treatment with MC. Therefore it is suggested that MC not only affects the spermatogonial cells but also interferes the steroidogenic capacity of the male gonad.

The present study was undertaken to examine the role of testosterone over MC induced changes in testicular histology.

Closed colony bred sexually immature male rats (30 days age), selected for the present investigation, were housed at a constant lighting schedule of 12 hr light per day and fed on a standard rat diet. Twentyfour such animals were taken and equally divided into 3 groups and treated as follows:

Group 1—MC (500 µg/Kg, ip) in distilled water (400 µg/ml) every alternate day for 30 days started from 30 days of age together with oil vehicle in the same amount as that of group 2.

Group 2—MC as that of group 1 together with testosterone propionate (1 mg/animal, SC) in oil (2 mg/ml) every day.

Group 3—Only distilled water and oil vehicle in the same amount as that of group 1 and group 2, and treated as control.

The animals were sacrificed by decapitation 24 hr after the last injection, testes were removed, weighed, fixed in Bouin’s fluid and stained with PAS and haematoxylin for microscopic examination.

On histological examination of the testes, control sections showed normal pattern of cellular association of spermatogonial cells in relation to spermatogenesis—consisting of spermatogonia, different stages of spermatocyte and spermatid along with mature sperm and Sertoli cells at the peripheral layer of the tubule. Mature spermatoids were lined around the lumen of tubules and a few residual bodies were observed amongst them (Fig. 1).
After treatment with MC most of the tubules were appeared to reduced significantly the number of spermatogonia, spermatocyte (resting, primary, secondary) and spermatid together with a good number of formation of multinucleated giant cells and pyknotic cells and vacuols. Some tubules were also appeared to have only Sertoli cells and practically devoid of any tubular epithelium (Fig 2). Administration of MC together with testosterone propionate resulted in a profound improvement in the overall testicular histology (presence of spermatogonia, different stages of spermatocytes, spermatid in the maximum tubules and mature spermatozoa in a fair number of tubules) and a minute microscopical observation showed a more or less normal spermatogenic activity as that of control rats when it is compared with the rats treated with only MC.

Relative testicular weights (mg) after treatment with MC alone and together with testosterone propionate (mean ± SE) are as follows: Control, 1378 ± 496, MC, 360 ± 49, MC + testosterone, 851.25 ± 85.3

The data clearly showed that testicular weight reduced to a large extent in MC treated rats and on the other hand the gaining of testicular weight towards normal was observed in MC treated rats followed by testosterone propionate administration. Therefore it is suggested that testicular degeneration in MC treated rats is not only due to the damage of the germinal material in the spermatogonia but also the importance of testosterone involvement in this aspects should be considered.

References
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7. Adler I-D, Mutat Res, 21 (1973) 20