Ultrastructural Analysis of Rat Testes After Gossypol Acetic Acid (GAA) Treatment

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Summary. The present investigations were carried out to show the histological and ultrastructural alterations in rat testes 10 weeks after gossypol acetic acid treatment (dose: 30 mg gossypol acetic acid/kg/day). The morphological findings in the interstitial compartment were compared with the data from studies carried out to investigate the testosterone biosynthesis in gossypol acetic acid treated rats. No morphological changes in the epididymal and vasal epithelia were found; however, the germinal epithelial cells showed vacuolisation, pycnosis, disconnections of junctions, cytolysis and exfoliation of germ cells from the epithelium. The Sertoli cells were affected, too. Gossypol acetic acid seemed to stimulate the physiological activity pathologically; cellular organelles as mitochondria, endoplasmic reticulum, lysosomal vacuoles, pigment granules and nuclei were either enlarged in size and number or malformed in shape. The cellular contact was often restricted to spots or completely disconnected. If gossypol acetic acid was administered for a longer period of time some Sertoli cells were found to be unable to withstand the toxic stimulus, and the cells became necrotic too. In contrast to the toxic process in the germinal and Sertoli cells the Leydig cell compartment did not show any changes in fine structure, and therefore testosterone biosynthesis is presumed to be intact.

Key words: Gossypol acetic acid (GAA), Germinal epithelium, Sertoli cell, Leydig cell.

Introduction

Considerable interest in gossypol, a phenolic compound isolated from cotton seeds, as a male antifertility agent has arisen around the world since the paper of Chinese scientists [18] was published.

In the recent numerous experiments have been carried out to establish the metabolic and physiological principles of this drug [1, 6, 13, 5, 14, 19, 14b, 20, 22, 26, 27].

In summary, the present literature confirms the capacity of gossypol acetic acid (GAA) to induce infertility in male animals; however, the precise mechanism of action is not clear. Antifertility efficacy of GAA has been reported to be dose and time dependent [18, 4, 22, 26]. According to reports of Hoffer [11], Bardin [2], Wang [23] and Xue [28] GAA did not interfere with the intact pituitary-gonadal hormonal axis. In contrast, disturbances of the hormonal axis were, however, found by Hadley [9] and Lin [16].

The present investigations were carried out to show the histological and ultrastructural alterations in rat testes 10 weeks after GAA-treatment (dose: 30 mg GAA/kg/day). The morphological findings in the interstitial compartment were compared with the data from studies carried out to investigate testosterone biosynthesis in GAA-treated rats. In one of our previous communications we have reported the proven infertility of GAA-treated rats [26].

Materials and Methods

GAA powder (supplied by courtesy of Dr. Sheldon Segal, Rockefeller Foundation, New York, USA) was suspended in 1% aqueous carboxymethyl cellulose-NA solution and applied orally to 20 male Wistar rats (260–420 g body weight) by stomach intubation. Ten animals were fed with suspendant only (Placebo group) and nine animals were used as an untreated control group. The animals were caged singly under standard conditions (24 °C room temperature, 12:12 dark:light interval). A commercial diet (AltrominR-H) and water were available ad libitum. GAA was kept in a brown bottle at 4 °C, and exposed to light for as short a time as possible because of its photosensitivity. The suspension was thoroughly agitated each time before filling the syringe. Fresh suspensions were prepared daily because there is evidence that gossypol might be unstable over an extended period of time.

At certain intervals the fertility rate of the experimental and control animals was proved by matings with females of proven fertility. The mating time was 14 days. For body-weight calculations the animals were weighed twice a week throughout the experimental period.

On the 43rd day of the experiment two animals of each group were vasectomized unilaterally under ether inhalation anesthesia.
Fig. 1. A weakly GAA affected tubule showing the normal progress of spermatogenesis except spermiation. The luminal space is filled with detached germinal cells (magn.: 160:1)

Fig. 2–4. Progressive steps of epithelial disarrangement and tubular wall condensation (magn.: 800:1)

Abbreviations: Ac = Acrosomal complex, AL = Annulatae lamellae, aL = autophagic lysosome, BL = Basal lamina, Cj = Cell junction, cM = convoluted Membrane, cMt = condensed Material, dCe = distal Centriol, Ep = End piece, Er = Endoplasmic reticulum, lcs = Intercellular space, Lpd = Lipid droplet, LLsd = Later Spermatid, Ly = Lysosome, Mi = Mitochondria, Mp = Middle piece, N = Nucleus, nSpc = necrotic Spermatocyte, pAr = post Acrosomal ring, pCe = proximal Centriol, PiGr = Pigment Granule, pNc = post Nuclear cuff, rEr = rough Endoplasmic reticulum, sMc = smooth Muscle cell, Spc = Spermatocyte, Spg = Spermatogonium, Ste = Sertoli cell, Stcn = Sertoli cell nucleus, Tw = Tubular wall, Va = Vacuole

Vital and stained smears of the outdropping seminal fluid were prepared to evaluate the motility rate and the morphological appearance of the spermatozoa.

After cessation of treatment on day 70 the animals were anaesthetized by ether inhalation. Blood was taken by cardiac puncture, centrifuged and the serum was used for hormone analysis (testosterone, LH and FSH). The hormone analyses for testosterone and for LH and FSH were run in duplicate and in triplicate for the gonado-tropins. The reproductive organs were carefully excised, weighed, and prepared for histological and electron microscopical investigations.

For light microscopy parts of the reproductive organs were fixed in Boulin's solution and after dehydration embedded in paraffin. The sections (6–8 μm) were stained either in Azan or in Hematoxylin-Eosin.

For electron microscopy small pieces were fixed in 1.5% glutaraldehyde, buffered in 175 mM cacodylate-NA solution (pH 7.3) for 1 h. Postfixation was done in a 1% aqueous solution of OsO₄ for 2 h. After stepwise dehydration in ethanol the samples were embedded in Epon® epoxy resin. Semithin and ultrathin sections were prepared on the Reichert microtome OM U3. The stained ultrathin