A structural and cytochemical study of the effects of gossypol on the epithelial cells of the guinea-pig seminal vesicle

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Summary. Gossypol acetic acid, given to guinea-pigs at a concentration of 10 mg/kg daily for up to 7 weeks, exerted an inhibitory effect on the secretory activity of the glandular cells of the seminal vesicles. The diminished secretory function was associated with a decrease in thiamine pyrophosphatase (TPPase) (EC 3.6.1.6) activity in the luminal border and the Golgi cisternae. There was no increase in cytoplasmic filaments, and mitochondrial damage was not observed. The most striking effect was on the basal lamina, where the lamina densa increased significantly in thickness, while the lamina lucida decreased marginally in thickness. This was coupled with an increase in proteoglycan content on both sides of the lamina densa. It would appear that gossypol reduces the luminal secretory function on the one hand, while it increases the basal secretory activity on the other, thus modifying the structure of the basal lamina. The functional significance of these changes remains unknown.

Keywords: gossypol; seminal vesicle; guinea-pig; structure

Introduction

There has been a large number of publications on the use of gossypol for regulation of male fertility, contributing a major step in the search for a male contraceptive (Liu, 1957; Wang & Lei, 1979; Wu & Shi, 1979; Liu *et al.*, 1981; Xue, 1981; Prasad & Diczfalusy, 1983). More recent studies have demonstrated that gossypol also exhibits antifertility activity in male rodents, rabbits and monkeys (Xue, 1981; Hoffer, 1982, 1983; Prasad & Diczfalusy, 1983; Xue *et al.*, 1983). Most of the basic and clinical studies carried out in China and elsewhere have used gossypol or gossypol acetic acid. The resulting damage to the testes seems to be specific, affecting the spermatids at certain stages of development, and also mature spermatozoa (Dai *et al.*, 1978; Hadley *et al.*, 1981; Xue, 1981). On the other hand, very little work has been done on the effect of this drug on the male accessory sex glands. Earlier reports from China indicated that gossypol has no effect on the structure of and amount of those enzymes related to steroid hormone synthesis in the interstitial cells. Moreover, no change in the size, weight and morphology of the accessory sex glands or the concentration of testosterone was reported (Xue, 1981). In contrast to these reports, Wong & Tam (1983, 1984, 1985, 1988) have reported that gossypol acetic acid has an inhibitory effect on the secretory activity of the seminal vesicle and the prostate, while others have reported that gossypol significantly reduces the blood testosterone concentration (Lin *et al.*, 1981; Hahn *et al.*, 1981; Liang *et al.*, 1981). The present study was undertaken to examine the structural and cytochemical changes of the seminal vesicle after gossypol treatment. Thiamine pyrophosphatase (TPPase) (EC 3.6.1.6), a marker enzyme located in the Golgi, was chosen to monitor the Golgi activities of the cells after gossypol treatment.

Materials and Methods

Five male guinea-pigs weighing 0.8–1.0 kg each, were kept in standard laboratory conditions of 14 h light/10 h dark. They were force fed with gossypol acetic acid in Tween-80 at a concentration of 10 mg/kg body weight daily for up to 7 weeks. Three control animals were kept under identical conditions and fed with the carrier alone.

Anaesthesia was induced by an intraperitoneal injection of an excess dose of pentobarbitone sodium. The animals were cannulated at the thoracic aorta, with the inferior vena cava cut. The vascular system was then flushed with 300 ml heparinized saline. This was then followed by 500 ml fixative containing 2 mM-MgCl₂, 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M-cacodylate buffer at pH 7.2. After perfusion, the seminal vesicles were removed and fixed in the same fixative for a further 2–4 h at 4°C, then post-fixed with 1% osmium tetroxide for 1–4 h, dehydrated and embedded in Taab for transmission electron microscopy. At least 5 blocks from each animal were cut. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 300 electron microscope.

For cytochemical demonstration of thiamine pyrophosphatase (TPPase) activity, tissue was cut with a tissue chopper to a thickness of 50–75 µm immediately after perfusion. The sections were incubated for TPPase activity in a medium prepared according to the method of Novikoff & Goldfischer (1961), using cocarboxylase as substrate for 2 h, at 37°C. Substrate omission in the incubating medium served as control for TPPase activity.

After incubation, the tissue was rinsed and post-fixed in 1% osmium tetroxide for 1 h, and stained *en bloc* with uranyl acetate before embedding for examination by electron microscopy. No further staining was performed on these sections.

Results

Morphological changes

The fine structure of the guinea-pig seminal vesicles has been described by others (Veneziale *et al.*, 1974; Tse & Wong, 1980), and only those features pertinent to the present study will be given here. The epithelium consists of tall columnar cells resting on a smooth continuous basement membrane, separating the epithelium from the loose lamina propria which contains sparse collagen and cells. The epithelial cells are richly endowed with granular endoplasmic reticulum (GER), typical dense-cored secretory granules and a few short microvilli (Fig. 1).

Some cells appeared normal even after 7 weeks of treatment, while others underwent mild degrees of alteration. The Golgi apparatus appeared to be slightly smaller and many cells had fewer and smaller secretory granules (Figs 2 & 3). Despite the decrease in size, the secretory granules retained their normal dense-cored appearance. Clear granules, which are a prominent feature seen in the prostate gland of similarly-treated guinea-pigs (Wong & Tam, 1988), were not observed in the seminal vesicles.

The GER profiles appeared less well organized and were less compactly arranged. There was no concurrent increase in filamentous content and the mitochondria appeared unaffected in the seminal vesicle (Fig. 3). There was, however, an increase in lipid content in the cells, as lipid globules were more frequently found (Fig. 4).

Marked changes were observed in the epithelial–stromal junction, however. In the normal untreated seminal vesicle, the relatively smooth basal lamina separating the epithelia from the

Fig. 1. Electron micrograph showing the apical portion of epithelial cells of the seminal vesicle from a control animal. Note the typical dense cored secretory granules (SG), and well developed GER $\times 29\,540$; bar = 1 µm.

Fig. 2. The secretory granules of the epithelial cells decrease in number and size 7 weeks after gossypol treatment. The Golgi (G) also decreases in size, but the changes in GER in these cells are not very distinctive. Note also that the mitochondria (M) appear normal. $\times 18\,200$; bar = 1 µm.

Fig. 3. Micrograph showing the small Golgi with forming granules (arrows). $\times 24\,760$; bar = 1 µm.

stromal tissue measured about 80 nm in thickness (Fig. 5). It is divided more or less equally between the lamina densa (an electron-dense layer) and the lamina lucida (an electron-translucent layer). In gossypol-treated animals, patchy changes in the thickness and structure of the basal lamina were observed. The lamina densa often increased in thickness to 120–180 nm compared to about 40 nm in the control, while the lamina lucida often became less distinctive, ranging from 10 to 30 nm (Figs 6 & 7). In some sections, a fine filamentous network was seen extending from the stromal surface of the lamina densa (Fig. 7). In some tissues, after prolonged fixation in osmic acid, regularly spaced dense granules were found associated with both sides of the lamina densa (Fig. 8). These granules are believed to be proteoglycans made visible by osmic acid fixation (Revel & Hay, 1963; Hay, 1981). Granules present on the epithelial side were of 30 nm in diameter, while those on the stromal side were about 50 nm. In places these granules were closely packed on the surface of the lamina densa so as to form a continuous layer (Fig. 9). This is in contrast to the normal untreated animals in which they are regularly spaced at intervals of 60 nm (unpublished observation).

TPPase activities

Normal controls. In the normal vesicle, the reaction products were found in 2–3 trans-Golgi cisternae (Fig. 10). The reaction was intense, and the reactive products completely filled the lumen of the cisternae concerned. Other sites of positive activity were found in the luminal (Fig. 11) and lateral cell membranes (Figs 10, 12), and in some lysosomes (Fig. 13). Reactive products were also observed in some secretory granules (Fig. 11), mainly restricted to the periphery of the granules.

Gossypol-treated animals. In these guinea-pigs, TPPase activity was persistently found to be less than that in the normal controls. Only sparse patchy deposition of reactive products was found in the Golgi, luminal and lateral borders (Figs 14 & 15). The lateral borders often showed minimal (Fig. 14) or no activity. TPPase activity in lysosomes was also reduced, and very few lysosomes showed a positive reaction (Figs 15 & 16). Occasional secretory granules were found with scanty TPPase deposition. Because of the difficulties in quantifying the cytochemical results, care was taken to ensure that only sections from superficial tissue of the blocks were examined to avoid any possible differences in reactivity of the enzyme which might have arisen from a difference in distance of substrate diffusion.

Discussion

The results of the present investigation confirm our earlier studies that gossypol depresses the secretory activity of the male accessory sex glands including the prostate gland (Wong & Tam, 1988). This is reflected in the reduction in size of the Golgi apparatus and the number of secretory

Fig. 4. Micrograph showing an increase in lipid (L) content after gossypol treatment. $\times 13\,975$; bar = 1 μm .

Fig. 5. Micrograph of the epithelial-stromal interface showing the basal lamina of cells from a control animal. Note the typical basal lamina (~80 nm) with roughly equal thickness of lamina densa (LD) and lamina lucida (LL). $\times 30\,650$; bar = 1 μm .

Fig. 6. The micrograph shows the basal lamina from a treated animal. The lamina densa (LD) greatly increases in thickness (120 nm) while the lamina lucida (LL) decreases to 10–30 nm. $\times 21\,730$; bar = 1 μm .

Fig. 7. Micrograph showing the tremendous increase in thickness in the lamina densa (150 nm). A fine filamentous (F) network can be traced from the stromal surface of the lamina densa and merges with the underlying connective tissue. $\times 36\,580$; bar = 1 μm .

granules. The mechanism is unknown. It has been reported in several animal species that gossypol affects serum androgen concentrations (Hahn *et al.*, 1981; Liang *et al.*, 1981; Donaldson *et al.*, 1985). It would seem likely that perturbation of endocrine function might also occur in the guinea-pig, and that the structural changes observed in the seminal vesicle may be secondary to the altered hormonal levels.

TPPase is an enzyme located in several trans-cisternae and works in concert with galactosyl-transferase to allow transfer of galactose to glycoprotein in the Golgi apparatus. TPPase catalyses the reaction to convert uridine diphosphate (UDP) to uridine monophosphate (UMP) and inorganic phosphate. The function of TPPase is, therefore, to avoid accumulation of UDP, thus preventing the inhibition of galactosyltransferase activity by UDP. TPPase activity therefore reflects the traffic through the Golgi cisternae and, hence, the secretory activity of the cells (Roth & Berger, 1982). The presence of TPPase-reactive products in the luminal and lateral borders is puzzling. TPPase activity has been reported to be present in selected regions of the cell membrane (Castel & Dellman, 1980; Laszlo & Bodor, 1981) other than the Golgi. The observation of TPPase reactive products in the membrane of some secretory granules indicates a possible link between the Golgi and TPPase of the luminal membrane. By the same token, the presence of TPPase reactive products in the lysosomes may also indicate their origin from the Golgi cisternae.

After gossypol treatment, the secretory granules not only became smaller in size, but also in number. Their morphology, however, remained similar to that of the control animals, i.e. a dense core surrounded by a loosely-fitted membrane. Wong & Tam (1988) reported that gossypol modifies the secretory granules in guinea-pig prostate gland, resulting in the formation of a second type of 'clear granule' in the lateral and dorsal lobes. Similar 'clear granules' were, however, not found in the present study of the seminal vesicles.

On the other hand, gossypol has been shown to cause damage to mitochondria in a number of tissues (Xue, 1981; Hoffer, 1982, 1983; Oko & Hrudka, 1982; Tanphaichitr *et al.*, 1984; Hu *et al.*, 1986; Robinson *et al.*, 1986), including the dorsal prostate and the coagulating gland of the guinea-pig (Wong & Tam, 1988). However, similar damage was not observed in the seminal vesicles. This confirms again that there might be differences in susceptibility in mitochondrial damage by gossypol. For the accessory sex glands of the guinea-pig, mitochondria of both the lateral prostate (Wong & Tam, 1988) and the seminal vesicle are more resistant to gossypol while the dorsal lobe of the prostate and the coagulating gland, like other tissues, are more susceptible to gossypol damage (Xue, 1981; Hoffer, 1982; Eliasson & Virji, 1983).

The most striking changes in the seminal vesicle after gossypol treatment appear to be in the basal lamina (Figs 6, 7, 8 & 9). There was a dramatic increase in thickness of the basal lamina from about 80 nm in the normal control to about 200 nm after gossypol treatment. The increase was

Fig. 8. This micrograph shows in addition to the greatly increased basal lamina (~180 nm), dense granules on both sides of the lamina densa (arrows). The granules in the lamina lucida measure about 30 nm while those on the outer side of the lamina densa are about 50 nm. These granules are believed to be the proteoglycans, made visible by prolonged fixation in osmic acid. $\times 29\,170$; bar = 1 μm .

Fig. 9. This micrograph shows the dense granules on both sides of the lamina densa forming a continuous layer (arrows). Note also the layer of granules on the stromal surface of the lamina densa. $\times 26\,580$; bar = 1 μm .

Fig. 10. Epithelial cells showing the distribution of the TPPase reactive products in the control animal. Note that the enzyme is concentrated mainly in the Golgi (G), where the dense products fill completely the 2–3 trans cisternae. $\times 37\,300$; bar = 1 μm .

Fig. 11. Micrograph showing the reactive products in the apical border in a control animal. Several secretory granules (arrows) also show positive activity. $\times 21\,350$; bar = 1 μm .

Fig. 12. Micrograph showing the presence of reactive products along the lateral membrane in a control animal. $\times 27\,760$; bar = $0.5\,\mu\text{m}$.

Fig. 13. Micrograph showing three lysosomes positively reacting with TPPase activity in a control animal. $\times 23\,290$; bar = $1\,\mu\text{m}$.

Fig. 14. Micrograph showing the supranuclear cytoplasm of the cell. The TPPase reactive products become greatly reduced in the gossypol-treated animal. The reactive products become patchy in Golgi (G), the luminal and also the lateral borders (arrows). $\times 19\,430$; bar = $1\,\mu\text{m}$.

Fig. 15. Micrograph showing minimal TPPase activities in the Golgi but negative in a secondary lysosome (lower left). $\times 19\,700$; bar = $1\,\mu\text{m}$.

Fig. 16. Several lysosome-like structures are shown, but only one shows enzyme activities. $\times 26\,800$; bar = $1\,\mu\text{m}$.

mainly in the lamina densa where it increased from the normal $40\,\text{nm}$ to as much as $180\,\text{nm}$. The lamina lucida, on the other hand, became slightly thinner ($10\text{--}30\,\text{nm}$ compared to $40\,\text{nm}$ in the normal control). However, these changes in the basal lamina were not uniform: relatively standard thickness was observed in some regions while in others the lamina densa was variously increased to $120\text{--}180\,\text{nm}$. The effects of gossypol on the basal lamina has not been reported before, and the significance of such changes to the epithelial cells is not known.

In the kidney, the glomerulus has a greatly thickened basal lamina where it serves, among other functions, as a filtration barrier. Basal lamina thickening is a prominent feature in diabetes (Osterby, 1980; Rohrbach *et al.*, 1982; Martinez-Hernandez & Amenta, 1983), which may alter the function of the basal lamina, resulting in development of the nephrotic syndrome (Martinez-Hernandez & Amenta, 1983). The exact reason for basal lamina thickening in our present study is not known. There seemed to be an increase in density of proteoglycan granules in the thickened basal lamina, at times to the point of forming a continuous layer on both sides of the lamina densa (Fig. 9). The increase in proteoglycans is accompanied by an increase in thickness of lamina densa (presumably resulting from increased synthesis of type IV collagen and laminin). This indicates that, under the effect of gossypol, synthesis of both proteoglycans and type IV collagen is also increased. If this interpretation is correct, then gossypol appears to depress the secretory activity of the epithelial cells at the luminal border, but raise the secretory activity along the basal border, resulting in thickening of the basal lamina.

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