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MORPHOLOGICAL EVALUATION OF THE TESTES IN ADULT GUINEA PIGS (*Cavia porcellus*, L.) TREATED WITH OXAMNIQUINE INCORPORATED INTO SYNTHETIC PHOSPHOLIPID (DIMYRISTOIL PHOSPHATIDYLCHOLINE) VESICLES

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ABSTRACT

The aim of this study was to examine the effects of the potent schistosomicide oxamniquine on the testicular morphology of adult guinea pigs following incorporation of the drug into synthetic phospholipid–DMPC vesicles. The guinea pigs were allocated to one of four groups: a) controls (no vesicles or oxamniquine), b) vesicle-treated controls which received DMPC (80 mM), c) treatment with oxamniquine (15 mg/kg) incorporated into DMPC, and d) treatment with oxamniquine diluted in mineral oil. Morphological evaluation showed that the testicular structure in the groups treated with DMPC vesicles and with oxamniquine incorporated into DMPC was similar to the control group. However, guinea pigs that received only oxamniquine had testes with a variety of morphological alterations. These findings indicate that when oxamniquine was administered alone it caused testicular damage, but when incorporated into synthetic phospholipidic vesicles, the side effects were suppressed.

Key words: Guinea pig, liposomes, oxamniquine, testis

INTRODUCTION

Liposomes are stable microscope vesicles formed by phospholipids and similar amphipathic lipids. The lipid bilayers in liposomes are similar in structure to those in cell membranes, so that liposomes provide a simple analogy to living cells. Since cell membranes vary widely in their composition and function between species and tissues, it is possible to produce different types of liposomes with widely varying properties.

Liposomes have been extensively used as transporter molecules for the systemic administration of drugs [1,4,8]. One advantage of using liposomes is their high selectivity for diseased tissues, which in turn increases the efficiency of drug delivery and reduces its side effects. Little is known about the interaction of liposomes with the environment or about the possible effect of such an interaction on the well-being of the organism.

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Maierhofer [22] reported that any type of liposome would be adequate for the incorporation of liposoluble compounds, *in vitro* and *in vivo*, but that the multilamellar vesicle type was the best choice because it allowed the gradual release of the incorporated drug. Clinical and animal studies have confirmed the ability of liposomes to encapsulate and effectively deliver a wide variety of drugs, including antibiotics, antineoplastics, steroids, soluble bronchodilators, nucleotides and peptides [33].

The absence of specificity in pharmacologically active agents is an obstacle to their effective use in research and medicine [12]. Fielding [7] reported that the ability of liposomes to contain, transport and release therapeutic agents had a wide range of clinical applications. The simplest, and perhaps most overlooked, pharmaceutical use is simply to act as a nontoxic vehicle for insoluble drugs [20]. More sophisticated applications involve the use of liposomes as prolonged drug release reservoirs, or for localising a drug within the body to avoid or target specific tissues or subcellular sites [7].

The tissue distribution of liposome-associated agents has generally been examined using preparations given intravenously. Because of their biocompatibility, liposomes are suitable for virtually every route of administration [7]. Studies in the rat [13] and mouse [16,18] have shown that, in addition to the liver and spleen, a variety of other organs such as kidney, lungs, skeletal muscle and brain take up some of the injected liposomes. The properties, production and therapeutic applications of liposomes have been reviewed elsewhere [7,15,17].

Oxamniquine has been used successfully in the treatment of infections by *Schistosoma mansoni*, with curing in about 95% of human cases. The mechanism of action involves alkylation of the macromolecule, which leads to irreversible inhibition of nucleic acid synthesis by the parasite [2,27-30].

Agents with an alkylating action affect mainly organs in which there is a high cell turnover, such as skin, bone marrow and the gastrointestinal tract, but can also act in the male gonad to cause the destruction of germ cells, especially spermatogonial cells [35,38].

Green *et al.* [11] studied the effects of the schistosomicide hicanthone on spermatogenesis of rats and showed that the drug, when used at a high dose (80 mg/kg/5 days), caused atrophy of the testicles and seminiferous tubules which resulted in sterility. In post-natal testicular maturation in guinea pigs, a therapeutic dose of oxamniquine (15 mg/kg) produced cytohistological alterations in the seminiferous epithelium which started when the guinea pigs were 15 days old and continued until they were 75 days old [5]. No alterations were observed in the diameters of the seminiferous tubules.

In this study, we examined the testicular morphology of adult guinea pigs treated with oxamniquine incorporated into synthetic phospholipid vesicles.

MATERIAL AND METHODS

Twenty adult male guinea pigs (*Cavia porcellus*, L.), were housed at 22ºC on a 12 h light/dark cycle. Oxamniquine (6-hydroxymethyl-2-isopropyl-7-nitro-1,2,3,4-tetraquinoleine, Pfizer Laboratories, Brazil) was administered at a dose of 15 mg/kg. Dimyristoil phosphatidylcholine (DMPC, Sigma Chemical Co., St. Louis, MO, USA) was used to produce multilamellar vesicles as described by Paula and Shreier [26] and Nogueira (Y.Y. Nogueira, Doctoral thesis, University of São Paulo, Ribeirão Preto 1996).

The animals were weighed and distributed into four groups : a) controls (no vesicles or oxamniquine), b) vesicle-treated controls which received 0.5 ml of multilamellar vesicles of DMPC (80 mM) prepared in phosphate buffer, pH 7.0, c) treatment with 0.5 ml of oxamniquine (15 mg/kg) incorporated into DMPC vesicles, and d) treatment with oxamniquine (15 mg/kg) diluted in mineral oil. All treatments were given as a single dose intraperitoneally, five days before killing the guinea pigs by ether inhalation, as described by Camargo [5].

After killing, the testicles were removed and fixed in Bouin solution for 24 h and then dehydrated and embeded in Paraplast (Oxford Labware, St. Louis, MO, USA). Sections 7 µm thick were stained by hematoxylin-eosin (H.E.) and periodic acid Schiff/hematoxylin (PAS/H). Photomicrographs were obtained using an Olympus BX-60 microscope.

RESULTS

In control guinea pigs (Fig. 1A), the seminiferous tubules had a well-defined epithelial cytoarchitecture, with type A, intermediate and type B spermatogonia, primary spermatocytes predominantly in the pachytene stage, round and elongated spermatids, and sperm arranged periluminally in the spermiation phase. The nuclei of Sertoli cells were located in the basal region of the germinal epithelium.

The testicular structure of guinea pigs treated with DMPC vesicles in the absence or presence of oxamniquine (Figs. 1B and 2A, respectively), showed seminiferous tubules with morphological characteristics similar to the control group. In these animals, there were no changes in the interstitial tissue or in the structure of the Leydig cells.

Guinea pigs that received only oxamniquine (Fig. 2B), had testicular structure different from the other groups. The morphological alterations observed included depletion of the seminiferous epithelium, with damage to all germinative elements in the tubules, intraepithelial vacuolization, disorganization of the spermatogenic cytoarchitecture and tubular atrophy. As a consequence of this atrophy, the intertubular space increased. In interstitial tissue, there was an infiltration of connective tissue cells. Histological analysis of this tissue showed that there was an increase in the plasmocyte population, which suggested an inflammatory process in the testis of guinea pigs treated with oxamniquine (Fig. 2B).

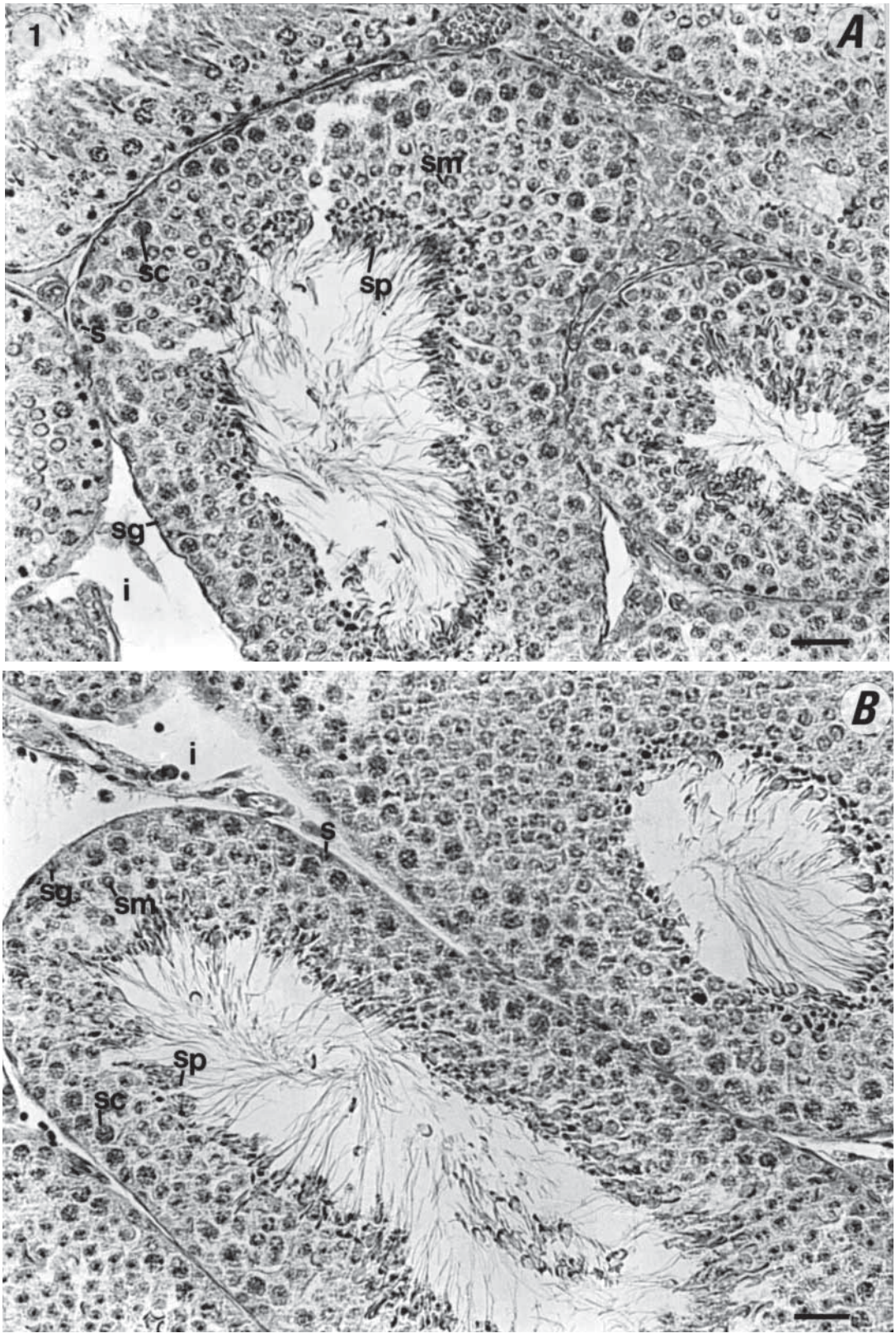

Figure 1. Cross-sections of guinea pig seminiferous tubules. (**A**) control, (**B**) treated with DMPC. The general architecture of the germinal epithelium has a normal appearance in these groups. i = interstitial tissue; s = Sertoli cell; sc = primary spermatocyte; sg = spermatogonia; sm = spermatid; sp = spermatozoa. Bar = 30 µm.

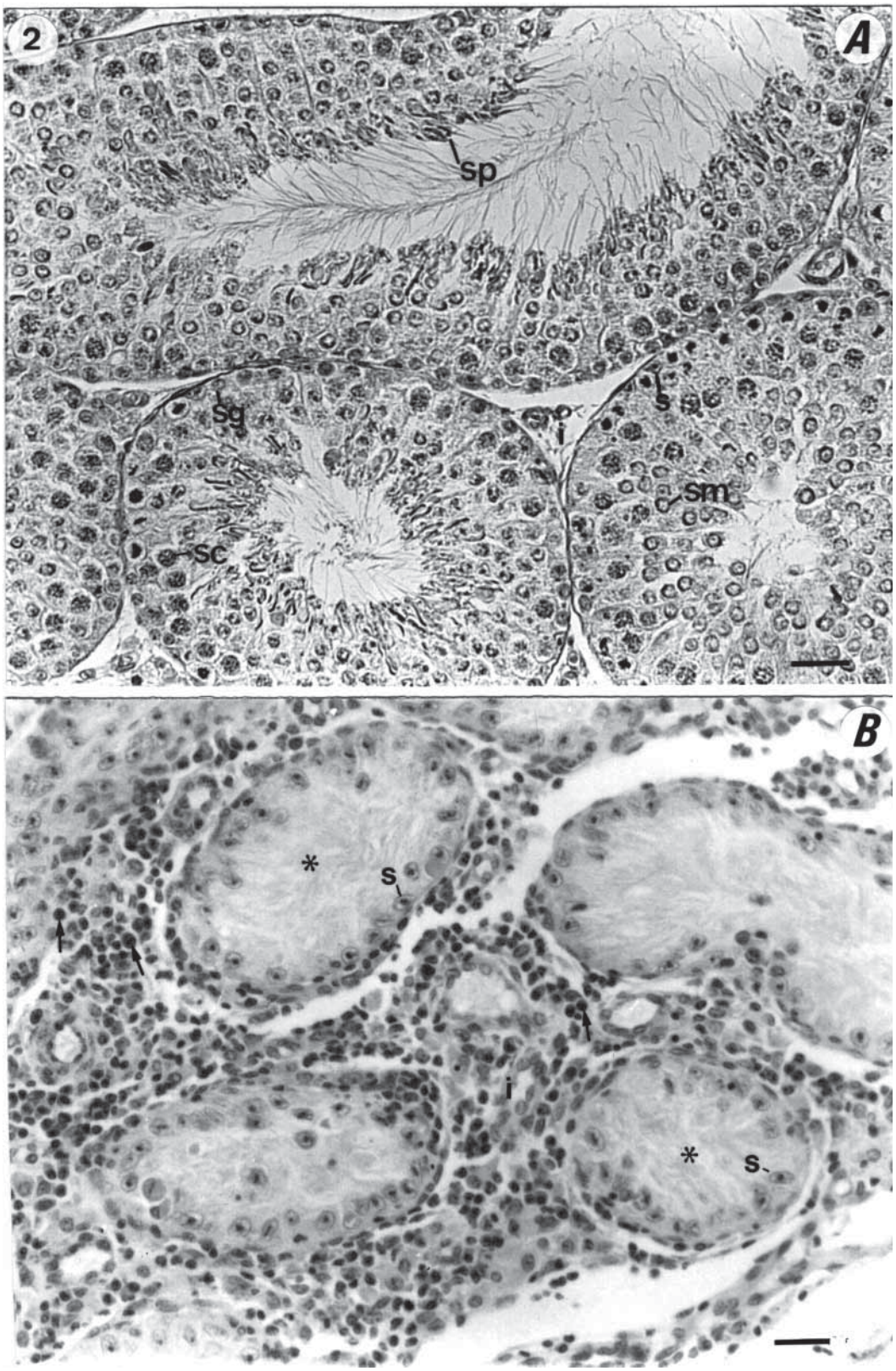

Figure 2. Cross-sections of guinea pig seminiferous tubules. (**A**) treated with DMPC and oxamniquine, (**B**) treated with oxamniquine. Note the normal spermatogenic architecture and the normal appearance of the intertubular tissue in the testis of a guinea pig treated with DMPC and oxamniquine. In the group treated with oxamniquine, note the atrophic tubules containing only Sertoli cells (*) and the interstitial tissue infiltrated by connective cells, especially plasmocytes (arrows). i = interstitial tissue; s = Sertoli cell; sc = primary spermatocyte; sg = spermatogonia; sm = spermatid; sp = spermatozoa. Bar = 30 μm.

DISCUSSION

Although there are numerous reports of drugs which affect fertility, there are no studies describing the morphological alterations in male gonads, caused by drugs incorporated into liposomes. Segal *et al.* [36] reported that whereas large liposomes were slowly disrupted at the site of injection (rat testis) to release the entrapped material, small liposomes and their contents were rapidly absorbed from the tissue into blood and lymphatic circulation and then partially recovered in the liver and spleen, as well as in lymph nodes draining the injected tissue.

Nogueira (Y.Y. Nogueira, Doctoral thesis, University of São Paulo, Ribeirão Preto 1996), used the Stern-Volmer constant for fluorescence probes of pyrene and sodium 4-(1-pyrene) butyrate (PBA) in DMPC vesicles to demonstrate the incorporation of oxamniquine (a fluorescence quencher) into the lipidic bilayer. The strong hydrophobic nature of the pyrene resulted in its total incorporation into the lipid bilayer of the vesicles, whereas the fluorescence probe of PBA, which was more hydrophilic than pyrene, was orientated with its polar region to the aqueous phase.

Leandro and Gremião [19] reported that *S. mansoni* had a high affinity for phospholipid, which can be incorporated into the parasite's structure. The targeting of oxamniquine in the liver using liposomes, can reduce the extent of metabolism and prolong the action of this drug, thereby increasing its efficiency and reducing the damage caused to host tissue. In patients, the alkylating action of oxamniquine [2,6,31,32], frequently produces side effects in organs with intense cell proliferation, as well as teratogenic effects [3,9,34].

Our histological results showed that incorporating oxamniquine into synthetic phospholipid vesicles, prevented the several morphological alterations caused by the drug when given alone.

In the latter group, the toxicity of oxamniquine was confirmed by the absence of germinative cells in several seminiferous tubules where the drug acts on spermatogonial multiplication and the subsequent stages of development. The spermatogonial cytotoxicity of other alkylating agents is also well documented in the literature [14,21,23,35]. The danger of testicular destruction by alkylating agents is that the germinative epithelium may be reduced to a single layer of Sertoli cells while Leydig cells remain intact [37]. The morphological alterations seen in the testes of guinea pigs that received only oxamniquine were similar to those described by Camargo [5] and by others who studied different alkylating agents [10,24,25,35,39].

Our results indicate that the incorporation of oxamniquine into DMPC liposomes protected the germinative cells, because the vesicles acted as an efficient biological membrane and suppressed the side effects of the drug. A similar approach could be of therapeutic use in treatments involving this drug or others which are capable of adversely affecting fertility.

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