Cimetidine-induced vascular cell apoptosis impairs testicular microvasculature in adult rats

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Summary. Cimetidine, an H_2 receptor antagonist used for treatment of gastric ulcers, exerts antiandrogenic and antiangiogenic effects. In the testes cimetidine impairs spermatogenesis, Sertoli cells and peritubular tissue, inducing apoptosis in the myoid cells. Regarding the importance of histamine and androgens for vascular maintenance, the effect of cimetidine on the structural integrity of the testicular vasculature was evaluated. Adult male rats received cimetidine (CMTG) and saline (CG) for 50 days. The testes were fixed in buffered 4% formaldehyde and embedded in historesin and paraffin. In the PAS-stained sections, the microvascular density (MVD) and the vascular luminal area (VLA) were obtained. TUNEL method was performed for detection of cell death. Testicular fragments embedded in Araldite were analyzed under transmission electron microscopy. A significant decrease in the MVD and VLA and a high number of collapsed blood vessel profiles were observed in CMTG. Endothelial cells and vascular muscle cells were TUNEL-positive and showed ultrastructural features of apoptosis. These results indicate that cimetidine induces apoptosis in vascular cells, leading to testicular vascular atrophy. A possible antagonist effect of cimetidine on the H_2 receptors and/or androgen receptors in the vascular cells may be responsible for the impairment of the testicular microvasculature.

Key words: H_2 receptor antagonist, Microvascular density, Vascular atrophy, Cell death, Smooth muscle cell

Introduction

In the human testes, blood vessels consist of arterioles, metarterioles, capillaries and venules, which interconnect Leydig cell clusters to the adjacent seminiferous tubules and play an important role in the distribution and transport of hormones, nutrients, oxygen and other factors from the interstitium to the seminiferous tubules (Ergün et al., 1997). In this context, it has been suggested that dysfunction in the vasculature, for example, the reduction of blood flow may affects spermatogenic process, resulting in marked decrease in sperm production (Bergh et al., 2001).

It is known that histamine induces vasoconstriction and vasodilatation by acting on both H_1 and H_2 receptors, respectively, located in blood vessels (Reinhardt and Ritter, 1979; Gross, 1981; Gross et al., 1981; Heltianu et al., 1982; Edvinsson et al., 1983; Saari, 1986; Guslandi, 1994). Furthermore, regarding the presence of androgen receptors in vascular smooth muscle cells (Vornberger et al, 1994; Welsh et al., 2010), the testicular blood vessels seem to be controlled by testosterone and are involved in endocrine and paracrine control of spermatogenesis (Bergh and Damber, 1992).

Cimetidine, an antacid and anti-ulcer drug, is a potent H_2-receptor antagonist, competing with histamine for H_2 receptors located on gastric parietal cells (Clayman, 1977). Among other effects, cimetidine competitively blocks receptors for dihydrotestosterone (DHT) in the pituitary and hypothalamus (Knigge et al., 1983) and other tissues that require DHT (Winters et al., 1979), causing an antiandrogenic effect (Winters et al., 1979; Pereira, 1987). Studies in rodents have shown that cimetidine causes testicular changes, including significant reduction of the seminiferous tubule diameters, irregular tubules showing disorganized

epithelium, loss of germ cells by apoptosis and sloughed germ cells filling the tubular lumen (Sasso-Cerri et al., 2001; Sasso-Cerri and Miraglia, 2002; Sasso-Cerri and Cerri, 2008; Beltrame et al., 2011). Furthermore, cimetidine induces peritubular myoid cell death (França et al., 2000; Sasso-Cerri and Miraglia, 2002) and structural alterations in the Sertoli cell-basement membrane interface leading to Sertoli cell apoptosis (Sasso-Cerri and Cerri, 2008). Some of these effects have been associated with the antiandrogenic action of cimetidine (Sasso-Cerri et al., 2001; Sasso-Cerri and Miraglia, 2002) as well as to a direct toxic effect on the peritubular tissue (França et al., 2000). It has also been described that cimetidine presents an antiangiogenic effect in the ulcer granulation tissue. Since histamine binds to H_2 receptors in the endothelial cells and stimulates vascular endothelial growth factor (VEGF) production (Ghosh et al., 2001), the antiangiogenic effect of cimetidine results from the blockade of histamine H_2 receptors present in the blood vessels (Tsuchida et al., 1990). So, the antiangiogenic action of cimetidine has favored the treatment of cancer, suppressing the growth of several tumors by inhibiting tumor-associated angiogenesis (Tomita et al., 2003; Natori et al., 2005).

In this study, we purpose to investigate the structural and morphometrical parameters of the testicular blood vessels in cimetidine-treated rats, relating the vascular structural changes to possible mechanisms of action of cimetidine.

Materials and methods

Animals and treatment

Animal care and experimental procedures were conducted following the national law on animal use. The protocol of this study was approved by the Ethical Committee for Animal Research of São Paulo Federal University, Brazil (UNIFESP/EPM).

Ten 100-day-old Holtzman male rats (*Rattus norvegicus albinus*) were maintained in plastic cages under 12h light/12h dark cycle at controlled temperature (23±2°C), with water and food provided *ad libitum*. The animals were distributed into two groups containing five animals each: cimetidine (CMTG) and control (CG). The animals from CMTG received daily intraperitoneal injections of 100 mg of cimetidine (Hycimet®, Hypofarma, Brazil) per kg b.w. and the control animals received saline solution. All the animals received the treatment for 50 consecutive days. As in previous studies (França et al., 2000; Beltrame et al., 2011) cimetidine was used in this study to produce testicular damage in rats, and not to attempt to mimic their pharmaceutical use in humans.

Histological procedures

The animals were anaesthetized with chloral hydrate (400 mg/Kg of body weight) and the testes were removed and fixed for 48h at room temperature in 4% formaldehyde freshly prepared from paraformaldehyde (MERK, Germany) buffered at pH 7.4 with 0.1 M sodium phosphate. Subsequently, the testes were dehydrated in graded ethanol and embedded in glycol methacrylate (Historesin Embedding Kit, JUNG, Germany) and paraffin. The historesin sections were submitted to Periodic Acid-Schiff (PAS) method, according to Cerri and Sasso-Cerri (2003). The PAS method is useful for the identification of the basal laminae surrounding the smooth muscle cells of arteriole/venules and underlying endothelial cells, facilitating the identification and morphometrical analyses of the profiles of these blood vessels. The paraffin sections, 6 μm thick, were adhered to silanized slides and submitted to the TUNEL (Terminal deoxynucleotidyl-transferase mediated dUTP Nick End Labelling) method for detection of cell death. Some testicular fragments were processed for analysis under transmission electron microscopy (TEM).

TUNEL method

The TUNEL method was performed as previously described (Sasso-Cerri and Miraglia, 2002; Sasso-Cerri and Cerri, 2008). After treatment of sections with 20 μg/ml proteinase K (Sigma-Aldrich Chemical Co., St. Louis, USA), the sections were immersed in 3% hydrogen peroxide for peroxidase inactivation and, subsequently, incubated in equilibration buffer for 20 min. The sections were incubated in a humidified chamber at 37°C in TdT enzyme (Terminal deoxy-nucleotidyl Transferase) for 1h and in anti-digoxigenin-peroxidase for 30 min. The reaction was revealed with 0.06% 3.3'-diaminobenzidine tetrahydrochloride (DAB - Sigma-Aldrich Chemical Co., St. Louis, USA) and counterstained with Carazzi's hematoxylin. Sections of involuting mammary gland provided by the manufacturer of the Kit were used as positive controls for the TUNEL method. Negative controls were incubated in a TdT enzyme-free solution.

Transmission Electron Microscopy (TEM)

The specimens were fixed for 16h in a mixture of 4% formaldehyde (freshly prepared from para-formaldehyde) and 5% glutaraldehyde buffered at pH 7.2 with 0.1 M sodium cacodylate. After washings in 0.1 M sodium cacodylate at pH 7.2 the specimens were transferred to sodium cacodylate-buffered 1% osmium tetroxide at pH 7.2 for 1h. The specimens were then immersed in 2% aqueous uranyl acetate for 1.5h, dehydrated in graded concentrations of ethanol, treated with propylene oxide and then embedded in Araldite. Semithin sections stained with 1% toluidine blue were examined in a light microscope and suitable regions were carefully selected for trimming of the blocks. Ultrathin sections were collected on grids, stained in

alcoholic 2% uranyl acetate and lead citrate and examined in a transmission electron microscope (Philips-CM 100).

Morphometrical analysis

The images were captured by a camera (DP-71, Olympus) attached to a light microscope (Olympus, BX-51). For each animal, three non-serial testicular sections were used and, in each section, approximately 17 fields were randomly chosen, totalling 50 fields per animal. In the interstitial tissue, the capillary profiles (cap), arteriole/venule profiles (a/v) and the total blood vessel profiles were quantified in a determined testicular area (18.3 mm^2) per animal at x860. Thus, the microvascular densities (MVD(cap), MVD(a/v), and total MVD) were obtained (Galindo-Moreno et al., 2010). To avoid the overestimation caused by the edge effect, two borders of the frame were systematically considered "forbidden", and the vessel profiles hit by these borders were not quantified (Gundersen, 1977).

With the aim of evaluating the effect of the treatment in the vascular structure, the vascular luminal area (VLA) was measured in each counted blood vessel profile. Thus, the vascular luminal area (VLA) was measured, at x1,720, in a total of 3,000-3,600 blood vessel profiles (capillary and arteriole/venule) per group. The capillaries and collapsed vessels were identified based on the presence of endothelial cells and PAS-positive basal lamina. These analyses were performed using an image analysis system (Image Pro-Express 6.0, Olympus). The areas corresponding to the lumen of arteriole/venule and capillary profiles were classified into four categories, as shown in Table 1, and the frequency of these vessels, according to the categories, was obtained.

Statistical analysis

Statistical analysis of data was performed using the software Jandel Statistical SigmaStat 2.0. The differences between the groups were analyzed by the one-way ANOVA followed by Student's t-test. The significance level considered was $p \leq 0.05$.

Results

Light microscopy

In the testes of animals from CG, the interstitial tissue was normally distributed around the seminiferous tubules and showed numerous blood vessel profiles with evident lumen (Fig. 1A,D). Generally, the arterioles/venules showed several concentric layers of smooth muscle cells, containing typical elliptical nuclei, surrounded by a PAS-positive basement membrane (Fig. 1D).

In the heterogeneously distributed interstitial tissue from CMTG, scarce blood vessel profiles were noted;

moreover, the majority of these blood vessels were collapsed (Fig. 1B,C) and several arteriole/venule profiles showed irregular shape, with smooth muscle cells forming a strong PAS-positive monolayer. In this layer, small and irregular nuclei and strongly basophilic nuclear fragments were observed (Fig. 1F). Some partially collapsed capillary profiles showed endothelial cell nuclei with strongly basophilic chromatin; small nuclear portions near the nuclei were also observed (Fig. 1E). TUNEL-positive vascular cells were found in the endothelium of capillaries, arterioles and venules, as well as in the muscular layer of arterioles and venules (Fig. 1G-L).

Transmission Electron Microscopy (TEM)

In the interstitial tissue of the testes of the animals from CG, profiles of blood capillaries, venules and arterioles with normal appearance and well-defined vascular lumen were observed (Figs. 2A, 3A). In the capillaries, the nuclei of endothelial cells were aligned along the major axis of the cell, and the vascular lumen was evident. In the venules and arterioles the endothelial cells surrounded a large lumen and were apposed to a regular and evident subendothelial layer. The smooth muscle cells showed normal nucleus with regularly distributed chromatin (Fig. 3A).

Table 1. Classification of arteriole/venule and capillary profiles according to the vascular luminal area (VLA).

Arterioles/Venules	Capillaries
Collapsed	Collapsed
Small (VLA≤49.99 μm^2)	Small (VLA≤39.99 μm^2)
Medium (50≤VLA≤599.99 μm^2)	Medium (40≤VLA≤299.99 μm^2)
Large (VLA≥600 μm^2)	Large (VLA≥300 μm^2)

Table 2. Total MVD, MVD of arterioles/venules (a/v) and MVD of capillaries (cap) of rats from CG and CMTG.

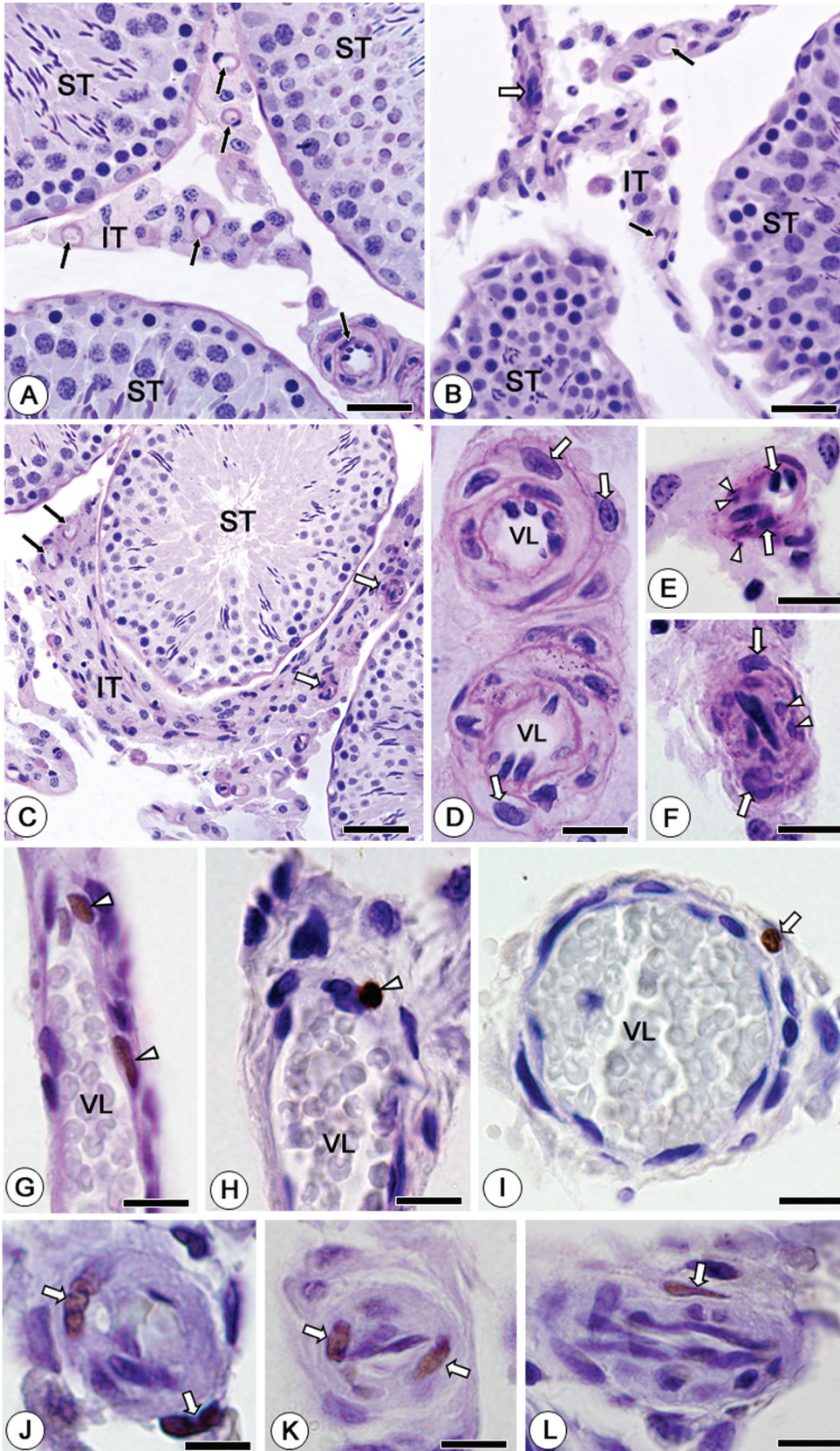| Animals	MVD		
	Total	a/v	cap
CG1	39.09	6.23	32.87
CG2	43.13	7.01	36.12
CG3	42.23	7.18	35.05
CG4	37.24	6.56	30.68
CG5	40.16	6.06	34.10
(X±SD)	40.37±2.37	6.61±0.48	33.76±2.10
CMTG1	38.81	4.94	33.88
CMTG2	36.79	4.99	31.80
CMTG3	32.98	5.27	27.71
CMTG4	30.57	6.73	23.84
CMTG5	27.99	6.79	21.20
(X±SD)	33.43±4.42*	5.74±0.94	27.68±5.29*

*$p \leq 0.05$

Fig. 1. Photomicrographs of testicular sections of rats from CG (**A and D**) and CMTG (**B, C, E-L**) submitted to PAS method (**A-F**) and to TUNEL method (**G-L**). In **A**, the interstitial tissue (IT) shows several blood vessel profiles with evident lumen (arrows). In **B and C**, scarce blood vessel profiles are observed (black arrows) in the interstitial tissue (IT); some of them are collapsed (white arrows). Seminiferous tubules (ST). In **D**, arteriole profiles with evident vascular lumen (VL) and concentric layers of smooth muscle cells with typical elliptical nuclei (arrows) are surrounded by PAS-positive basement membrane, in magenta. In **E**, a partially collapsed blood vessel profile shows endothelial cell nuclei with strongly basophilic chromatin (arrows); small portions of chromatin near the nuclei are observed (arrowheads). In **F**, a collapsed arteriole profile with irregular shape contains smooth muscle cells forming a strong PAS-positive monolayer, in which small irregular nuclei (arrows) and nuclear fragments (arrowheads) are observed. In **G**, a capillary profile with TUNEL-positive endothelial cells (arrowheads). In **H and I**, venule profiles with TUNEL-positive nuclei in the endothelium (arrowhead) and muscular layer (arrow). In Figs. **J-L,** collapsed arteriole profiles with TUNEL-positive smooth muscle cells (arrows). Vascular lumen (VL). Bars: A, B, 35 μm; C, 55 μm; D-F, 15 μm; G-I, L, 10 μm; J, 8 μm; K, 9 μm.

In the testes of animals from CMTG, obliterated or collapsed capillaries, arterioles and venules were found. In these blood vessels, portions of endothelial cells were protruding from the endothelium and obliterating partially or almost totally the vascular lumen (Figs. 2B,C, 3B,C). In some endothelial cells the whole nucleus was composed of a homogeneous mass of condensed chromatin, while in others, condensed clumps of chromatin were observed in the nuclear periphery (Fig. 2B,C). In some arteriole profiles the endothelial cells were protruding towards the vascular lumen (Fig. 3C) and the surface of endothelial cells in contact with the subendothelial layer was also folded (Fig. 3D). In both arterioles and venules the smooth muscle cells showed an irregular nucleus with peripheral condensed chromatin and portions of condensed chromatin in the cytoplasm (Fig. 3B,C).

Morphometrical results

Microvascular density (MVD) and vascular luminal area (VLA)

A significant reduction in the total MVD was observed in CMTG in comparison to CG (Table 2).

Table 3. Vascular luminal area per arteriole/venule profile (VLA/$_{a/v}$) and vascular luminal area per capillary profile (VLA/$_{cap}$) of rats from CG and CMTG.

Groups	VLA/$_{a/v}$	VLA/$_{cap}$
CG	891.86±227.57	120.7±22.91
CMTG	448.37±62.73*	75.02±11.43*

Values expressed in μm^2; X±SD; *p≤0,05

Although MVD(a/v) was intensely decreased in the animals from CMTG, this reduction was not statistically significant. Moreover, MVD(cap) decreased significantly (p≤0.05) in CMTG (Table 2).

A significant reduction in the VLA was also observed in CMTG (Table 3). The vascular luminal areas of arteriole/venule profiles (VLA/a/v) and capillary profiles (VLA/cap) was reduced significantly (49.7% and 37.8%, respectively) in CMTG.

Frequency of blood vessel profiles according to the vascular luminal area

The frequency of blood vessel profiles classified according to four categories is demonstrated in figures 4 and 5.

The CMTG showed a significant increase in the frequency of collapsed arteriole/venule profiles (158%). A significant increase in the frequency of arteriole/venule profiles with small vascular luminal area was also observed. The frequencies of arteriole/venule profiles with medium and large vascular luminal area were reduced significantly in the CMTG, in comparison to CG (Fig. 4).

Similarly to arteriole/venule, a significant increase (130%) was observed in the frequency of collapsed capillary profiles. The frequency of small capillaries increased significantly (54%) in the CMTG while the frequency of capillaries with medium and large vascular luminal area was reduced significantly (29% and 53%, respectively) in this group (Fig. 5).

Discussion

The use of historesin sections stained by PAS method provided an excellent resolution of the vascular
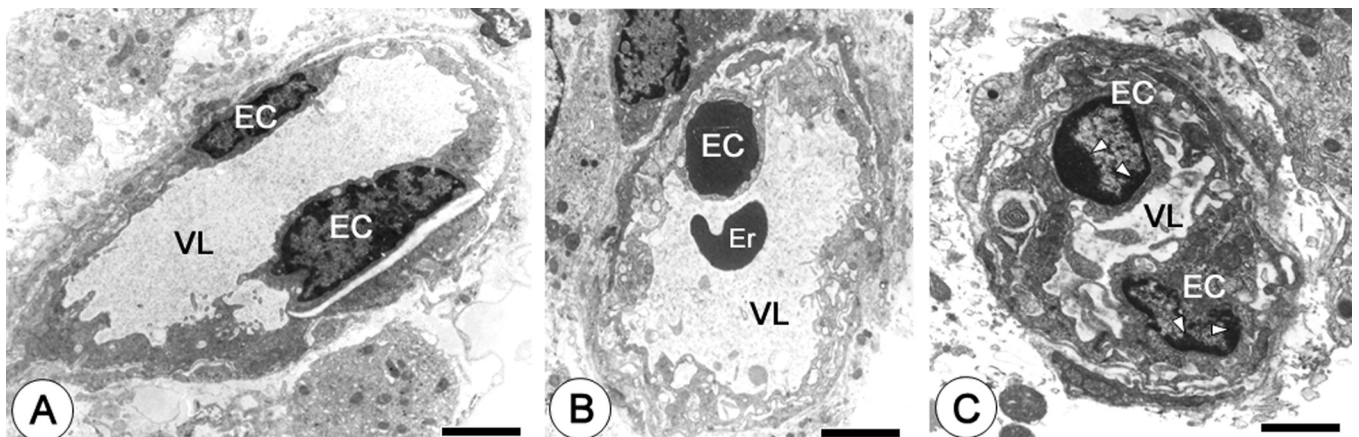

Fig. 2. Electron micrographs of testicular blood capillary profiles of rats from CG **(A)** and CMTG **(B and C)**. In **A**, capillary with evident lumen (VL) and endothelial cells (EC), whose nuclei are aligned and show normal aspect. In **B**, the endothelial cell nucleus (EC) is composed of a mass of electrondense chromatin. Erythrocyte (Er); Vascular lumen (VL). In **C**, a partially collapsed capillary shows abnormal endothelial cells (EC), with irregular nuclei and peripheral condensed chromatin (arrowheads). Vascular lumen (VL). Bars: A, 1.5 μm; B, 3 μm; C, 1 μm.
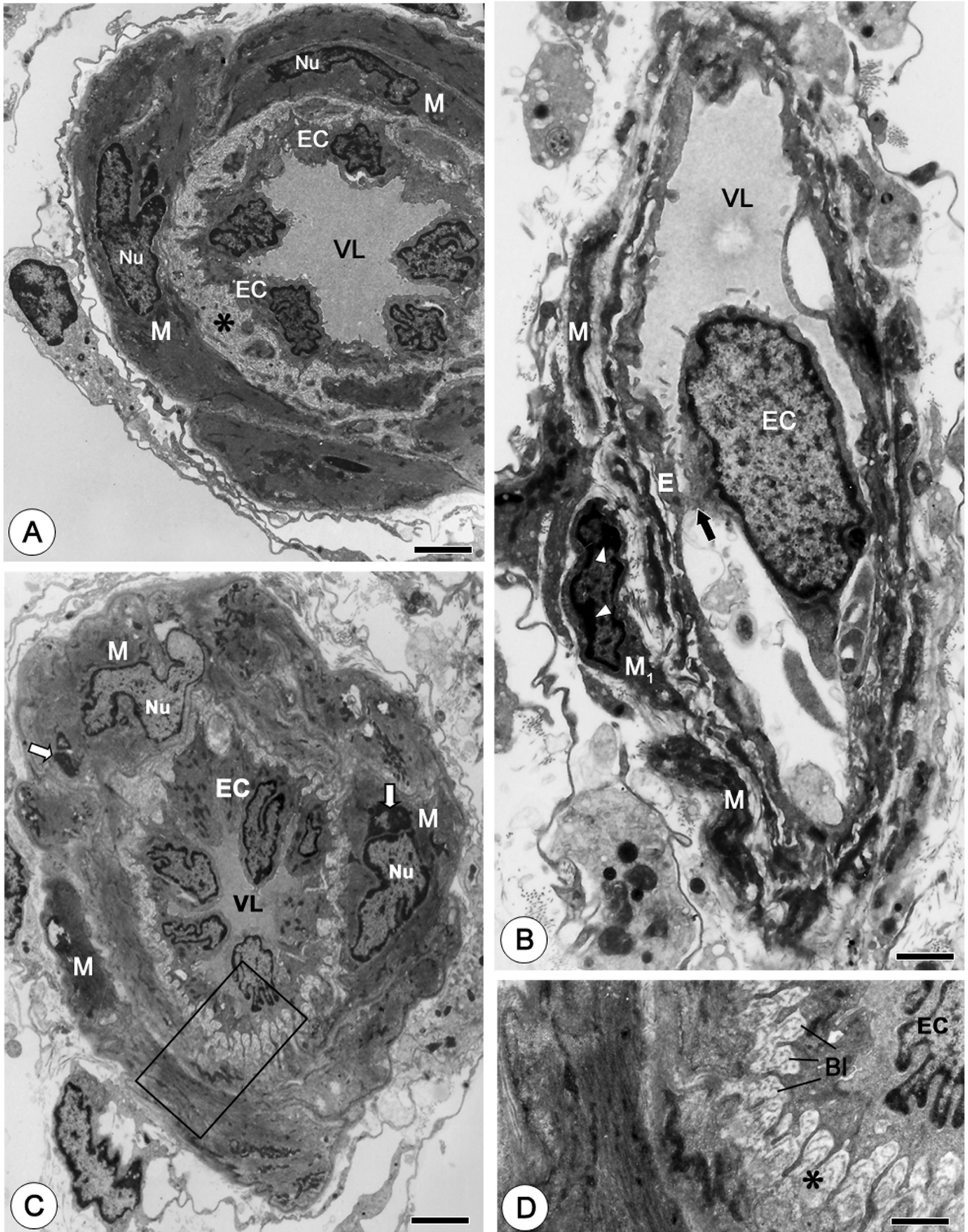
Fig. 3. Electron micrographs of testicular blood vessel profiles of rats from CG (**A**) and CMTG (**B-D**). In **A**, a normal arteriole with several endothelial cells (EC) and muscular layer (M) shows nuclei (Nu) with regularly distributed chromatin. The subendothelial layer (asterisk) and the vascular lumen (VL) are evident. In **B**, the venule shows a portion of an endothelial cell (EC) protruding from the endothelium (arrow) and obliterating the vascular lumen (VL). Muscle fiber portions (M) underlying the endothelium (E) are observed. In one of them (M1) the nucleus shows irregular outline and condensed clumps of chromatin in the nuclear periphery (arrowheads). In **C**, arteriole in atrophic process shows muscle cells (M) with irregularly outlined nuclei (Nu) and portions of condensed chromatin (white arrows). The endothelial cells (EC) are protruding towards the vascular lumen (VL), which is partially obliterated. In **D** (magnified portion of Fig. C), endothelial cell (EC), basal lamina (Bl) and subendothelial layer (asterisk) are folded. Bars: A, 3 μm; B, 0.7 μm; C, 1 μm; D, 0.5 μm.

structures analyzed and was useful for the morphometrical analyses, including the minimal capillary and collapsed blood vessel profiles. In the cimetidine-treated rats the significant reduction in the density of blood vessels (MVD) and in the vascular luminal area (arterioles/venules and capillaries) associated with the increased number of collapsed blood vessel profiles and to the apoptotic aspects of the vascular cells (under light and electron microscopy) indicate that cimetidine causes testicular vascular atrophy. This effect was confirmed by the analysis of frequency of arteriole/venule and capillary profiles according to the vascular luminal area. This analysis revealed that the significant increase in the frequency of both arterioles/venules with reduced vascular luminal area and collapsed arterioles/venules was due to atrophy

Fig. 4. Frequency (%) of arteriole and venule profiles (a/v), classified according to the vascular luminal area (VLA), from CG and CMTG. *$p \leq 0.05$

Fig. 5. Frequency (%) of capillary profiles (cap), classified according to the vascular luminal area (VLA), from CG and CMTG. *$p \leq 0.05$

of arterioles/venules with medium and large luminal areas. This is reinforced by the fact that the frequency of these vessel profiles (medium and large) was reduced significantly; the same process was observed in the capillaries.

It has been demonstrated that vascular atrophy occurs concomitantly with apoptosis in vascular cells (Meeson et al., 1996; Cho et al., 1997). In the cimetidine-treated rats, the analysis under light microscope showed vascular cells with apoptotic features. Moreover, either TUNEL-positive endothelial and/or muscle cells were found. The TUNEL method alone is not sufficient to confirm cell death by apoptosis. However, the typical structural features of apoptosis observed under light microscopy and TEM, such as condensed chromatin in the nuclear periphery, nuclear fragments and cellular shrinkage, have been conclusive for identification of this classical type of cell death (Cerri, 2005) and confirm that the endothelial and muscle cells undergo apoptosis.

In the testis, it has been demonstrated that cimetidine induces apoptosis in the peritubular myoid cells (França et al., 2000; Sasso-Cerri and Miraglia, 2002), indicating that smooth muscle cells are susceptible to cimetidine treatment. The significant changes induced by cimetidine in the testicular vasculature may be related, at least in part, to the effect of this drug on the smooth muscle cells of arterioles/venules. There is evidence that vascular muscle cell apoptosis induces vascular endothelium alterations, changes in the blood flow and death in other cell types (Clarke et al., 2006). During arterial remodeling, due to vascular injury, smooth muscle cell apoptosis is followed by vasoconstriction, resulting in the reduction of vascular diameter and luminal area (Cho et al., 1997). Therefore, either angiogenesis or vascular atrophy depend on the equilibrium between vascular proliferation and apoptosis (Mallat and Tedgui, 2000). The loss of this homeostasis significantly affects the dimensions of the vascular luminal area (McCarthy and Bennett, 2000). Thus, regarding the arteriole/venule profiles, the reduction of the vascular lumen, the vascular atrophy and, then, the reduction in the testicular MVD are due to cimetidine-induced smooth muscle cell apoptosis.

The vascular changes caused by cimetidine may be due to its antagonist effect on the H_2 receptors. The smooth muscle cells of blood vessels express histamine H_1 e H_2 receptors (Reinhardt and Ritter, 1979; Gross, 1981; Edvinsson et al., 1983). The binding of histamine to H_1 receptors leads to vascular smooth muscle contraction while the binding to H_2 receptors leads to muscle relaxation (Gross et al., 1981; Saari, 1986). The response of smooth muscle, under histamine action, is influenced by the extracellular calcium (Ca^{2+}) concentration (Vohra, 1979; Mikkelsen, 1995). In vas deferens, the activation of H_2 receptors by histamine inhibits the muscle contraction response due to a decrease in the Ca^{2+} influx necessary for contraction. This histamine effect (inhibition of muscle contraction)
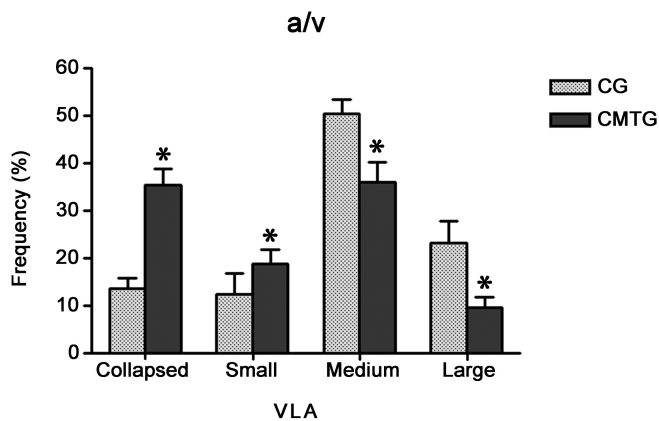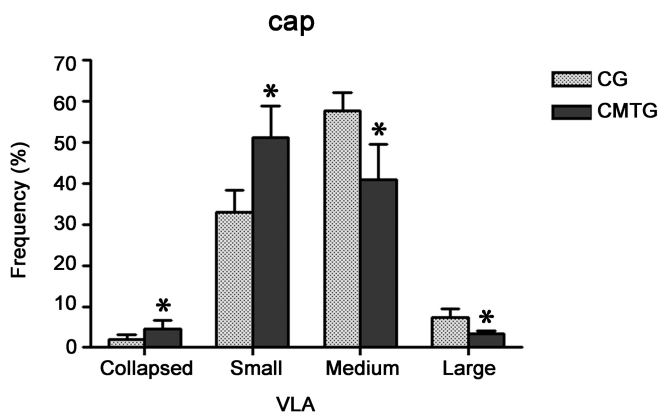
is reduced or totally antagonized by cimetidine (Vohra, 1979; Vohra, 1981). With the aim of verifying the vasomotion response by histamine control, Saari (1986) also demonstrated an increase in the contractile response of blood vessels after H_2 receptors inhibition by cimetidine. Thus, it is possible that cimetidine affects intracellular calcium homeostasis in the smooth muscle cells, leading to contraction of the testicular blood vessels and apoptosis of the vascular cells. It has been demonstrated that Ca^{2+} influx plays an important role in the apoptotic process of different cellular types (McConkey et al., 1989; McConkey and Orrenius, 1996; Sasaki et al., 2007). The excess of calcium ions seems to be one of the main routes involved in the induction of programmed cell death (Rizzuto et al., 2003). Cheng et al. (2003) showed that simvastatin, a hypolipidemic drug used to control elevated cholesterol, induces apoptosis in the smooth muscle cells due to increased Ca^{2+} levels and, subsequently, caspase activation. Therefore, it should be considered that cimetidine-induced vascular atrophy due to apoptosis in the muscle cells of the testicular arterioles/venules could be related to Ca^{2+} influx increase due to the antagonist effect of cimetidine on the H_2 receptors present in these cells. This possible effect is reinforced by the fact that H_2 receptors are also found in endothelial cells (Gross, 1981; Heltianu et al., 1982). In the present study, TUNEL-positive endothelial cells of arteriole/venule and capillary profiles were also found. Moreover, a significant reduction in the luminal area and density of capillaries was also related to apoptosis in the endothelial cells. In these vascular cells, histamine binds to H_2 receptors and stimulates VEGF production (Ghosh et al., 2001). As well as being a mitogenic factor, VEGF is also considered a survival factor of endothelial cells, since it inhibits apoptosis (Mallat and Tedgui, 2000). Therefore, further studies are necessary to confirm whether endothelial cells apoptosis is related to the inhibitory effect of cimetidine on VEGF expression.

Androgen receptors (ARs) have been detected in the smooth muscle cells of testicular arterioles (Vornberger et al., 1994); thus, these blood vessels seem to be controlled by testosterone (Bergh and Damber, 1992; Welsh et al., 2010). In smooth muscle cells, testosterone inhibits the type L calcium channels (Scragg et al., 2004, 2007). In the rat vas deferens, 5α-DHT inhibits Ca^{2+} influx through the voltage-dependent calcium channels and, then, inhibits smooth muscle contraction (Lafayette et al., 2008). As discussed previously, the increased calcium influx stimulates caspase cascade, leading to apoptosis (McConkey and Orrenius, 1996). Therefore, since cimetidine seems to exert an antiandrogenic effect, inhibiting ARs (Winters et al., 1979; Knigge et al., 1983), a possible antiandrogenic effect of cimetidine on the testicular vasculature should also be considered.

In conclusion, cimetidine treatment causes significant reduction in the luminal area of blood vessel profiles, leading to vascular atrophy and a significant reduction in testicular microvascular density. These vascular alterations are due to apoptosis of smooth muscle cells and endothelial cells. The cause of cimetidine-induced vascular cell apoptosis should be investigated, focusing on a possible interference on the androgen and/or H_2 receptors. Further studies are necessary to elucidate whether the histopathological disorders caused by cimetidine in the seminiferous tubules can be related to the impairment in the blood supply.

References

Beltrame F.L., Caneguim B.H., Miraglia S.M., Cerri P.S. and Sasso-Cerri E. (2011). Vitamin B12 supplement exerts a beneficial effect on the seminiferous epithelium of cimetidine-treated rats. Cells Tissues Organs 193, 184-194.

Bergh A. and Damber J.E. (1992). Immunohistochemical demonstration of androgen receptors on testicular blood vessels. Int. J. Androl. 15, 425-434.

Bergh A., Collin O. and Lissbrant E. (2001). Effects of acute graded reductions in testicular blood flow on testicular morphology in the adult rat. Biol. Reprod. 64, 13-20.

Cerri P.S. (2005). Osteoblasts engulf apoptotic bodies during alveolar bone formation in the rat maxilla. Anat. Rec. 286A, 833-840.

Cerri P.S. and Sasso-Cerri E. (2003). Staining methods applied to glycol methacrylate embedded tissue sections. Micron 34, 365-372.

Cheng G., Shan J., Xu G., Huang J., Ma J., Ying S. and Zhu L. (2003). Apoptosis induced by simvastatin in rat vascular smooth muscle cell through Ca^{2+}-calpain and caspase-3 dependent pathway. Pharmacol. Res. 48, 571-578.

Cho A., Mitchell L., Koopmans D. and Langille B.L. (1997). Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. Circ. Res. 81, 328-337.

Clarke M.C.H., Figg N., Maguire J.J., Davenport A.P., Goddard M., Littlewood T.D. and Bennett M.R. (2006). Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. Nat. Med. 12, 1075-1080.

Clayman C.B. (1977). Evaluation of cimetidine (Tagamet): An antagonist of hydrochloric acid secretion. JAMA 238, 1289-1290.

Edvinsson L., Gross P.M. and Mohamed A. (1983). Characterization of histamine receptors in cat cerebral arteries in vitro and in situ. J. Pharmacol. Exp. Ther. 225, 168-175.

Ergün S., Kiliç N., Harneit S., Paust H.J., Ungefroren H., Mukhopadhyay A., Davidoff M. and Holstein A.F. (1997). Microcirculation and the vascular control of the testis. Adv. Exp. Med. Biol. 424, 163-180.

França L.R., Leal M.C., Sasso-Cerri E., Vasconcelos A., Debeljuk L. and Russel L.D. (2000). Cimetidine (Tagamet) is a reproductive toxicant in male rats affecting peritubular cells. Biol. Reprod. 63, 1403-1412.

Galindo-Moreno P., Padial-Molina M., Fernández-Barbero J.E., Mesa F., Rodríguez-Martínez D. and O'Valle F. (2010). Optimal microvessel density from composite graft of autogenous maxillary cortical bone and anorganic bovine bone in sinus augmentation:

influence of clinical variables. Clin. Oral Impl. Res. 21, 221–227.

Ghosh A.K., Hirisawa N. and Ohuchi K. (2001). Enhancement by histamine of vascular endothelial growth factor production in granulation tissue via H_2 receptors. Br. J. Pharmacol. 134, 1419-1428.

Gross P.M. (1981). Histamine H_1- and H_2-receptors are differentially and spatially distributed in cerebral vessels. J. Cereb. Blood. Flow. Metab. 1, 441-446.

Gross P.M., Harper A.M. and Teasdale G.M. (1981). Cerebral circulation and histamine. 1. Participation of vascular H_1- and H_2-receptors in vasodilatory responses to carotid arterial infusion. J. Cereb. Blood. Flow. Metab. 1, 97-108.

Gundersen H.J. (1977). Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. J. Microsc. 111, 219-227.

Guslandi M. (1994). Antiulcer drugs and gastric mucosal blood flow. Pharmacol. Res. 30, 93-98.

Heltianu C., Simionescu M. and Simionescu N. (1982). Histamine Receptors of the Microvascular Endothelium Revealed In Situ with a Histamine-Ferritin Conjugate: Characteristic High-affinity Binding Sites in Venules. J. Cell. Biol. 93, 357-364.

Knigge U., Dejgaard A., Wollesen F., Ingerslev O., Bennett P. and Christiansen P.M. (1983). The acute and long term effect of the H_2-receptor antagonists cimetidine and ranitidine on the pituitary-gonadal axis in men. Clin. Endocrinol. 18, 307-313.

Lafayette S.S.L., Vladimirova I., Garcez-Do-Carmo L., Monteforte P.T., Neto A.C. and Jurkiewicz A. (2008). Evidence for the participation of calcium in non-genomic relaxations induced by androgenic steroids in rat vas deferens. Brit. J. Pharmacol. 153, 1242-1250.

Mallat Z. and Tedgui A. (2000). Apoptosis in the vasculature: mechanisms and functional importance. Br. J. Pharmacol. 130, 947-962.

McCarthy N.J. and Bennett M.R. (2000). The regulation of vascular smooth muscle cell apoptosis. Cardiovasc. Res. 45, 747-755.

McConkey D.J. and Orrenius S. (1996). The role of calcium in the regulation of apoptosis. J. Leukoc. Biol. 59, 775-783.

McConkey D.J., Nicotera P., Hartzell P., Bellomo G., Wyllie A.H. and Orrenius S. (1989). Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca^{2+} concentration. Arch. Biochem. Biophys. 269, 365-370.

Meeson A., Palmer M., Calfon M. and Lang R. (1996). A relationship between apoptosis and flow during programmed capillary regression is revealed by vital analysis. Development 122, 3929-3938.

Mikkelsen E.O. (1995). Calcium channel blockers (calcium antagonists). Background, effects and use. Ugeskr. Laeger. 157, 3750-3754.

Natori T., Sata M., Nagai R. and Makuuchi M. (2005). Cimetidine inhibits angiogenesis and suppresses tumor growth. Biomed. Pharmacother. 59, 56-60.

Pereira O.C. (1987). Some effects of cimetidine on the reproductive organs of rats. Gen. Pharmacol. 18, 197-199.

Reinhardt D. and Ritter E. (1979). Hypothermia-induced potentiation of histamine H_2-receptormediated relaxation and cyclic AMP increase in the isolated mesenteric artery of the rabbit. Agents Actions 9, 9-14.

Rizzuto R., Pinton P., Ferrari D., Chami M., Szabadkai G., Magalhães P.J., Di Virgilio F. and Pozzan T. (2003). Calcium and apoptosis: facts and hypotheses. Oncogene 22, 8619-8627.

Saari J.T. (1986). Characterization of the coronary vascular response to histamine in rabbit hearts using cimetidine. Pharmacology 32, 80-89.

Sasaki H., Watanabe F., Murano T., Miyashita Y. and Shirai K. (2007). Vascular smooth muscle cell apoptosis induced by 7-ketocholesterol was mediated via Ca^{2+} and inhibited by the calcium channel blocker nifedipine. Metabolism 56, 357-362.

Sasso-Cerri E. and Cerri P.S. (2008). Morphological evidences indicate that the interference of cimetidine on the peritubular components is responsible for detachment and apoptosis of Sertoli cells. Reprod. Biol. Endocrinol. 6, 18.

Sasso-Cerri E. and Miraglia S.M. (2002). *In situ* demonstration of both TUNEL-labeled germ cell and Sertoli cell in the cimetidine-treated rats. Histol. Histopathol. 17, 411-417.

Sasso-Cerri E., Giovanoni M., Hayashi H. and Miraglia S.M. (2001). Morphological alterations and intratubular lipid inclusions as indicative of spermatogenic damage in cimetidine-treated rats. Arch. Androl. 46, 5-13.

Scragg J.L., Jones R.D., Channer K.S., Jones T.H. and Peers C. (2004). Testosterone is a potent inhibitor of L-type Ca^{2+} channels. Biochem. Biophys. Res. Commun. 318, 503-506.

Scragg J.L., Dallas M.L. and Peers C. (2007). Molecular requirements for L-type Ca^{2+} channel blockade by testosterone. Cell Calcium 42, 11-15.

Tomita K., Izumi K. and Okabe S. (2003). Roxatidine- and cimetidine-induced angiogenesis inhibition suppresses growth of colon cancer implants in syngeneic mice. J. Pharmacol. Sci. 93, 321-330.

Tsuchida T., Tsukamoto Y., Segawa K., Goto H. and Hase S. (1990). Effects of cimetidine and omeprazole on angiogenesis in granulation tissue of acetic acid-induced gastric ulcers in rats. Digestion 47, 8-14.

Vohra M.M. (1979). A postsynaptic inhibitory histamine H_2-receptor in the mouse isolated vas deferens. Agents Actions 9, 319-326.

Vohra M.M. (1981). Species differences in histamine receptors in the vas deferens. Agents Actions 11, 208-214.

Vornberger W., Prins G., Musto N.A. and Suarez-Quian C.A. (1994). Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134, 2307-2316.

Welsh M., Sharpe R.M., Moffat L., Atanassova N., Saunders P.T.K., Kilter S., Bergh A. and Smith L.B. (2010). Androgen action via testicular arteriole smooth muscle cells is important for Leydig cell function, vasomotion and testicular fluid dynamics. PLoS One 5, e13632.

Winters S.J., Banks J.L. and Loriaux D.L. (1979). Cimetidine is an antiandrogen in the rat. Gastroenterology 76, 504-508.