Histol Histopathol (2002) 17: 411-417

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

In situ demonstration of both TUNEL-labeled germ cell and Sertoli cell in the cimetidine-treated rats

E. Sasso-Cerri and S.M. Miraglia

Department of Morphology, Federal University of São Paulo (UNIFESP), São Paulo (SP), Brazil

Summary. Cimetidine has caused dysfunction in the male reproductive system. In the rat testis, intratubular alterations and loss of peritubular tissue due to peritubular myoid cell death by apoptosis have been recently shown. Thus, the aim of this study is to evaluate which cells of the seminiferous epithelium have been affected and/or died by apoptosis after the treatment with cimetidine.

For this purpose, an experimental group containing five male albino Wistar rats received intraperitoneal injections of cimetidine (50 mg/kg body weight) during 52 days. The testes were fixed with 4% buffered formaldehyde and were embedded in paraffin. For detection of DNA breaks (apoptosis) in the cells of the seminiferous epithelium, the testicular sections were treated by the TUNEL method (Apop-Tag Plus Peroxidase Kit).

In the tubules affected by cimetidine, altered peritubular tissue, including the presence of TUNEL labeling in the myoid peritubular cells, were usually found. In these tubules, the seminiferous epithelium exhibited low density of germ cells and TUNEL-positive labeling in the germ cells of the basal compartment. The concomitant staining in both germ cells of the basal compartment and late spermatids suggest a sensitivity of these cells in the damaged tubules. Besides germ cells, TUNEL-positive Sertoli cells were also found in the injured seminiferous tubules. Thus, a relationship between dying germ cells and Sertoli cell damage and/or death must be considered in tubules where peritubular tissue has been affected by toxicants.

Key words: Apoptosis, Testis, Cimetidine, Germ cells, Sertoli cell

Offprint requests to: Estela Sasso-Cerri, Rua Estado de Israel, 907, ap.52 - Vila Clementino, Cep: 04022-002, São Paulo, SP, Brazil. Fax: 55 11 5576-4263. e-mail: scerri.morf@epm.br

Introduction

Cimetidine has been widely utilized in human for treatment of duodenal ulcer (Ippoliti et al., 1978). This drug competes with dihydrotestosterone (DHT) in ventral prostate (Winters et al., 1979) and causes male sexual dysfunction (Peden et al., 1981; Wang et al., 1982), thus, it has been referred to as a weak non-steroidal anti-androgen (Winters et al., 1979; Peden et al., 1981; Wang et al., 1982).

In the testis, cimetidine has caused significant reductions of testicular weight (Pereira, 1987; Sasso-Cerri et al., 2001), tubular diameters (Gill et al., 1991; França et al., 2000; Sasso-Cerri et al., 2001), and seminiferous epithelium height (Gill et al., 1991; França et al., 2000). França et al. (2000) demonstrated a significant reduction of tunica propria (peritubular tissue) in the testis of cimetidine-treated rats. In addition, the presence of apoptotic peritubular cells and disordered basal lamina around apparently normal seminiferous tubules suggests that the peritubular cell was the primary target affected by cimetidine (França et al., 2000). In the seminiferous epithelium, loss of germ cells and lack of contact between Sertoli cells and spermatids have been also described in the cimetidine-treated rats. Moreover, an increased concentration of intratubular lipid inclusions and failure of spermatid release have been related to degradation of late spermatids which were probably phagocytosed by Sertoli cells (Sasso-Cerri et al., 2001).

Several factors provide or prevent cell death in the testis (reviewed in Blanco-Rodríguez, 1998). Testicular injuries caused by hormonal disturbance (Blanco-Rodríguez and Martínez-García, 1996, 1998) or testicular toxicants (Richburg and Boekelheide, 1996; Richburg et al., 1999) usually lead to germ cell apoptosis. These experimental models have been widely utilized for the knowledge of the control mechanisms involved in cell death during the spermatogenic process. The treatment with MEHP (Mono-(2-Ethylhexyl) phthalate), for example, causes Sertoli cell damage and, consequently, programmed cell death in the germ cells that cannot be supported by the Sertoli cells (Richburg

and Boekelheide, 1996; Richburg et al., 1999; Lee et al., 1999).

Taking into account that peritubular myoid cell is affected by cimetidine in the rat testes (França et al., 2000) and that these cells seem to stimulate Sertoli cell activity (Thompson et al., 1995), a consecutive damage in both Sertoli cells and germ cells is expected in the affected tubules by cimetidine. Thus, we proposed to evaluate which cells of the seminiferous epithelium are affected and/or undergo cell death in the tubules affected by cimetidine. For this purpose, cell death in the seminiferous epithelium has been detected in the testicular sections of cimetidine-treated rats by *in-situ* 3´-end labeling of fragmented DNA (TUNEL method).

Materials and methods

Animals and treatment

Ten male albino Wistar rats - provided by the Center of Development of Experimental Models of the Federal University of São Paulo - aged 90 days and weighing 300g were kept under 25 °C temperature, standard lighting conditions (12-h light/dark cycle), and water and food *ad libitum*. The animals were distributed into two groups (control and experimental) containing five animals each. The experimental group received intraperitoneal injections of aqueous solution of 50 mg/kg body weight/day cimetidine (Tagamet, SmithKline Beecham) for 52 consecutive days - the period pertinent to the duration of spermatogenesis in adult Wistar rats (Huckins, 1965). Principles of laboratory animal care (NIH publication 85-23, 1985) and national laws on animal use were observed.

Tissue preparation and TUNEL labeling

Twenty-four hours after ending the treatment, the testes of the anaesthetized rats were fixed with 4% buffered formaldehyde (Sasso-Cerri et al., 2001), dehydrated and embedded in paraffin. Testicular sections (5 µm thick) were deparaffinized, hydrated and pretreated with 20 µg/ml proteinase K (Oncor-Protein Digesting Enzyme) for 15 min at 37 °C. The sections were immersed in 3% hydrogen peroxide for 10 minutes, and DNA 3'-end labeling of apoptotic cells was performed by the Apop-Tag Plus Peroxidase Kit (Oncor, Inc. Gaithersburg, MD) as previously described (Cerri et al., 2000). After washes in PBS and in the "equilibration" buffer, the sections were incubated in TdT enzyme (Terminal deoxynucleotidyl Transferase) for 1 hr in a humidified chamber, at 37 °C. Subsequently, the sections were immersed in "stop wash" buffer for 15 min, washed in PBS and incubated in anti-digoxigenin-peroxidase for 30 min, at 37 °C. After several washes in PBS, the reaction was revealed by DAB (3.3'-diaminobenzidine tetrahydrochloride) and finally, the sections were counterstained with Mayer's hematoxylin. Negative controls were processed in the same manner, except that the TdT enzyme was substituted by distilled water. Moreover, involuting mammary gland sections (provided by Apop-Tag Kit/Oncor, Inc. Gaithersburg) were also used as positive controls for the TUNEL method. The testicular sections were examined and photographed by an OLYMPUS BX-50 photomicroscope.

Results

In the testicular sections of the animals from the control group, the seminiferous tubules exhibited rare cells of the germinal lineage labeled by the TUNEL method. On the other hand, numerous structures exhibiting TUNEL-positive staining (brown-yellow) were usually found in the seminiferous tubules of the treated rats. The mammary gland sections (positive controls) also showed a specific staining in several structures, but in the negative controls no color reaction was detected after omitting the TdT.

The seminiferous tubules from cimetidine-treated rats showed low density of germ cells and TUNEL-positive staining in the germ cells of the basal compartment (Fig. 1A-F). In general, these tubules presented altered peritubular tissue in which the myoid cells were absent or exhibited TUNEL-positive labeling (Figs. 1E,F, 2I). At almost all stages of the seminiferous cycle (II-XIII), the most frequent TUNEL-positive germ cells were spermatogonia, preleptotene and leptotene spermatocytes (Fig. 1B-F). In tubules exhibiting TUNEL-positive pre-leptotene or leptotene spermatocytes, spermatogonia were rarely found. Spermatids (steps 16-19) were also absent in the III-VII tubules showing TUNEL-positive labeling in the germ cells of the basal compartment (Fig. 1B). On the other hand, some tubules (IX-XII), in which spermatids were present, a concomitant TUNEL-positive labeling in both elongating spermatids and germ cells of the basal compartment was also observed (Figs. 1C-E). TUNEL-positive labeling in the pachytene to diplotene spermatocytes was not observed in the less injured tubules (Figs. 1A-F). The highly damaged seminiferous tubules containing Sertoli cells and rare cells of the germinal lineage showed TUNEL-positive staining in round and elongating spermatids likewise in pachytene and diplotene spermatocytes (Fig. 2A-E). Some of these labeled germ cells surrounded by a vacuole-like structure were adjacent to Sertoli cell nuclei (Fig. 2A-D).

In tubules at stages IX-X, some TUNEL-positive spermatids were detached from Sertoli cells (Figs. 1C, 2F); sometimes these tubules also showed TUNEL-positive Sertoli cell nuclei abnormally positioned in the lumen (Fig. 2F). In general, these nuclei exhibited an evident nucleolus associated to a juxtanucleolar portion - typical of a Sertoli cell. Either in the apparently normal or maximally injured tubules, some Sertoli cell nuclei were dislocated from the tunica propria to the adluminal compartment (Fig. 1B, 2A,B,D). In the maximally injured tubules, Sertoli cell nuclei exhibiting TUNEL-

positive labeling were often observed (Fig. 2G,H). Occasionally, these cells were very close to TUNEL-negative Sertoli cell nucleus (Fig. 2H). In the maximally damaged tubules showing irregular outline, in which peritubular cells were TUNEL-positive, no cell of the seminiferous epithelium was adjacent to the altered

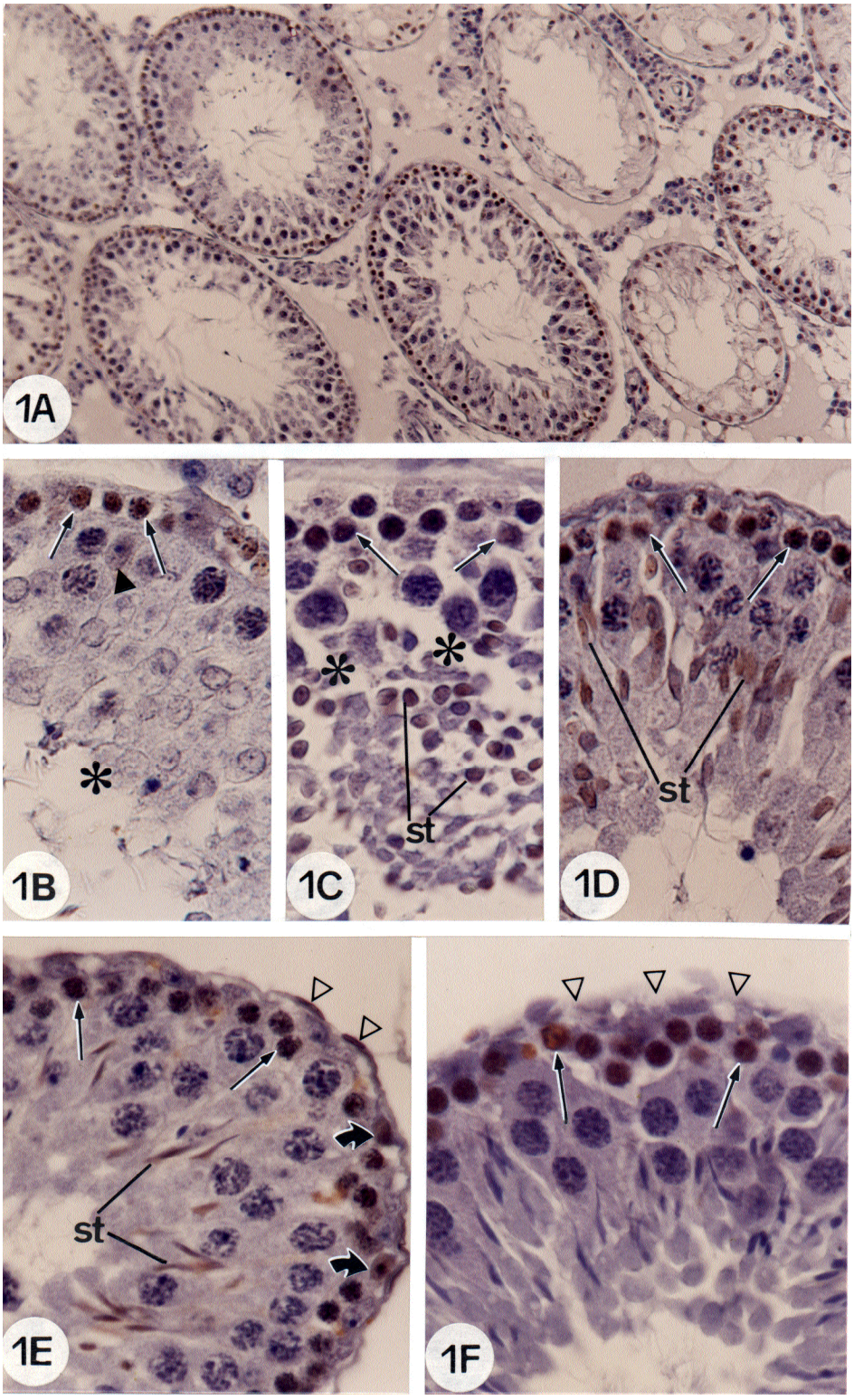

Fig. 1. Photomicrographs of testicular sections of cimetidine-treated rats exhibiting *in-situ* labeling of apoptotic cells by the TUNEL method. **A.** Seminiferous tubules show loss of germ cells and TUNEL-positive labeling in the germ cells of the basal compartment. x 140. **B.** A tubule at stage VI exhibits loss of elongated spermatids at step 18 (asterisk) and TUNEL-positive labeling in the B spermatogonia (arrows). A Sertoli cell nucleus dislocated from the tunica propria is also observed (arrowhead). x 600. **C and D.** TUNEL-positive leptotene spermatocytes (arrows) and spermatids (st) are observed in the tubules at stages IX **(C)** and X **(D)**. In **C**, some spaces among germ cells (asterisks) indicate detachment of germ cells from Sertoli cells. x 600. **E.** TUNEL-positive labeling in both zygotene spermatocytes (thin arrows) and spermatids (st) are exhibited in a tubule at stage XII. In addition, spermatogonia are not found and some Sertoli cells (thick arrows) as well as peritubular myoid cells (arrowheads) are also labeled by the TUNEL method. x 600. **F.** In a stage XII tubule, only zygotene spermatocytes are TUNEL-positive (arrows). The altered peritubular tissue including absence of peritubular myoid cells are also observed (arrowheads). x 600

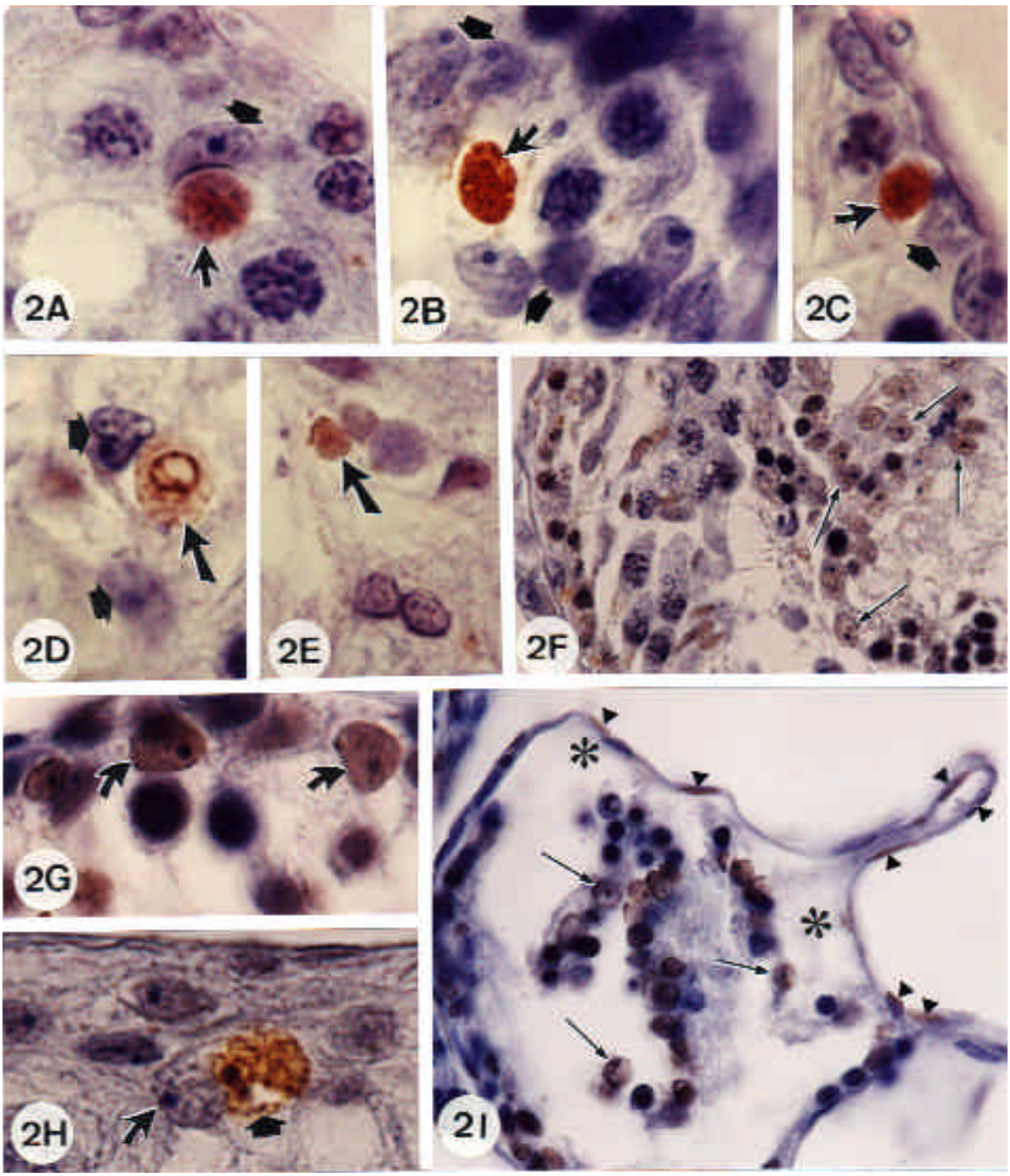

Fig. 2. Photomicrographs of testicular sections of cimetidine-treated rats exhibiting *in situ* labeling of apoptotic cells in several damaged seminiferous tubules. **A and B.** TUNEL-positive pachytene spermatocytes (thin arrows), surrounded by a vacuole-like structure, are adjacent to one **(A)** or several **(B)** Sertoli cell nuclei (thick arrows). Note that the Sertoli cell nuclei are dislocated from the tunica propria. x 1,450. **C.** In the seminiferous tubule containing Sertoli cells and rare germ cells, a TUNEL-positive germ cell, probably a preleptotene spermatocyte (thin arrow), is surrounded by a vacuole-like structure and is adjacent to a Sertoli cell nucleus (thick arrow). x 1,450. **D and E.** TUNEL-positive round and step 9 spermatids (thin arrows), respectively. In **D**, the spermatid is next to Sertoli cell nuclei (thick arrows) in the adluminal compartment. x 1450. **F.** A disordered seminiferous tubule at stage X showing TUNEL-positive germ cells (i.e. leptotene spermatocytes and spermatids) and numerous TUNEL-positive Sertoli cell nuclei abnormally positioned in the tubular lumen (arrows). x 600. **G, H and I.** Maximally damaged seminiferous tubules containing Sertoli cells and rare cells of the germinal lineage. In **G**, TUNEL-positive Sertoli cell nuclei are adjacent to the tunica propria (arrows). In **H**, a strongly TUNEL-positive Sertoli cell nucleus dislocated from the tunica propria (thick arrow) is in close contact with a TUNEL-negative Sertoli cell nucleus (thin arrow). x 1,450. **I.** Tubule exhibiting irregularly outlined tunica propria and several TUNEL-positive peritubular cells (arrowheads). Note that no cell of the seminiferous epithelium is adjacent to this portion of the tunica propria (asterisks). Among the dislocated germ cells, some TUNEL-positive Sertoli cells are also observed (arrows). x 600

portion of the tunica propria. In addition, together with the germ cells localized in the tubular lumen, some TUNEL-positive Sertoli cell nuclei were found (Fig. 2I).

Discussion

Cimetidine has been referred to as a weak non-steroidal antiandrogenic drug (Winters et al., 1979; Peden et al., 1981; Wang et al., 1982) because it antagonizes dihydrotestosterone (DHT) and occupies androgen receptors in the accessory sex organs (Winters et al., 1979). In the testis, intratubular morphological changes (Gill et al., 1991; Sasso-Cerri et al., 2001) together with significant reduction of tubular diameter at stages VII-IX (Sasso-Cerri et al., 2001) have been related to a possible interference of cimetidine in the tubular androgenization of the treated rats (Gill et al., 1991; Sasso-Cerri et al., 2001). A significant reduction of peritubular tissue and the loss of peritubular myoid cells by apoptosis in tubules exhibiting normal aspect have indicated that these cells are the primary target affected by cimetidine action in the rat testis (França et al., 2000). In the present study, the presence of disordered tunica propria (where peritubular myoid cells were absent or exhibited TUNEL-positive labeling) may be associated with a concomitant or consecutive loss of germ cells via apoptosis and Sertoli cell death in the tubules affected by cimetidine. This is supported by the occurrence of TUNEL-positive labeling in the germ cells of the basal compartment (i.e. spermatogonia and preleptotene/leptotene spermatocytes), in spermatids and in Sertoli cells. The TUNEL method has been widely used and has provided valuable information about apoptosis *in situ*. After some tests made in different tissues for detection of apoptosis by TUNEL, no false-positive reaction has been detected in the testis (Pulkkanen et al., 2000). Our results are in agreement with these observations since no labeling was verified in the testicular sections used as negative controls.

In contrast to other mechanisms of cell death such as necrosis, apoptotic germ cells are rapidly phagocytosed by Sertoli cells preventing an inflammatory response (Allan et al., 1992). Thus, the absence of germ cells in some tubules could reinforce the fact that these cells died via apoptosis and were phagocytosed by Sertoli cells. As previously shown, failure of spermatid release together with an increased concentration of intratubular lipid inclusions have been related to phagocytosis of these germ cells by Sertoli cells (Sasso-Cerri et al., 2001). Our results showed TUNEL-positive germ cells adjacent to Sertoli cell nuclei in both basal or adluminal compartments. In addition, these cells were usually surrounded by a vacuole-like structure. Thus, a suggestive phagocytosis of apoptotic germ cells by Sertoli cells may be considered in the cimetidine-treated rats. Similar ultrastructural features, have also been associated with phagocytosis of germ cells *in vivo* (Mori et al., 1997).

Considering that cimetidine causes peritubular myoid cell death as well as morphological alterations in the basal lamina of the tunica propria (França et al., 2000), an abnormal activation of cell death may have occurred in the germ cells that were in direct contact with the basal lamina. On the other hand, TUNEL-positive labeling in zygotene to diplotene spermatocytes as well as in round spermatids (stages II-VIII) was not observed in the less damaged tubules. This may be due to the fact that leptotene spermatocytes pass through the blood-testis barrier which separates the basal from the adluminal compartments (Russell et al., 1990); in the latter, these cells continue the meiotic prophase (zygotene to diplotene) being protected from the immune system (reviewed in Heindel and Treinen, 1989). It is interesting to note that TUNEL-positive labeling in the spermatids (steps 9-12) was observed in tubules where germ cells of the basal compartment were also dying. Spermatids (step 9-10) are rarely seen dying during normal spermatogenesis (Blanco-Rodríguez and Martínez-García, 1996). TUNEL-positive spermatids were not found in the testicular sections from the control rats or in the negative controls from cimetidine-treated rats. It is important to emphasize that in the tubules at stages III-VII, late spermatids (steps 16-19) were not found. Thus, the loss of these cells was probably due to an apoptotic pathway in spermatids at early stages (steps 9-12). Some interference in the survival factors required by the elongating spermatids might have occurred during the cimetidine treatment. Considering that several IX-XII tubules exhibited positive labeling only in the germ cells of the basal compartment, it might be concluded that the cells of the basal compartment seem to be the primary germ cells to undergo cell death in these tubules. An indirect influence from the apoptotic germ cells of the basal compartment mediated by Sertoli cells might be considered. This is supported by the presence of a specific control of germ cell death involving extracellular regulatory signals, as previously proposed (Packer et al., 1995; Blanco-Rodríguez and Martínez-García, 1997).

It is known that vimentin filaments may be responsible for both Sertoli cell-germ cell attachment and positioning of the Sertoli cell nucleus (Aumuller et al., 1992). Detachment of spermatids (step 9) from the Sertoli cells was usually observed in the cimetidine-treated rats (Sasso-Cerri, 2001). In the present study, these detached spermatids were also positive to the TUNEL labeling. After Sertoli cell injury by MEHP (Mono-(2-Ethylhexyl)phthalate), vimentin filaments collapse around the nucleus and the Sertoli cell-germ cell physical interaction is disrupted (Richburg and Boekelheide, 1996). Subsequently, programmed cell death is induced in the detached germ cells by the Fas system (Richburg et al., 1999). Our results showed Sertoli cell nuclei dislocated from the tunica propria. In addition, TUNEL-positive Sertoli cell nuclei were also seen in basal and adluminal compartments as well as in the tubular lumen. The occurrence of apoptosis in Sertoli cells is not commonly observed (Billig et al., 1995; Lin

et al., 1997; Mori et al., 1997). *In vitro* studies have demonstrated that FSH and testosterone appear to protect Sertoli cells against apoptosis, but not in the absence of the basement membrane (Dirami et al., 1995). Morphological changes in the basal lamina adjacent to Sertoli cells have been described in the cimetidine-treated rats (França et al., 2000). Considering that the basement membrane seems to be essential for the maintenance and survival of Sertoli cells (Dirami et al., 1995), it might be suggested that an interference in the functional or structural integrity of the Sertoli cells may have occurred in the tubules affected by cimetidine. This is supported by the fact that labeled Sertoli cell nuclei were dislocated from the tunica propria in tubules where irregular outline as well as dying peritubular cells were found. Considering the possibility that germ cell loss takes place when Sertoli cell function is altered (Lee et al., 1999), the loss of the germ cells verified in the present study can be directly related to the Sertoli cell damage and/or death.

Increased FSH and reduced testosterone levels have been associated with the increase of germ cell apoptosis in damaged testis with MEHP (Brinkworth et al., 1995). Although significant changes in the levels of plasma testosterone have not been verified after treatment with cimetidine (Winters et al., 1979; França et al., 2000; Sasso-Cerri et al., 2001), a significant increase of the FSH levels has been observed (Wang et al., 1982; França et al., 2000). It is known that peritubular myoid cells present androgen receptors (Vornberger et al., 1994). In addition, these cells, together with the extracellular matrix components (i.e. basement membrane), are able to stimulate the production of Androgen Binding Protein (ABP) by Sertoli cell (Thompson et al., 1995). Besides this, considering that: 1) ABP bind to testosterone as well as to DHT with high affinity (reviewed in Heindel and Treinen, 1989); 2) DHT has shown to be indispensable for the restoration of spermiogenesis (O'Donnell et al., 1996) and for Sertoli cell activity (Lamb et al., 1981); 3) cimetidine competes for DHT (Winters et al., 1979), and 4) high FSH levels are associated with intratesticular decreases of inhibin, testosterone (Weinbauer et al., 1989) and ABP (Kerr et al., 1979); it seems to be reasonable to suggest that the survival factors required for the maintenance of the seminiferous epithelium, mainly for Sertoli cells, might be affected by cimetidine. Thus, a consecutive disturbance in the feedback control for FSH secretion can explain the increased FSH levels as previously observed (Wang et al., 1982; França et al., 2000).

Based on the results of the present study, it can be concluded that programmed cell death in the germ cells of the basal compartment and in the elongating spermatids together with the absence of late spermatids suggest that these cells are the most sensitive to the cimetidine action. It is probable that damage and death of the Sertoli cells can be, at least in part, responsible for the loss of germ cells via apoptosis in the treated rats with cimetidine. Although Sertoli cell apoptosis has rarely been described under normal or abnormal conditions, disrupted spermatogenesis, in which peritubular tissue damage has occurred, should be investigated taking into account possible Sertoli cell alteration and/or death.

Acknowledgments. The authors thank Drs. Paulo S. Cerri and Wilson S. Sasso for their advice in the preparation of the manuscript.

References

Allan D.J., Harmon B.V. and Roberts S.A. (1992). Spermatogonial apoptosis has three morphologically recognizable phases and shows no circadian rhythm during normal spermatogenesis in the rat. Cell Prolif. 25, 241-250.

Aumuller G., Schulze C. and Viebahn C. (1992). Intermediate filaments in Sertoli cells. Microsc. Res. Tech. 20, 50-72.

Billig H., Furuta I., Rivier C., Tapanainen J., Parvinen M. and Hsueh J.W. (1995). Apoptosis in testis germ cells: developmental changes in gonadotropin dependence and localization to selective tubule stages. Endocrinology 136, 5-12.

Blanco-Rodríguez J. (1998). A matter of death and life: the significance of germ cell death during spermatogenesis (Review). Int. J. Androl. 21, 236-248.

Blanco-Rodríguez J. and Martínez-García C. (1996). Spontaneous germ cell death in the testis of the adult rat takes the form of apoptosis: assessment of cell types that exhibit the ability to undergo apoptosis during spermatogenesis. Cell Prolif. 29, 13-31.

Blanco-Rodríguez J. and Martínez-García C. (1997). *in vivo* analysis of germ cell apoptosis reveals the existence of stage-specific ´social` control of germ cell death in the seminiferous epithelium. Int. J. Androl. 20, 373-379.

Blanco-Rodríguez J. and Martínez-García C. (1998). Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormone suppression by short-term oestradiol treatment of rats. Int. J. Androl. 21, 109-115.

Brinkworth M.H., Weinbauer G.F., Schlatt S. and Nieschlag E. (1995). Identification of male germ cells undergoing apoptosis in adult rats. J. Reprod. Fertil. 105, 25-33.

Cerri P.S., Freymüller E. and Katchburian E. (2000). Apoptosis in the early developing periodontium of rat molars. Anat. Rec. 258, 136-144.

Dirami G., Ravindranath N., Kleinman H.K. and Dym M. (1995). Evidence that basement membrane prevents apoptosis of Sertoli cells *in vitro* in the absence of known regulators of Sertoli function. Endocrinology 135, 4439-4447.

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

Gill M., Sareen M.L. and Sanyal S.N. (1991). Effect of H2-receptor antagonists, cimetidine and ranitidine on reproductive functions in male mice. Indian J. Exp. Biol. 29, 900-906.

Heindel J.J. and Treinen K.A. (1989). Physiology of the male reproductive system: endocrine, paracrine and autocrine regulation. Toxicol. Pathol. 17, 411-445.

Huckins C. (1965). Duration of spermatogenesis in pre and postpuberal Wistar rats. Anat. Rec. 151, 364 (Abstract).

Ippoliti A.F., Sturdevant R.A.L., Isenberg J.I., Binder M., Camacho R., Cano R., Cooney C., Kline M.M., Koretz R.L., Meyer J.H., Samloff I.M., Schwabe A.D., Strom E.A., Valenzuela J.E. and Wintroub R.H. (1978). Cimetidine versus intensive antacid therapy for duodenal ulcer. Gastroenterology 74, 393-395.

Kerr J.B., Rich K.A. and Kretser D.M. (1979). Effects of experimental cryptorchidism on the ultrastructure and function of the Sertoli cell and peritubular tissue of the rat testis. Biol. Reprod. 21, 823-838.

Lamb D.J., Tsay Y.H., Steinberger A. and Sanborn B.M. (1981). Sertoli cell nuclear transcriptional activity: stimulation by follicle stimulating hormone and testosterone *in vitro*. Endocrinology 108, 1020-1026.

Lee J., Richburg J.H., Shipp E.B., Meistrich M.L. and Boekelheide K. (1999). The Fas system, a regulator of testicular germ cell apoptosis, is differentially up-regulated in Sertoli cell versus germ cell injury of the testis. Endocrinology 140, 852-858.

Lin W.W., Lamb D.J., Wheeler T.M., Lipshultz L.I. and Kim E.D. (1997). In situ end-labeling of human testicular tissue demonstrates increased apoptosis in conditions of abnormal spermatogenesis. Fertil. Steril. 68, 1065-1069.

Mori C., Nakamura N., Dix D.J., Fujioka M., Nakagawa S., Shiota K. and Eddy E.M. (1997). Morphological analysis of germ cell apoptosis during postnatal testis development in normal and Hsp70-2 Knockout mice. Dev. Dyn. 208, 125-136.

O'Donnell L., Stanton P.G., Wreford N.G., Robertson D.M. and McLachlan R.I. (1996). Inhibition of 5α-reductase activity impairs the testosterone-dependent restoration of spermiogenesis in adult rats. Endocrinology 137, 2703-2710.

Packer A.I., Besmer P. and Bachvarova R.F. (1995). Kit ligand mediates survival of type A spermatogonia and dividing spermatocytes in postnatal mouse testes. Mol. Reprod. Dev. 42, 303-310.

Peden N.R., Boyd E.J.S., Browning M.C.K., Saunders J.H.B. and Wormsley K.G. (1981). Effects of two histamine H2-receptor blocking drugs on basal levels of gonadotrophins, prolactin, testosterone and oestradiol-17ß during treatment of duodenal ulcer in male patients. Acta Endocrinol. 96, 564-568.

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

Pulkkanen K.J., Laukkanen M.O., Naarala J. and Yla-Herttuala S. (2000). False-positive apoptosis signal in mouse kidney and liver detected with TUNEL assay. Apoptosis 5, 329-333.

Richburg J.H. and Boekelheide K. (1996). Mono-(2-ethylhexyl)Phthalate rapidly alters both Sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. Toxicol. Appl. Pharmacol. 137, 42-50.

Richburg J.H., Nañez A. and Gao H. (1999). Participation of the Fas-signaling system in the initiation of germ cell apoptosis in young rat testes after exposure to Mono-(2-Ethylhexyl)Phthalate. Toxicol. Appl. Pharmacol. 160, 271-278.

Russell L.D., Ettlin R.A., Sinha Hikim A.P. and Clegg E.D. (1990). Histological and histopathological evaluation of the testis. Cache River Press. Clearwater.

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.

Thompson E.W., Blackshaw A.W. and Raychoudhury S.S. (1995). Secreted products and extracellular matrix from testicular peritubular myoid cells influence androgen-binding protein secretion by Sertoli cell in culture. J. Androl. 16, 28-35.

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.

Wang C., Lai C.L., Lam K.C. and Yeung K.K. (1982). Effect of cimetidine on gonadal function in man. Br. J. Clin. Pharmacol. 13, 791-794.

Weinbauer G.F., Bartlett J.M.S., Fingscheidt U., Tsonis C.G., Kretser D.M. and Nieschlag E. (1989). Evidence for a major role of inhibin in the feedback control of FSH in the male rat. J. Reprod. Fertil. 85, 355-362.

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

Accepted December 19, 2001