Mast Cell distribution and micro-anatomical location after bilateral uterine artery ligation in mature rabbits

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Abstract

To determine the effect of bilateral uterine artery ligation (BUAL) on mast cell (MC) distribution, 24 mature female rabbits were studied. The rabbits were ovariohysterectomized on day 23, 43 or 63 following BUAL. Histological sections were stained with toluidine blue to determine the MC distribution. In rabbits that had undergone BUAL, MCs were present extensively in the helium of the treated ovaries, in dense groups close to the blood vessels. In the control group, MCs were observed at a medium density around the blood vessels and the number of MCs in the reproductive tract was significantly ($P \leqslant 0.01$) lower than in the test groups. This study demonstrates that, after BUAL, the distribution and numbers of MCs differ between different parts of the reproductive system.

Introduction

Mast cells (MCs), basophils, platelets and endothelial cells are well known sources of histamine in different organs (Jones *et al*., 1994; Krishna *et al*., 1989). Histamine has been reported to regulate blood flow and vascular permeability in the reproductive tract (Krishna *et al*., 1989; Reibiger and Spanel-Borowski, 2000). Previous *in vitro* and *in vivo* studies have hypothesized that there is an association between MC degranulation and consequently activation, angiogenesis and neovascularization (Folkman, 1982; Varayound *et al*., 2004). This hypothesis is partially supported by the close micro-anatomical association between MCs, the vasculature and the recruitment of these cells during tumor growth; wound healing and inflammatory processes (Benítez–Bribiesca *et al*., 2001). Angiogenesis refers to the growth of new blood vessels from pre-existing microcirculation and requires endothelial migration, proliferation and stabilization (Varayound *et al*., 2004). In rabbits, the ovaries possess a dual blood supply, involving ovarian and uterine arteries. Bifurcation of the uterine artery creates the utero-ovarian branch, which supplies the tip of the uterine horns and the oviduct, and forms an anastomosis with a primary branch of the ovarian artery. Thus, the uterus and the ovary are linked by a vascular junction (Kuscu *et al*., 2002). Procedures such as total hysterectomy, unilateral hysterectomy, tubal ligation and total salpingectomy can destroy parts of the tube and block the link between the uterus and the ovary (Tiars *et al*., 2001; Ozdamer *et al*., 2005; Kim *et al*., 2007; Petri Nahás *et al*., 2005), reducing the ovarian blood supply and potentially resulting in a decreased ovulation rate (Ahn *et al*., 2002; Kelekci *et al*., 2004). These procedures can also shorten the life span of the corpus luteum and cause pathological changes in both animal (Ulf *et al*., 2000; Ozdamer *et al*., 2005) and human (Salmon, 1999; Kim *et al*., 2007) hormones.

The first purpose of the present study was to evaluate the effect of experimental ischemia, which can be induced by bilateral uterine artery ligation (BUAL), on the distribution and population of MCs in the luminal structures and ovaries of rabbits. Our other aim was to evaluate the probable correlation between MC micro-anatomical location and angiogenesis after BUAL.

Materials and Methods

Twenty-four albino rabbits aged 5–6 months were used. The rabbits were obtained from the animal centre of the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The animals were acclimatized in an environmentally controlled room (temperature 17–23°C; relative humidity 50–70%; 12 h light/12 h dark). Food and water were given *ad libitum*. The rabbits were divided into two groups: test and control (sham operated).The test group was subdivided into three groups of six rabbits each: ovariohysterectomy was performed on day 23 after BUAL in group A, on day 43 in group B and on day 63 in group C. The vulvas of these animals were observed daily for color intensity, based on the rapid and consistent reddish/purple, to determine the day of surgery (day 0) in the test groups (Ozdamer *et al*., 2005). Approval for this study was gained from Urmia University veterinary medicine animal care and ethics committee.

Surgical method for bilateral uterine artery ligation

Test and control rabbits were anesthetized with ketamine 5% (Trittau Co, Germany) 35 mg/kg and xylazine 2% (Woerden Co, Netherlands) 5 mg/kg administered intraperitoneally.

After anesthesia, the rabbits were positioned in dorsal recumbency with their hind limbs restrained in extension. A midline incision (2–3 cm) was made through the skin, between the umbilicus and the cranial rim of the pelvis (pelvic symphysis), and the isolated structures were inspected carefully. The uterine arteries were bilaterally ligated (using 0-2 silk) close to the bifurcation of the uterine horns. No arterial ligation was made in the control rabbits.

Ovariohysterectomy

Ovariohysterectomy was performed 23, 43 and 63 days after the ligation in test groups A, B and C, respectively. The animals were anesthetized as described previously. After incision, the body of the uterus was isolated and clamps were placed. The ovarian pedicles were isolated and ligated, as were the uterine artery and vessels supplying the broad ligament. Finally, the organs were dissected out and the remaining stumps were checked for hemorrhages before they were placed back through the incision.

Cell count

A 100 square ocular micrometer was used to determine the distribution of MCs in preparations stained with toluidine blue, counting in a high power field (400×). Cells were counted within 18 areas per tissue, selected from the cortex and medulla of the ovary, the endometrium, myometrium and perimetrium of the uterine horns, and the tunica mucosa, submucosa and serosa of the test and control groups. Mean MC numbers within the area covered by 100 square ocular micrometers were determined, using 40× objective enlargements. The MC density in each site was recorded as MCs/mm^2.

Statistical analysis

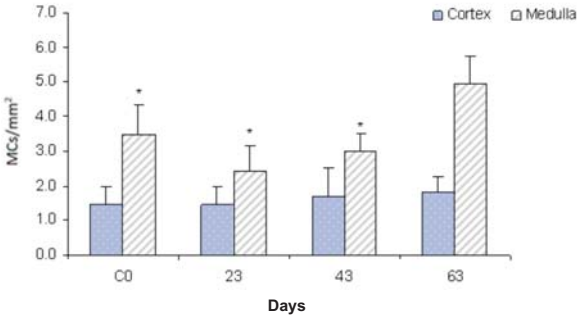
All results are presented as means ± SD. Differences in MC numbers and distribution between groups were analyzed by two-way analysis of variance followed by a Bonferroni test, using GraphPad Prism 4.00 software. $P < 0.05$ was considered significant.

Results

MC distribution in ovaries

Mcs of various sizes and appearance were observed in sections stained with toluidine blue. Their shapes ranged from oval or flat spindle-like. In almost all sample, the cytoplasm of the MCs was homogeneously stained with metachromatic dye. MCs were located in the medulla of the ovaries in both the test groups and the controls. They were especially abundantly around the blood vessels. The population of MCs was smaller in the ovaries of groups A and B than in group C (observed 63 days after surgery) (Figures 1 and 2).

Figure 1: Mast cell (MC) distribution in the cortex and medulla of ovaries in the control and test groups. All data are means ± SD. Asterisks indicate significant ($P < 0.05$) difference between controls and days 23 and 43 after bilateral uterine artery ligation (BUAL), and day 63 after BUAL.



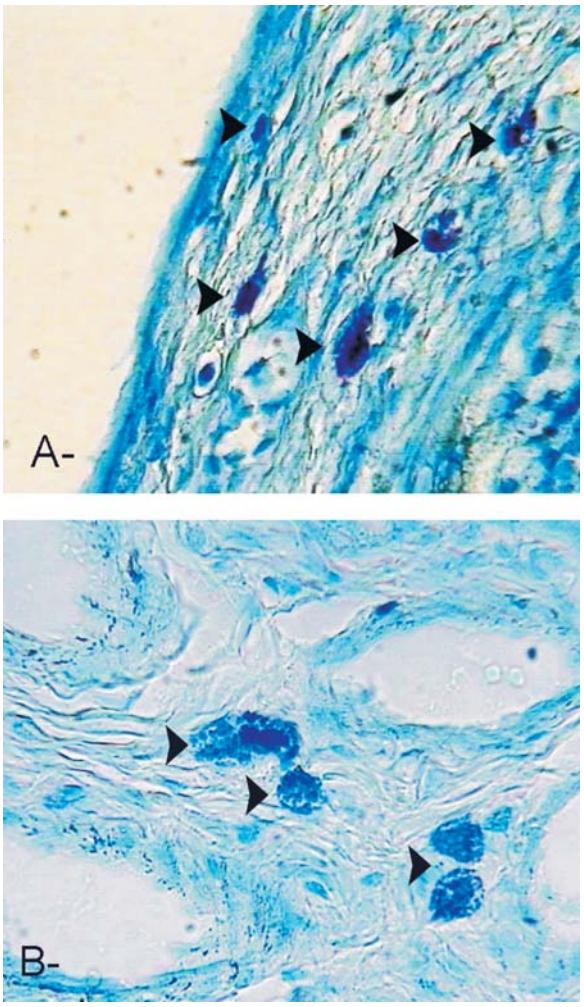


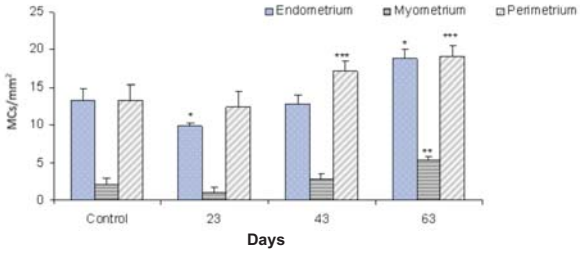
Figure 2: Cross-section from ovary 63 days after bilateral uterine artery ligation. **(A)** Cortex of the ovary; arrows indicate mast cells (MCs). **(B)** Medulla of the ovary; arrows indicate dense MCs close to the blood vessels.

MC distribution in uterine horns

Compared with the test groups, MCs were observed at a low density around the endometrial blood vessels in the control group. Importantly, the MCs in the control group were located evenly throughout the endometrium, whereas the MCs in the test groups were located densely around the newly generated capillaries in preference to other parenchymal regions of the endometrium. Twenty-three days after BUAL, MCs were decreased in the endometrium of group A rabbits, although the difference was not statistically significant. By contrast, the rabbits in group C showed a marked elevation in the MC population per mm^2 of endometrium.

Light microscopy showed that MC numbers were decreased significantly in the myometrium of the uterine horns in group A, whereas groups B and C had increased MC populations in the myometrium. Most of the cells were located around the myometrial blood vessels. All of the animals in both test and control groups showed the highest MC populations in the perimetrium of the uterine horns. The rabbits in group C had significantly ($P < 0.05$) greater numbers of MCs per mm^2 of tissue than the other test and control animals (Figures 3 and 4).

Figure 3: Mast cell (MC) distribution in the endometrium, myometrium and perimetrium of the uterine horns in control and test groups. All data are means ± SD. Asterisks indicate significant *(P < 0.05)* differences in the three layers of the uterine horns between the test groups and the controls. There are significant *(P < 0.05)* differences in the three layers between all test groups.



MC distribution in oviduct

MC numbers were decreased in the three layers of the oviduct on day 23 after surgery compared to numbers in the control rabbits. This difference was most significant ($P < 0.05$) in tunica serosa. The test animals showed greater numbers of MCs per mm^2 in all three layers compared to those in the control animals. The rabbits in group C had more MCs in the oviduct than the other test groups and the controls (Figure 5).

Discussion

The present study indicated marked differences between the numbers of MCs and their distribution and micro-anatomical location in intact rabbits and rabbits that had undergone BUAL. It is well known that the degranulation of MCs by various secretagogues causes

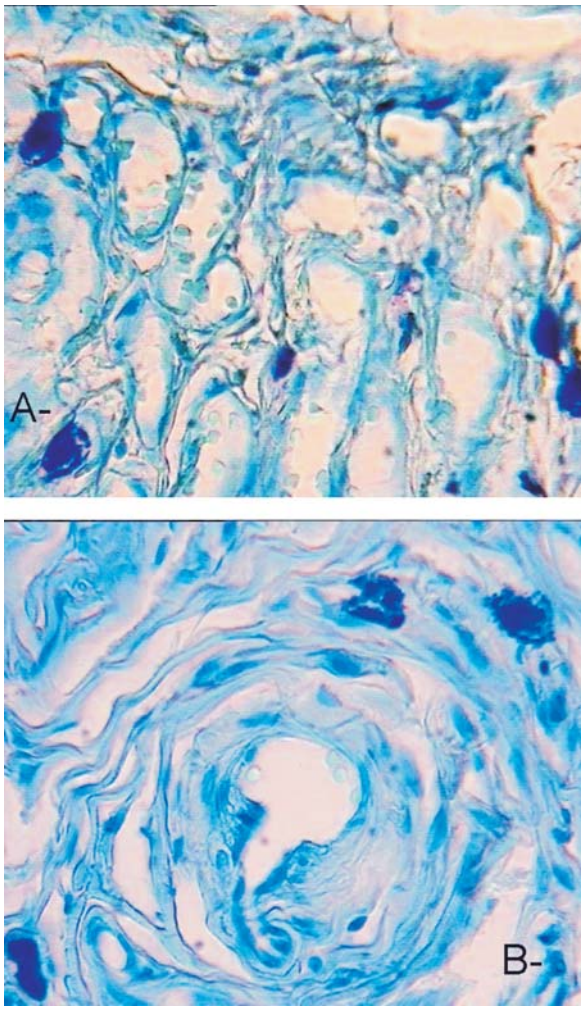
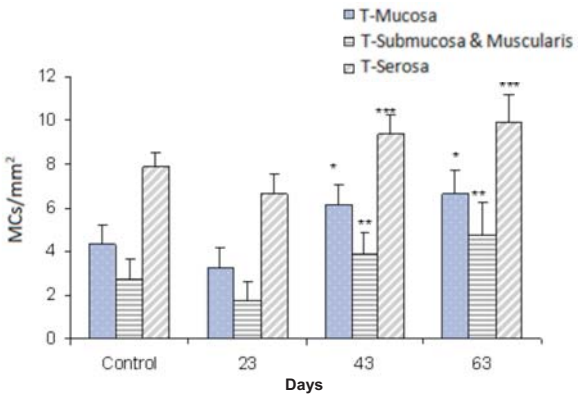


Figure 4: Cross-section from uterine horn 63 days after bilateral uterine artery ligation. **(A)** Mast cells (MCs) are abundant around capillaries in the tunica serosa. **(B)** MCs are located densely around medium size artery in the tunica serosa of the uterine horn.

Figure 5: Mast cell distribution in the tunica mucosa, the tunica submucosa and muscularis and the tunica serosa of the oviducts in control and test groups. All data are means ± SD. Asterisks indicate significant ($P < 0.05$) differences in the three layers between 43 and 63 days after bilateral uterine artery ligation and the control group.



the release of potent angiogenic factors, including vascular endothelial growth factor, basic fibroblast growth factor and interleukins (ILs) such as IL-1 and IL-6 (Benítez–Bribiesca *et al*., 2001; Ying *et al*., 1991). Along with basophiles and endothelial cells, MCs also have a vital role in regulating blood flow and vascular permeability in the ovarian and luminal structures of the reproductive tract (Jones *et al*., 1994) and are important in follicular development (Reibiger and Spanel-Borowski, 2000). As reported from previous studies, there is an association between MC degranulation and activation, and angiogenesis and neovascularization (Varayound *et al*., 2004). In accordance with such reports, we demonstrated high densities of MCs around the blood vessels in rabbits that had undergone BUAL. Previous studies have shown that MCs in the hamster ovary are found exclusively around the blood vessels of the medulla, indicating that these cells participate in gonadotropin-induced preovulatory events (Krishna *et al*., 1986). In intact rats, MCs are absent from the theca externa of the Graafian follicles and the corpus luteum, whereas the MC count in the medulla has been reported to change with the phase of the estrous cycle, from a maximum during estrus, through moderate numbers in metestrus to a minimum in proestrus (Holte *et al*., 1999; Bath and Parshad, 2000; Wehrenberg *et al*., 1977). Interestingly, in the present study MCs in the ovaries of the rabbits that had undergone BUAL increased with time independent of the phase of the estrous cycle. This suggests that MCs might have roles in different pathways controlling blood flow in the ovaries and luminal structures of the reproductive tract.

MCs are main source of vasoconstrictor secretagogues in various organs (Webb, 1998; Yildirim, 2003), including histamine and serotonin. BUAL may have led to lower blood flow, and MCs may thus have participated in physiological pathways that normalized the ischemic conditions. In the light of this hypothesis, we found that MC numbers increased time-dependently, possibly as a result of ischemic conditions in the rabbits that had undergone BUAL. Reibiger and Spanel–Borowski (2003) observed deposition of MCs in the adventitia of thick-walled muscular arteries in the ovaries of cattle, leading to the suggestion of an effect on smooth muscle. In the present study, MCs were abundant in the periphery of small to medium blood vessels in the ovarian medulla and the luminal structures of the reproductive tract of rabbits that had undergone BUAL. The medulla of the ovaries, the perimetrium of the uterine horns and the tunica serosa of the oviducts are the regions with the most blood vessels; thus, there would be expected to be more MCs here than in the other regions of these organs. In corroboration of this, greater populations of MCs were observed around the blood vessels in these regions compared to the other layers in the intact rabbits, whereas, surprisingly, MCs were elevated in all of the above-mentioned organs after BUAL. This situation was time dependent; accordingly, the rabbits in group C showed the greatest and widest MC population and distribution. Varayound and co-authors (2004) examined the distribution of MCs in the perivascular zone of the rat uterus during pregnancy; they found that MCs were located around the blood vessels and suggested that these cells are important in the regulation of vascular permeability. According to Hiromatsu and Toda (2003), when rats are not pregnant MCs are at a medium density around the vessels in the endometrium of the uterus. In contrast, in the present study MCs increased in number and distribution dependent on time after BUAL.

In conclusion, MC distribution and histologic localization depend on physiologic and pathologic conditions, and in the present study we demonstrated that, after BUAL, the numbers and distribution of MCs vary between different parts of the reproductive system.

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