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A. MACHOWSKA, A. SZLACHCIC, M. PAWLIK, T. BRZOZOWSKI,
S. J. KONTUREK, W.W. PAWLIK

THE ROLE OF FEMALE AND MALE SEX HORMONES IN THE HEALING PROCESS OF PREEXISTING LINGUAL AND GASTRIC ULCERATIONS

Department of Physiology, Jagiellonian University Medical College, Cracow, Poland

Studies in different animal species and in humans have suggested that sex hormones influence gastric acid secretion and contribute to the integrity of the oral and gastroduodenal mucosa but the effect of male and female sex hormones on the healing of the preexisting ulcers in the oral cavity and stomach have not been studied. We compared the effects of major male hormone, testosterone, and female hormone, progesterone, on the healing of lingual and gastric ulcers induced by acetic acid technique in male rats with intact or removed testicles (testectomy) and female rats with intact or removed ovaries (ovariectomy). The gastric acid secretion was determined in rats with gastric ulcers equipped with chronic gastric fistula (GF). Rats were sacrificed at day 7 upon ulcer induction; the ulcer area was measured by planimetry and the lingual and gastric blood flow (GBF) was determined by H_2-gas clearance method and venous blood was collected for determination of plasma gastrin and plasma proinflammatory cytokine interleukin (IL)-1β levels. Gastric acid output from GF rats was significantly reduced while plasma gastrin was significantly enhanced in testectomized animals as compared to those in intact control rats and these effects were reversed by supplementation of testectomized animals with testosterone. The area of lingual and gastric ulcers in placebo-control rats decreased significantly at day 7 and this effect was significantly accelerated by testectomy or ovariectomy. In contrast, testosterone significantly delayed ulcer healing while producing a significant fall in the gastric blood flow and lingual blood flow determined at the margin of these ulcers. Treatment with progesterone significantly accelerated ulcer healing and increased the gastric and lingual blood flow at margin of these ulcers. Testosterone applied alone or supplemented in testectomized animals produced the significant increment in plasma IL-1β levels as compared to the respective levels of this cytokine in placebo-control animals. We conclude that: 1) major male (testosterone) and female (progesterone) sex hormones exhibit opposite effect on healing of preexisting ulcers in the oral cavity and stomach because testosterone markedly delayed while progesterone significantly accelerated this healing; 2) testosterone-induced delay in ulcer healing involves the fall in the gastric microcirculation at the margin of lingual and gastric ulcers and the excessive

production and release of proinflammatory cytokine IL-1β; and 3) testectomy improves the gastric ulcer healing due to inhibition of gastric acid secretion and the rise in plasma gastrin, which exerts gastroprotective, trophic and ulcer healing action on the gastric mucosa.

Key words: *testosterone, progesterone, ulcer healing, oral cavity, gastric acid secretion, blood flow, interleukin -1beta, gastrin*

INTRODUCTION

Gastric mucosa is continuously subjected to the action of various irritants, which may lead to the impairment of its integrity and to the development of acute and then chronic ulcerations. The etiology of gastroduodenal ulcers includes a number of pathological factors, mainly stress, inappropriate diet, strong gastric secretagogues (e.g. gastrin in gastrinoma), *Helicobacter pylori* (*H. pylori*) infection and the use of non-steroidal antiinflammatory drugs (NSAID) (1, 2). Similarly, the pathogenesis of ulcerations appearing in the oral mucosa is a complex one, since such alterations may develop in the course of numerous clinical entities such as bacterial, mycotic or viral diseases, trophic disorders, neoplastic diseases, blood diseases, auto-immunologic disorders, or they may originate from the action of mechanical, chemical, electrical or physical stimuli related to prosthesis (3 - 5).

The development of gastric ulcerations is linked to the changes that occur within hormonal cycles, especially those related to the sex hormones secretion. It is accepted that the incidence of gastric peptic ulcers increases among women in menopause. Moreover, burning sensation in the mouth, xerostomia, and recurring aphtae may also occur (6-9). The mechanism of these pathologies remains unknown but the secretion of estrogens, progesterone and androgens, that accompany *climacterium* were proposed to contribute to the enhanced ulcerogenesis in these patients (10). Within females at reproductive age, recurring ulcerations of oral mucosa were reported and this was attributed to the phase in the sexual cycle (11).

It is of interest that gastric lesions become less intense in most females during pregnancy. Bleeding and gastroduodenal ulcer perforations appear more rarely in comparison to patients in the *puerperium* (12). Moreover, it has been observed that peptic ulcers and chronic lip cracking occur more frequently among men than women at reproductive age.

Experimental evidence indicates that cysteamine-induced gastroduodenal ulcers significantly decreased in laboratory animals during pregnancy and lactation (18, 19). Moreover, ovariectomy or the administration or progesterone to female rats was shown to decrease the cysteamine-induced duodenal ulcerations (20). The mechanism of this protective effect include the modulatory role of female hormones on the vascular permeability and an increase in the mucus secretion. In contrast, 17β-estradiol exerted pro-ulcerogenic influence by aggravating cysteamine-

induced gastroduodenal ulcers suggesting that estrogens and its metabolites may differ from progesterone in their effect on ulcer formation and the process on ulcer healing (20). In another report both, progesterone and estrogenes attenuated the area of acute gastric lesions induced by aspirin and indomethacin (21).

Since the earlier clinical and experimental results on the role of sex hormones in the pathomechanism of gastroduodenal ulceration and those developed in oral cavity were conflicting (18-22), we decided to study whether male (testosterone) and female sex hormone (progesterone) influence healing process of experimental lingual and gastric ulcerations and accompanying changes in the gastric acid secretion and the blood flow at the ulcer margin. In addition, we wanted to check whether removal of male (testectomy) and female (ovariectomy) sex organs exerts any significant influence on the rate of healing of lingual and gastric ulcers and whether hormonal supplementation in the form of exogenous testosterone and progesterone to testectomized or ovariectomized rats could reverse the effect of these procedures on ulcer healing.

MATERIAL AND METHODS

The study was conducted on 120 Wistar rats of both sexes, weighing 200-250 g fasted 18 h prior to the study but allowed to the drinking water. All procedures described in this study were performed in accordance to the Declaration of Helsinki and were accepted by the Local Ethical Committee of Animal Care at the Jagiellonian University.

In 40 rat males the testectomy was performed according to the method described by Montoneri *et al.* (18) while 40 females underwent removal of ovaries (ovariectomy) according to the procedure proposed by Kelly and Robert (19). Briefly, the males were anaesthetized with Morbital (30 mg/kg i.p.) and then mounted on the operating table. A 1 cm incision was made at the tip of the *scrotum*. A precise, 7 mm incision opened the cremaster muscle. One cut enabled to access both testes, which were then removed using a blunt forceps, together with the *epididymidis, vas deferens*, and the testicular blood vessels, which were ligated, and then the testes were removed. Next, the remaining pieces of the *vas deferens*, the fat and the blood vessels were replaced in the scrotal sac using restorable suture. Skin was closed with non-resorbable suture material, which was removed 7 days after the operation.

For ovariectomy to be performed, rat females were anaesthetized in the same way as the males. A 10 mm incision was made in the lower part of the abdomen. The skin was separated from the underlying muscles, muscle fibers incised and a forceps placed at the boundary between the oviduct and the uterus. After removing the ovary and oviduct, the *uterus* was put back into the abdominal cavity and the incision was closed with sutures.

Production of gastric and lingual ulcers

Seven days later, gastric and lingual ulcers were induced the ovariectomized and non-ovariectomized females as well as the testectomized and non-testectomized males, according to our modification (23, 24) of the acetic acid method proposed by Okabe in Pfeifer (25). With the animals under ether anesthesia, the stomach was exposed and 75 µl of acetic acid was poured through the plastic mold (4.2 mm diameter) onto serosal surface of anterior wall of the stomach just proximal to the antral gland area for 25 sec in case of gastric ulcers (23) and through the plastic mold (4.2

mm in diameter) onto the serosal surface of the lingual mucosa for 25 sec in case of tongue ulcers (24). After the application of acetic acid the animals were allowed to recover from anesthesia and received only water on the day of operation (day 0). Then they were divided into various groups and received normal chow and water *ad libitum* for the next 7 days.

Gastric secretory studies

The alterations of gastric secretion during ulcer healing in rats treated with vehicle (saline) and testosterone with or without testectomy were tested in a separate group of 30 fasted rats with lingual and gastric acetic acid ulcers, surgically equipped two weeks earlier with chronic gastric fistulas (GF) as described in our previous studies (26,27). Control sham-operated rats with GF were also included, but instead of acetic acid, 75 µl of saline was applied to the serosal surface of the stomach for 25 s. Placebo (0.2% Tween) for dissolving of testosterone or testosterone (1 mg/kg-d) was injected intramuscularly (i.m.) to GF animals with or without lingual and gastric ulcers by the means of a metal orogastric tube and this treatment was continued throughout the period of 7 days. Testosterone (Sigma Chemical Co., USA) was prepared as a stock solution (100 mg/ml) by dissolving it in 0.2% Tween immediately before use. Testosterone was further diluted to the desired concentration with saline. After recovery from anesthesia (day 0) or at day 7 after ulcer induction, GF rats without and with gastric ulcers were placed in the individual Bollman cages to prevent coprophagy and to maintain the necessary restraint. Each GF was then opened, and the stomach rinsed gently with 5-8 ml of tap water at 37°C. Basal gastric secretion was collected for 120 min, during which time the rats received saline at a rate of 4 ml/h subcutaneously (s.c.). The gastric juice was collected every 30 min, the volume was measured, and then the acid concentration and outputs were determined and expressed as the output per 30 min as described previously (26, 27).

Experimental design

The animals with acetic-acid induced ulcers were divided into eight following groups: 1) control females (sham-operated); 2) control males (sham-operates) 3) testectomy alone, 4) ovariectomy alone; 5) testosterone (1 mg/kg-d i.m.) alone; 6) progesterone (25 mg/kg i.m. in a single *depot* dose) alone; 7) testectomy plus testosterone (1 mg/kg-day i.m.); 8) ovariectomy plus progesterone (25 mg/kg i.m. in a single *depot* dose).

Administration of hormones started after twenty-four hours following the induction of acetic-acid induced ulcer in oral cavity and the stomach and was continued for seven days afterwards.

Determination of lingual blood flow (TBF) and gastric blood flow (GBF) at margin of gastric ulcers and the plasma proinflammatory cytokine IL-1β levels

To evaluate the effects of testectomy or ovariectomy with or without sex hormones administered exogenously, the animals were lightly anesthetized with ether and the abdomen was opened and the stomach was exposed to assess the GBF and the tongue was exposed to determine the TBF at the margin of gastric and lingual ulcers, respectively, and in the intact non-ulcerated lingual and gastric mucosa using H_2-gas clearance technique as described in details in our previous studies (24, 26). A venous blood sample was withdrawn from vena cava into EDTA-containing vials in order to determine the plasma level of interleukin-1beta (IL-1β) by ELISA technique (BioSource International, Camarillo, CA, USA) (28, 29).

At day 7 upon ulcer induction, the stomachs were removed and pinned open for the determination of the area of gastric ulcers by planimetry (Morphomat, Carl Zeiss, Berlin, German) by two investigators under blinded conditions.

RESULTS

Effect of placebo and testosterone on gastric acid secretion during the ulcer healing in rats with or without testectomy

The results of gastric secretory studies in conscious rats equipped with gastric fistula with or without induction of gastric ulcers are presented in *Table 1*. In control rats without gastric ulcers, the basal acid output averaged 189 ± 26 μmol/30 min. Immediately after induction of gastric ulcers, the gastric acid output were significantly reduced by about 55% as compared with the respective values in control rats without ulcer induction (not shown). After 7 days, the values of gastric acid output in placebo-control rats were still significantly lower as compared to those obtained in rats without ulcers. Seven days following ulcer induction in testectomized animals, a further significant decrease in gastric acid output was observed as compared to that recorded in sham-controls. Testosterone alone failed to affect significantly the gastric acid secretion as compared to placebo-treated control animals. Supplementation of testectomized animals with testosterone resulted in a significant increase in gastric acid output as compared to that in rats with testectomy alone (*Table 1*).

Effect of placebo or testosterone administration on the healing of gastric and lingual ulcers and the accompanying changes in TBF and GBF at ulcer margin in testectomized and non-testectomized males

Figs 1 and *2* show the influence of placebo (saline) and testosterone on the area of lingual and gastric ulcers in testectomized and non-testectomized males. The area of lingual ulcer in placebo-treated control animals was significantly decreased from the initial 13,8 mm^2 immediately upon ulcer

Table 1. The influence of placebo (Tween) or intramuscularly injected testosterone treatment (1 mg/kg-day) in testectomized and non-testectomized animals or in the testosterone + testectomy combination on the level of plasma gastrin levels. Results are mean ± S.E.M. of 8 -10 rats. * $p < 0.05$ vs Placebo; $^{+}p < 0.05$ vs testectomy.

	GASTRIC ACID OUTPUT	PLASMA GASTRIN
	μmol/30 min	pmol/l
Placebo	166 ± 14	65 ± 8
Testectomy	104 ± 8*	103 ± 4*
Testosterone	180 ± 18	74 ± 5
Testectomy plus Testosterone	139 ± 10^{+}	63 ± 7^{+}

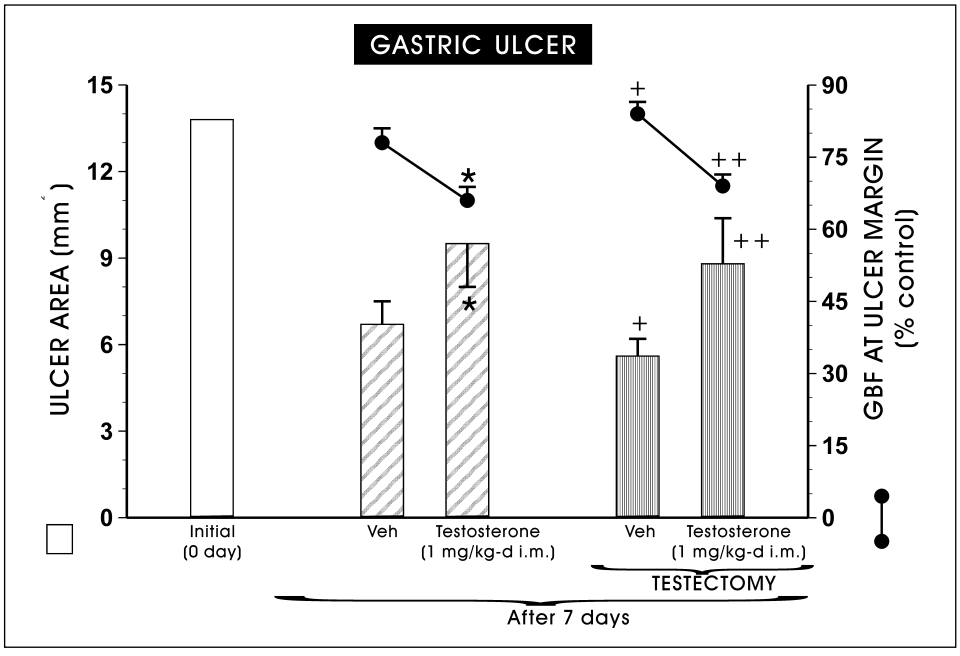

Fig. 1. The effect of placebo (0.2% Tween) or testosterone injected intramuscularly (i.m.) in the dose of 1 mg/kg-day alone or given the combination with testectomy on the area of gastric ulcer and gastric blood flow (GBF) at the ulcer margin at day 7 after ulcer induction. The results are presented as mean ± S.E.M. 6-8 individuals in each group; *p≤0.005 in comparison with the placebo-treated group; $^{+}$p≤0.05 in comparison with the placebo-treated non-testectomized animals; $^{++}$p≤0.05 in comparison with the placebo-treated testectomized animals.

induction to 9.8±1.6 mm^2 at day 7 upon ulcer induction and in case of gastric ulcers from 13.8 mm^2 at day 0 to 6.71±2.3 mm^2 at day 7 upon ulcer induction. The blood flow in the non-ulcerated part of oral mucosa reached the value of 44.5±2.6 ml/min in 100g of tissue while and the blood flow in the non-ulcerated gastric mucosa reached the value of 44.5±3.5 ml/min in 100 g of tissue and these values were considered as 100%. The lingual and gastric blood flow at the ulcer margin of placebo-treated animals were significantly decreased by about 13% and 23% respectively, in comparison with the respective values of blood flow in the non-ulcerated lingual and gastric mucosa. At day 7 upon ulcer induction daily administration of testosterone applied i.m., resulted in a significant increase in the area of lingual and gastric ulcers by about 50% and 45 %, respectively, and this was effect was accompanied by the significant decrease in the blood flow at the margin of lingual and gastric ulcers as compared to the corresponding values in the placebo-control animals. Testectomy alone resulted in a moderate, though significant decrease in the area of lingual and gastric ulcers, and this effect was accompanied by the significant increase in gastric and lingual blood flow in

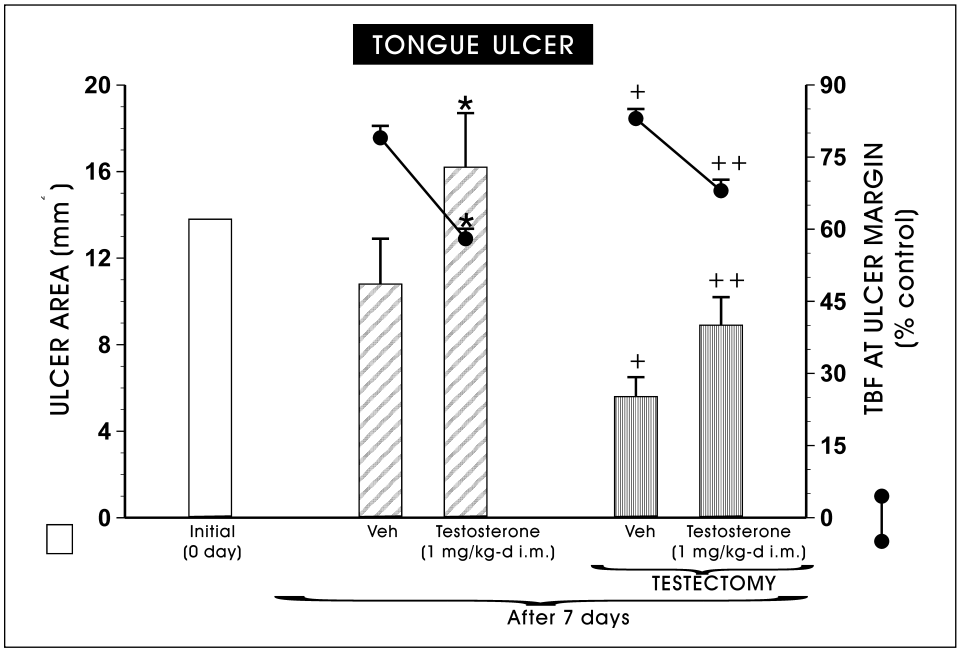

Fig. 2. The effect of placebo (0.2% Tween) or testosterone injected intramuscularly (i.m.) in the dose of 1 mg/kg-day alone or given the combination with testectomy on the area of lingual ulcer and lingual blood flow (TBF) at the ulcer margin at day 7 after ulcer induction. The results are presented as mean ± S.E.M. 6-8 individuals in each group; *$p \leq 0.005$ in comparison with the placebo-treated group; $^{+}p \leq 0.05$ in comparison with the placebo-treated non-testectomized animals; $^{++}p \leq 0.05$ in comparison with the placebo-treated testectomized animals.

the ulcerated area. The decrease in the ulcer area observed in animals with their testicles removed and accompanying rise in blood flow at the margin of lingual and gastric ulcers were eliminated in testectomized animals that received substitutive therapy with testosterone.

Effect of placebo or progesterone administration on the area of lingual and gastric ulcers and the alterations in GBF and TBF at margin of gastric and lingual ulcers in ovariectomized and non-ovariectomized females

Figs 3 and *4* show the effect of administration of placebo or progesterone on the ulcer area and the changes in the GBF and TBF at ulcer margin in rats with ovaries or in those in which the influence of ovariectomy was additionally studied. Similarly as in the case of males, the placebo-treated control females showed a significant decrease in the area of gastric ulcers from the initial 13.8 mm^2 to the value of 8.95±0.73 mm^2 at day 7 upon ulcer induction while the area of lingual ulcers was significantly decreased from 13.8 mm^2 to 9.37±1.14 mm^2 at the end of the study period. The blood flow in the non-ulcerated part of lingual

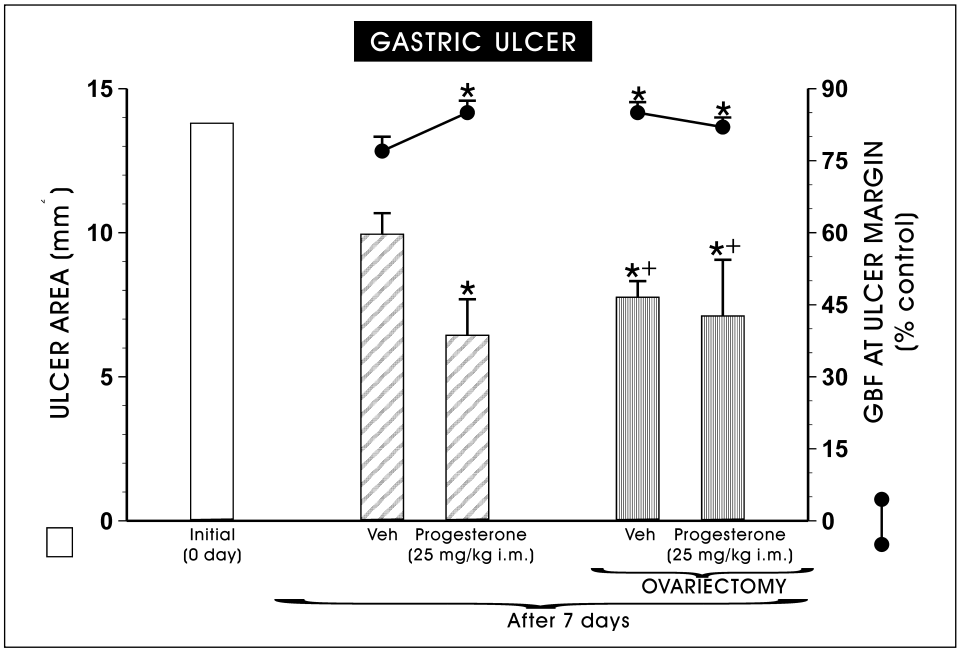

Fig. 3. The effect of placebo (0.2% Tween) or progesterone injected intramuscularly (i.m.) in a single *depot* dose of 25 mg/kg, with or without the combination with ovariectomy on the mean ulcer area and accompanying changes in the gastric blood flow (GBF) at the ulcer margin determined 7 days upon ulcer induction. Results are mean ± S.E.M. from 10-20 individuals in each group; *$p \leq 0.005$ in comparison with the placebo-treated control group; $^{+}p \leq 0.05$ in comparison with the placebo or progesterone treated ovariectomized animals.

mucosa of the tongue reached the value of 42.5±1.6 ml/min-100g of tissue, while analogical blood flow in the gastric mucosa reached the value of 40.2±2.8 ml/min-100 g of tissue and these values were considered as control value of 100%. At day 7 upon ulcer induction, the placebo-treated animals showed a significant decrease in both TBF and GBF at the ulcer margins by about 21% and 22%, respectively, as compared with the blood flow in the non-ulcerated tissue. A single *depot* dose of progesterone injected i.m., significantly decreased the area of lingual and gastric ulcers, by about 29% and 28%, respectively, and also significantly raised the TBF and GBF at margin of these ulcers as compared to the respective values recorded in the placebo-treated group. Ovariectomy by itself significantly decreased the area of lingual and gastric ulcers and this effect was accompanied by the significant rise in the TBF and GBF at the margin of these ulcers. The supplementation therapy with progesterone in ovariectomized animals significantly decreased the area of gastric and lingual ulcers and significantly raised the GBF and TBF at the margin of these ulcers in comparison to the values achieved in placebo- or progesterone-treated rats without ovariectomy.

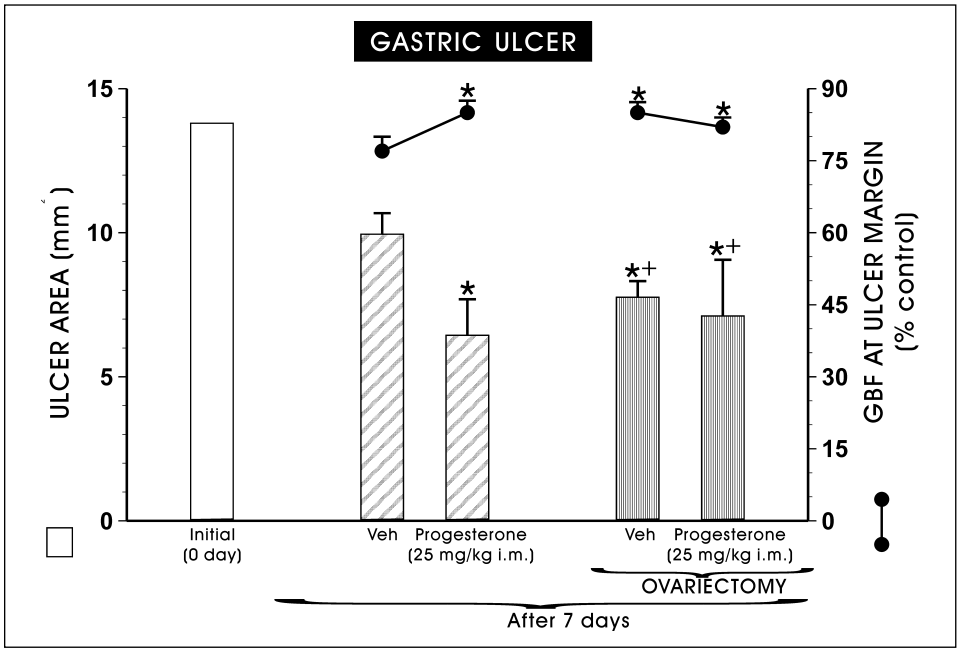

Fig. 4. The effect of placebo (0.2% Tween) or progesterone injected intramuscularly (i.m.) in a single *depot* dose of 25 mg/kg, with or without the combination with ovariectomy on the mean area of lingual ulcer and accompanying changes in the lingual blood flow (TBF) at the ulcer margin determined 7 days upon ulcer induction. Results are mean ± S.E.M. from 8-10 individuals in each group; *$p \leq 0.005$ in comparison with the placebo-treated control group; $^{+}p \leq 0.05$ in comparison with the placebo or progesterone treated ovariectomized animals.

Effect of placebo or testosterone administration on the plasma IL-1β levels in testectomized and non-testectomized animals

As shown in *Fig. 5*, testectomy alone failed to affect significantly the plasma IL-1β level as compared to that in placebo-treated animals. In contrast, testosterone administration which by itself resulted in a significant rise in the plasma IL-1β levels, when given to testectomized animals also significantly increased this plasma cytokine levels as compared to the corresponding values in the placebo-control or testectomized animals.

DISCUSSION

The results presented in this report indicate an involvement of sex hormones in reparative processes of the digestive tract, especially in the process of lingual and gastric ulcers healing. This notion is supported by our finding that application of major male sex hormone, testosterone, significantly delayed the healing

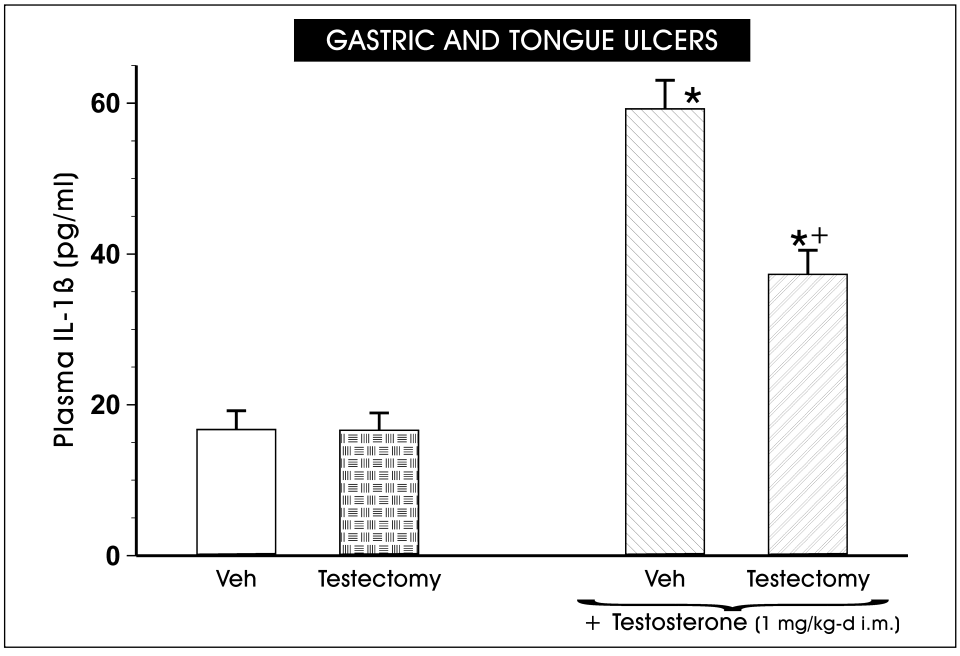

Fig. 5. The effect of testectomy with or without the supplementation therapy with testosterone (1 mg/kg-d i.m.) on the plasma IL-1β levels. Results are mean ± S.E.M. from 8-10 individuals in each group; *$p \leq 0.05$ in comparison with the placebo-treated control group and testectomy alone ; *+$p \leq 0.05$ in comparison with testectomy alone.

process of these ulcers, while progesterone, which is the major female sex hormone, accelerated the ulcer healing process both in the oral cavity and in the stomach. The importance of the sex hormones in the healing process are emphasized by our observations that testectomy and ovariectomy also accelerated the healing rate and blood flow on the margin of the lingual and gastric ulcers. Moreover, the administration of testosterone or the supplementation therapy with testosterone in testectomized animals, significantly delayed ulcer healing and eliminated the acceleration of ulcer healing caused by testectomy alone. These results suggest that the major male sex hormones, testosterone, delays the healing process of lingual and gastric ulcers which is in keeping with the epidemiological data in humans that ulcers develop in men more often than women, and the process of peptic ulcer disease in men is associated with much higher number of adverse symptoms and greater number of side effects than that in women.

Our results with the healing of lingual ulcers are relevant, in light of the known development of oral ulcers in the course of Behcet's disease, which depends upon the cycle of sex hormones and has been described mainly in men (11 - 17). The conception of Behcet's syndrome (*syndroma oculobuccogenitale*) covers a complex of three symptoms, with ulcerating type of lesions being the most predominant. The ulcerations appear in three areas of the body such as the eye,

the oral cavity and sex organs and are usually associated with the alterations within the digestive tract, skin lesions, arthritis, neurological changes and *thrombophlebitis*. The cause of this syndrome remains unknown, although it has been noted that this disease is 2-3 times more common among men than women and the initial symptoms appear among patients at the age of 20 up to 30 (11).

Research conducted in our study on female rats revealed that progesterone in contrast to testosterone, accelerated the healing of lingual and gastric ulcers in non-ovariectomized animals and in those who underwent ovariectomy, and significantly raised the blood flow at the edge of lingual and gastric ulcers. It is of interest that ovariectomized females showed greater sensitivity to the progesterone-induced acceleration of the healing process, especially with the respect of lingual ulcerations, suggesting that substitutive therapy with progesterone might be beneficial in the course of healing of lingual and gastric ulcers. Overall, our results emphasize the importance of female sex hormones, mainly progesterone, in the mechanisms of the healing process of lingual and gastric ulcers.

Our results are consistent with the observations made by Laszlo *et al.* (22), who have also shown the negative influence of testosterone on the formation of cysteamine-induced gastroduodenal ulcers and the enhancement of the vascular permeability in rats with gastroduodenal mucosal lesions, in which vascular leakage was additionally significantly augmented by treatment with testosterone. Moreover, testectomy or application of anti-androgen preparation, cyproterone acetate for 8 subsequent days - the time-duration of administration similar as in our present study, markedly decreased the magnitude of cysteamine-induced gastroduodenal lesions and the above mentioned vascular dysfunction (22).

The trophic influence of progesterone on damaged gastric mucosa is especially visible during pregnancy. Early-stage pregnancy rats, in contrast to late-stage pregnancy ones, demonstrate reduced reactions to the harmful influence of cysteamine (18). Moreover, the non-pregnant female and in addition, the male rats, which were subject to progesterone therapy exhibited the gastroduodenal resistance to the cysteamine-induced gastroduodenal lesions (18). Such effects were absent in animals pretreated with 17β-estradiol (18). It is likely that the protective activity of progesterone depends upon the increase in the production of mucus by gastric mucosa since an increase in the gastroduodenal mucus levels was observed in early-stage pregnancy females and animals pretreated with progesterone (18).

Previous observations in rats by Adeniyi *et al.* (30) suggested that the gonadectomy of male rats inhibited the secretion of gastric acid suggesting that testectomy resulting in suppression of the testosterone production has a protective effect on gastric mucosa through the attenuation of production of gastric juice. Moreover, the hydrochloric acid was considered to exert a deleterious influence on ulcer healing and predisposing the gastric mucosa to the development of cysteamine-induced gastric mucosa ulcerations (22, 30, 31). This is why we

tested whether gonadectomy in male rats with preexisting acetic acid ulcers affects gastric acid secretion in comparison with non-testectomized animals. We found that testectomy inhibited gastric acid, which can, at least in part, explain the acceleration of the healing process in these animals. Moreover, the plasma gastrin levels was significantly raised in testectomized rats, probably secondary to the inhibition of acid secretion, indicating that gastrin, which is known to exert trophic influence on the gastric mucosa, could also contribute to the increase in the ulcer healing observed in these animals. Our finding that gastric acid secretion was decreased in rats that underwent testectomy, is also consistent with the previous observations by Szabo *et al.* (31) and Bernardini *et al.* (32), but could not be sufficient to explain why lingual ulcers healed faster in these males, since in the oral cavity, the suppression of intragastric acidity does not play a major role. Along with the delay in ulcer healing in rats treated with testosterone, we observed a marked rise in the plasma IL-1β levels as compared to the corresponding values in the control group. Furthermore, supplementation therapy with testosterone in rats that underwent testectomy significantly raised the plasma IL-1β over that measured in testectomized animals, whose ulcer healing was delayed as compared with that observed in testectomized animals. The exact mechanism by which testectomy improves the ulcer healing but testosterone delays ulcer healing require future studies but so far, the acceleration of ulcer healing in the lingual in testectomized animals could be attributed to the improvement of the blood flow at the ulcer edge whereas testosterone induced prolongation of this healing could be due to the fall in the microcirculation around the ulcer possibly mediated by the increase in the plasma proinflammatory cytokine IL-1β level observed in these animals.

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Author's address: Prof. Tomasz Brzozowski, M.D., Department of Physiology, Jagiellonian University Medical College, ul. Grzegórzecka 16, 31-531 Krakow, Poland
E-mail: mpbrzozo@cyf-kr.edu.pl