Insight into the mechanism by which metoclopramide improves immune functions after trauma-hemorrhage

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Knöperl, Markus W., Martin K. Angele, Alfred Ayala, William G. Cioffi, Kirby I. Bland, and Irshad H. Chaudry. Insight into the mechanism by which metoclopramide improves immune functions after trauma-hemorrhage. Am J Physiol Cell Physiol 279: C72–C80, 2000.—Although studies have shown that prolactin (Prl) and metoclopramide (Mcp) administration restores the depressed cell-mediated immune functions after hemorrhage, the underlying mechanism responsible for the immunostimulatory effects of Mcp remains unknown. We hypothesized that Mcp improves immune responses by upregulating the secretion of Prl. To test this hypothesis, male C3H/HeN mice were subjected to sham operation or laparotomy (i.e., soft tissue trauma) and hemorrhagic shock (Hem; 35 ± 5 mmHg for 90 min) and then resuscitated. Plasma Prl levels were determined 30 min after Mcp (1 μg/g body wt sc at end of Hem) or vehicle (Veh) treatment in sham and Hem mice. The results indicate that plasma Prl levels increased significantly in Mcp-treated mice (sham-Veh 249.9 ± 5.3, Hem-Veh 229.9 ± 7.6, Hem-Mcp 596.9 ± 73.1 ng/ml, one-way ANOVA, P < 0.05 vs. Veh). To determine whether Mcp produces its salutary effects directly or indirectly via increased Prl secretion, splenocyte proliferation and splenocyte interleukin (IL)-2 and IL-3 release from untreated sham or Hem mice were determined in the presence of increasing concentrations of mouse Prl or Mcp. The addition of Mcp had no effect on splenocyte immune functions in vitro. However, the addition of Prl restored the hemorrhage-induced depressed splenocyte proliferation as well as splenocyte IL-2 and IL-3 release in vitro in a dose-dependent manner. Thus the beneficial effects of Mcp on immune functions after Hem appear to be mediated by Prl. Because Mcp increases plasma levels of the immunoenhancing hormone Prl, this agent should be considered a useful adjunct for the treatment of immunodepression in trauma victims.

rodent; inflammation; immunomodulators; in vivo animal models

IT IS WELL ESTABLISHED that major hemorrhage, even in the absence of any significant tissue trauma, produces a marked depression in both the specific and nonspecific cell-mediated immunity (5). This depression in various immune functions is apparent immediately after hemorrhage and persists despite adequate fluid resuscitation (4, 26). Recent studies indicate that administration of the pituitary peptide hormone prolactin after hemorrhagic shock restores the depressed cell-mediated immune functions and decreases mortality from subsequent sepsis (32, 33). Similarly, studies have shown that treatment of posthemorrhage animals with metoclopramide, a central dopamine antagonist, restored the depressed immune functions. However, the precise mechanism by which metoclopramide exerts its beneficial immunomodulatory effects under such conditions remains unknown. We hypothesized that metoclopramide improves the depressed immune functions after hemorrhage by upregulating the secretion of the immunoenhancing hormone prolactin from the anterior pituitary gland. In this regard, studies suggest that the increase in prolactin secretion is the result of metoclopramide’s central antagonism of dopamine receptors in the anterior pituitary (11). Dopamine, which is secreted in the hypothalamus, is thought to be an agent that inhibits the release of prolactin (11). Because prolactin and metoclopramide produce salutary effects on the depressed immune responses after hemorrhage, the aim of the present study was to determine whether the beneficial effects of metoclopramide are direct effects of metoclopramide on immune cells or whether they are mediated via increased secretion of the pituitary hormone prolactin.

MATERIALS AND METHODS

Animals. Inbred male C3H/HeN mice (Charles River Laboratories, Wilmington, MA), 7–8 wk old (24–26 g body wt), were used in this study. All procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital and Brown University.

Experimental groups. The animals were randomized into three groups for the measurement of prolactin plasma levels and macrophage immune functions after in vivo administra-
tion of metoclopramide. The animals in group 1 served as sham-operated animals that were neither hemorrhaged nor resuscitated and received 100 μl of saline vehicle subcutaneously after sham operation. Animals in groups 2 and 3 were subjected to the trauma-hemorrhage procedure (16), and animals in group 2 received 100 μl of saline vehicle, whereas animals in group 3 received 1 μg/ml body wt metoclopramide in 100 μl of saline vehicle subcutaneously at the end of the trauma-hemorrhage, i.e., immediately before resuscitation.

Each group consisted of seven to eight animals. For the in vitro experiments, cells were harvested from additional animals that were either sham operated or subjected to the trauma-hemorrhage procedure but did not receive metoclopramide.

**Trauma-hemorrhage procedure.** Mice in the trauma-hemorrhage (16) groups were lightly anesthetized with methoxyflurane (Metofane; Pitman Moore, Mundelein, IL) and restrained in a supine position, and a 2.5-cm midline laparotomy (i.e., soft tissue trauma induced) was performed, which was then closed aseptically in two layers using 6-0 Ethibond (Ethicon, Somerville, NJ). Both femoral arteries were then aseptically cannulated with polyethylene 10 tubing (Clay-Adams, Parsippany, NJ) using a minimal dissection technique, and the animals were allowed to awaken. Blood pressure was constantly monitored by attaching one of the catheters to a blood pressure analyzer (Digi-Med, Louisville, KY). Lidocaine was applied to the incision sites to provide analgesia during the study period. Upon awakening, the animals were bled rapidly through the other catheter to a mean arterial blood pressure of 35 ± 5 mmHg (mean arterial blood pressure prehemorrhage was 95 ± 5 mmHg), which was maintained for 90 min. At the end of that procedure, the animals were resuscitated with four times the shed blood volume in the form of lactated Ringer solution. Next, the catheters were removed, the vessels were ligated, and the groin incisions were closed. Sham-operated animals in group 1 underwent the same surgical procedure, which included ligation of both femoral arteries, but neither hemorrhage nor fluid resuscitation was carried out. There was no mortality observed in this model of trauma-hemorrhage.

**Blood, tissue, and cell harvesting procedure.** The animals were killed by methoxyflurane overdose at 30 min after trauma-hemorrhage and resuscitation for the assessment of prolactin plasma levels or at 2 h to obtain the spleen, peritoneal macrophages, and whole blood.

**Plasma collection and storage.** Whole blood was obtained by cardiac puncture and was placed in microcentrifuge tubes (Microtainer; Becton Dickinson, Rutherford, NJ). The tubes were then centrifuged at 16,000 g for 15 min at 4°C. Plasma was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored at −80°C until assayed.

**Cell line maintenance.** The IL-2-dependent CTLL-2 cells were prepared from the American Type Culture Collection and were maintained according to the supplier's directions. The interleukin (IL)-3-dependent FDC-P1 cells (a gift from Dr. Charles Janeway, Yale University, New Haven, CT) were maintained as previously described (13). The IL-6-sensitive murine B cell hybridoma (7TD1; a gift from Dr. Jacques Van Snick, The Ludwig Institute for Cancer Research, Brussels, Belgium) was maintained as previously described (12).

**Preparation of peritoneal and splenic macrophage culture.** Resident peritoneal macrophages were harvested at 2 h after sham operation or trauma-hemorrhage and resuscitation, and monolayers were established as previously described (1). Spleens were harvested aseptically, and splenic macrophage cultures were also established as previously described in detail (33). The monolayers (1×10⁶ cells/ml) were stimulated with 10 μg lipopolysaccharide W (from Escherichia coli 055:B5; Difco Laboratories, Detroit, MI)/ml Click's medium containing 10% heat-inactivated FBS (GIBCO BRL, Grand Island, NY) for 48 h at 37°C, 5% CO₂, and 90% humidity to yield a final concentration of 1×10⁶ cells/ml. The ability of the splenocyte cultures to produce lymphokines in response to a mitogenic challenge was assessed by incubation for 48 h (at 37°C, 5% CO₂, and 90% humidity) in the presence of 2.5 μg/ml concanavalin A (Con A; Pharmacia/LKB Biotech, Piscataway, NJ). After this incubation period, the cell suspension was centrifuged at 300 g for 15 min, and the supernatants were harvested, and stored at −80°C until assayed for IL-1β and IL-6.

**Splenocyte proliferation.** A second portion of the splenocyte suspension was placed in a 96-well microtiter plate (Corning Glass, Corning, NY) in aliquots of 100 μl. The cell's ability to proliferate in response to mitogenic stimulation with 0 (negative control) or 2.5 μg/ml Con A (Pharmacia/LKB Biotech) was measured by the [³H]thymidine incorporation technique as previously described (26). Briefly, after incubation for 48 h at 37°C, 5% CO₂, and 90% humidity, 1 μCi of the radionucleotide (specific activity 6.7 Ci/mmol; New England Nuclear, Wilmington, DE) was added to each well, and the cultures were incubated for another 16 h. The cells were then harvested on glass fiber filter mats, and the β-decay was detected by liquid scintillation counting, as previously described (19).

**In vitro treatment with prolactin/metoclopramide.** Mouse prolactin was a gift of the National Hormone and Pituitary Program from the National Institute of Diabetes and Digestive and Kidney Diseases and was prepared according to their instructions. Metoclopramide (4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxy benzamidine) monohydrate was obtained from Sigma Chemical (St. Louis, MO). At 2 h after the preparation of splenocyte cultures or splenocyte proliferation and stimulation with 2.5 μg/ml Con A, either mouse prolactin or metoclopramide dissolved in PBS was added to the culture media in increasing concentrations. The concentrations of prolactin or metoclopramide used for the in vitro experiments were selected to cover the range of plasma prolactin and metoclopramide levels that have been observed after in vivo administration of metoclopramide (2, 15, 27). The cultures were then incubated for a period of 46 h at 37°C, 5% CO₂, and 90% humidity to determine splenocyte proliferative capacity and the release of lymphokines in the supernatants.

**Assessment of lymphokine release.** IL-2 activity in the culture supernatants was determined as described previously (19) by the amount of proliferation of CTLL-2 cells, which only grow in a dose-dependent fashion in the presence of...
IL-2. IL-3 activity in the culture supernatants was detected as described previously (19) by adding serial dilutions of the supernatants to FDC-P1 cells (2.5 × 10^5 cells/ml), which grow in a dependent fashion in the presence of IL-3. IL-10 levels in the splenocyte supernatants were determined using sandwich ELISA. In brief, 96-well Nunc-Immuno Maxisorp plates were coated overnight with 4 μg rat anti-mouse IL-10 capture antibody (clone JES-5; Pharmingen, San Diego, CA/ml 0.1 M NaHCO3, pH 8.2. The plates were washed three times with PBS containing 0.05% Tween 20 (Sigma Chemical) and were blocked with PBS containing 20% FCS for 2 h. After being washed, 100 μl of the samples and standard and 5 ng/ml murine IL-10 (Pharmingen) were added to the plate and then incubated overnight (4°C). After repeated washings, the plates were incubated for 1 h with 100 μl of biotinylated monoclonal rat anti-mouse IL-10 (clone SXC-1; Pharmingen) at a concentration of 2 μg/ml at room temperature. For detection of IL-10, the plates were washed and incubated at room temperature for 30 min with avidin-peroxidase (diluted 1:400; Sigma Chemical). After being washed, 100 μl of 2,2'-azinobis(3-ethylbenzthiazolone) sulfonic acid/H2O2 substrate buffer were added to each well to initiate color development. The optical density at 405 nm for each well was then determined on a microplate reader. The concentration of IL-10 present in the samples was determined by interpolation using a standard curve produced with murine IL-10.

Assessment of monokine release. IL-1β levels in the macrophage supernatants were determined using sandwich ELISA, as described previously (16). IL-6 activity in culture supernatants was determined as previously described (16) by the amount of proliferation of the murine B cell hybridoma cell-line 7TD1, which only grows in a dependent fashion in the presence of IL-6 (12).

Determination of plasma prolactin levels. Plasma prolactin levels were determined using a commercially available rat prolactin enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). The rabbit antiserum to rat prolactin used in this kit exhibits ~40% cross-reactivity with mouse prolactin (data not shown). In brief, the plate, which is precoated with monoclonal anti-rabbit antibody, was washed repeatedly with washing buffer. Next, 50 μl of plasma samples, diluted 1/7 in EIA buffer (Cayman Chemical), and the standard (mouse prolactin, concentration 500 ng/ml, a gift of the National Hormone and Pituitary Program from the National Institute of Diabetes and Digestive and Kidney Diseases), were added in duplicate. After samples and standard were dispensed, 50 μl of acetylcholinesterase-linked rat prolactin tracer (Cayman Chemical) were added to each well. Next, 50 μl of polyclonal rabbit anti-rat prolactin antiserum (Cayman Chemical) were added to each well, and the plate was incubated for 18 h at room temperature. After repeated washings, 200 μl of Ellman’s reagent (Cayman Chemical) were added to each well, and the plate was incubated for 2 h in the dark at room temperature on an orbital shaker. The optical density at 405 nm was then determined on a microplate reader. The concentration of prolactin present in the samples was determined by interpolation against the standard curve produced with human prolactin enzyme immunoassay kit exhibits 40% cross-reactivity with mouse prolactin (data not shown).

Statistical analysis. The results are presented as means ± SE. One-way ANOVA followed by the Student-Newman-Keuls test as a post hoc test for multiple comparisons was used to determine the significance of the differences between experimental means. Rank Sum Test was used to determine the significance of differences observed between sham and trauma-hemorrhage after in vitro treatment with prolactin or metoclopramide. A P value of <0.05 was considered significant.

RESULTS

Peritoneal macrophages. Peritoneal macrophage IL-1β and IL-6 release capacity was significantly depressed in vehicle-treated animals at 2 h after trauma-hemorrhage compared with peritoneal macrophages from sham animals (Fig. 1). Treatment with a single dose of metoclopramide after trauma-hemorrhage restored the depressed peritoneal macrophage IL-1β and IL-6 release to levels observed in macrophages from sham-operated animals.

Splenic macrophages. Similar to peritoneal macrophage proinflammatory cytokine release, splenic macrophage IL-1β and IL-6 release at 2 h after trauma-hemorrhage was significantly depressed in vehicle-treated animals (Fig. 2). However, splenic macrophages harvested from animals treated with

![Fig. 1. Release of interleukin (IL)-1β (A) or IL-6 (B) by peritoneal macrophages harvested from mice at 2 h after sham operation or trauma (T)-hemorrhage (Hem) and treatment with metoclopramide (1 μg/g body wt sc) or saline vehicle in the presence of 10 μg/ml lipopolysaccharide W. IL-1β levels in macrophage supernatants were measured by sandwich ELISA technique specific for IL-1β, and IL-6 was measured by a specific bioassay (7TD1) for IL-6. Values are means ± SE of 6–8 animals in each group: #P < 0.05 vs. sham vehicle by ANOVA.]
metoclopramide subcutaneously after trauma-hemorrhage showed IL-1β and IL-6 release that was similar to the release from shams.

**Splenocyte proliferation.** At 2 h after trauma-hemorrhage, splenocyte proliferative capacity was significantly depressed compared with sham animals (Fig. 3A). The addition of mouse prolactin in vitro to the splenocyte cultures improved the depressed splenocyte IL-2 release from hemorrhaged mice in a dose-dependent manner, and the values were similar to shams at a concentration of 0.1 ng mouse prolactin/ml culture medium. However, the addition of metoclopramide to the splenocyte cultures had no stimulatory effect on the depressed IL-2 release capacity of splenocytes from hemorrhaged animals (Fig. 3B). Moreover, there was no effect of prolactin or metoclopramide addition on IL-2 release by splenocytes from sham-operated animals. Similar findings were observed with splenocyte addition in vitro was observed on proliferative capacity of splenocytes harvested from sham animals.

**Lymphokine release.** IL-2 release in splenocytes harvested from animals subjected to trauma-hemorrhage was significantly decreased compared with the release seen in splenocytes from sham-operated animals (Fig. 4A). The in vitro addition of mouse prolactin to the cultures improved the depressed splenocyte IL-2 release from hemorrhaged mice in a dose-dependent manner, and the values were similar to shams at a concentration of 0.1 ng mouse prolactin/ml culture medium. However, the addition of metoclopramide to the splenocyte cultures had no stimulatory effect on the depressed IL-2 release capacity of splenocytes from hemorrhaged animals (Fig. 4B). Moreover, there was no effect of prolactin or metoclopramide addition on IL-2 release by splenocytes from sham-operated animals. Similar findings were observed with splenocyte...
IL-3 release capacity, which was significantly depressed at 2 h after trauma-hemorrhage (Fig. 5A). In vitro addition of mouse prolactin led to a dose-dependent increase in splenocyte IL-3 release in cells from hemorrhaged animals, whereas no effect was observed on splenocytes from sham-operated animals. Similar to splenocyte IL-2 release, no significant effects on IL-3 release were observed in splenocytes cultured in the presence of various concentrations of metoclopramide (Fig. 5B).

Splenocyte IL-10 release increased at 2 h after trauma-hemorrhage (Fig. 6, A and B). This increase, however, was not statistically significant. The addition of increasing concentrations of mouse prolactin to the culture media led to a decreased release of IL-10 by splenocytes from hemorrhaged animals (Fig. 6A). However, the addition of metoclopramide to the culture media had no effects on IL-10 release by splenocytes from sham-operated or hemorrhaged animals (Fig. 6B).

**Prolactin plasma levels.** At 30 min after trauma-hemorrhage or sham operation and saline vehicle administration, no significant difference was observed in plasma prolactin levels between sham animals and animals subjected to trauma-hemorrhage (Fig. 7). However, treatment with metoclopramide after trauma-hemorrhage significantly increased plasma prolactin levels compared with vehicle-treated animals at 30 min after subcutaneous injection.

**DISCUSSION**

It is well established that major hemorrhage, even in the absence of any significant tissue trauma, produces a marked depression in both the specific and nonspecific cell-mediated immunity (5). Several studies indicate that this depression in cellular immune functions is apparent immediately after hemorrhage and persists despite adequate fluid resuscitation (4, 26). However, a number of pharmacological agents have been...
shown to improve the depressed immune functions after hemorrhagic shock and to decrease susceptibility to subsequent sepsis. In this regard, recent studies from our laboratory indicate that in vivo administration of the pituitary peptide hormone prolactin after hemorrhagic shock led to restoration of the depressed lymphocyte (32) and macrophage functions (33) and significantly improved the survival rate of animals subjected to a subsequent septic challenge (33). Additional studies have shown that treatment with metoclopramide, a central dopamine antagonist, restored the depressed splenocyte proliferation as well as splenocyte, splenic, and peritoneal macrophage immune functions after hemorrhage (30) and significantly improved the depressed cardiovascular and hepatocellular functions under those conditions (14). Because metoclopramide antagonizes dopamine’s inhibitory effects on prolactin secretion via specific receptors located in the anterior pituitary, we hypothesized that metoclopramide administration might improve immune responses after trauma-hemorrhage by upregulating the secretion of the immunoenhancing hormone prolactin from the anterior pituitary gland. The aim of the present study, therefore, was to determine the mechanism by which metoclopramide exerts its beneficial immunomodulatory effects after trauma-hemorrhage.

In the in vivo part of the study, animals were treated with metoclopramide or saline vehicle after sham operation or trauma-hemorrhage, and macrophage proinflammatory cytokine release was determined. The results indicate that the depressed splenic and peritoneal macrophage proinflammatory cytokine release after trauma-hemorrhage was increased in metoclopramide-treated animals to levels observed in sham-operated animals. These findings are in line with previous results from our laboratory that showed that metoclopramide treatment after simple hemorrhage restored the depressed splenic and peritoneal macrophage function (16). The present findings are of particular interest, since the salutary effects of metoclopramide on immune functions were observed in an animal model of combined soft tissue trauma and hemorrhagic shock. In this regard, it has previously been demonstrated that this combined trauma-hemorrhage model is characterized by more prolonged immunosuppression compared with simple hemorrhage in the absence of significant tissue trauma (29, 30). Therefore, the present findings that metoclopramide treatment led to a restoration of the depressed macrophage functions after trauma-hemorrhage indicate that this agent has immunorestorative effects even in the more severe model of trauma-hemorrhage.

Although the concentrations of circulating proinflammatory cytokines were not measured in this study, the findings from previous studies indicate that restoration of splenic and peritoneal macrophage cytokine release capacity due to immunomodulatory treatment...
was associated with decreased levels of circulating cytokines (17). In this regard, Kupffer cells have been identified to be the primary cellular source for circulating cytokines (21). Therefore, it could be speculated that treatment with metoclopramide might also reduce the concentrations of circulating proinflammatory cytokines via alterations in Kupffer cell cytokine productive capacity. Additional studies are, however, needed to determine the effects of metoclopramide treatment on proinflammatory cytokines.

In an attempt to address the question whether metoclopramide treatment increases the secretion of the pituitary peptide hormone prolactin, plasma levels of prolactin were determined after administration of metoclopramide. The results demonstrate that subcutaneous administration of metoclopramide in mice led to significantly elevated prolactin plasma levels. These findings are in agreement with the studies of other investigators who showed a close relationship between metoclopramide administration and pituitary release of prolactin in the circulation under conditions other than trauma-hemorrhage (6, 15). In this regard, it has been suggested that the increase in prolactin secretion is the result of metoclopramide’s central antagonism of pituitary dopamine receptors. Dopamine’s effect on specific receptors located in the anterior pituitary gland is considered to inhibit prolactin release and, therefore, drugs with dopamine antagonist activity appear to increase plasma prolactin levels by blocking the inhibitory effects of dopamine (11). It is worth noting that, after trauma-hemorrhage and vehicle administration, no decrease in circulating prolactin levels was observed. Therefore, it appears that the beneficial effects of metoclopramide treatment are not due to restoration of decreased plasma prolactin levels but rather are mediated by elevation of circulating prolactin to supraphysiological, pharmacologically active concentrations.

Based on the findings that metoclopramide administration restored the depressed splenic and peritoneal macrophage immune functions after trauma-hemorrhage and led to increased prolactin plasma levels, we investigated whether the immunoprotective actions of metoclopramide are direct effects of this agent on immune cells or indirectly mediated via increased circulating prolactin levels. To study this, splenocytes were harvested from untreated mice after trauma-hemorrhage or sham operation and were cultured in the presence or absence of increasing concentrations of metoclopramide or mouse prolactin. Although prolactin is a highly conserved molecule, mouse prolactin was used in the present study to minimize species-related differences in bioactivity (24). The results indicate that, after trauma-hemorrhage, splenocyte immune functions were significantly depressed compared with sham animals. Treatment of splenocytes in vitro with metoclopramide had no effects on proliferative capacity and splenocyte IL-2, IL-3, or IL-10 release, neither on cells from sham-operated animals nor from animals that underwent trauma-hemorrhage. However, the addition of mouse prolactin to the splenocyte cultures led to a dose-dependent restoration of the depressed splenocyte proliferative capacity as well as splenocyte IL-2 and IL-3 release of cells harvested from hemorrhaged animals. Moreover, prolactin treatment in vitro led to a significant reduction in the release of the immunosuppressive Th2-lymphokine IL-10. These findings suggest that the pituitary hormone prolactin directly restores immune functions of splenocytes harvested from posthemorrhage animals, whereas metoclopramide appears to have no direct effects on splenocyte immune functions. Our results, however, do not allow us to conclude whether the improved cytokine production seen in cells harvested from hemorrhaged animals and treated with prolactin in vitro is due to improvement of cell function or increased cell viability. Nonetheless, these findings suggest that the beneficial immunomodulatory effects of metoclopramide treatment after trauma-hemorrhage are mediated via up-regulation of the immunoenhancing hormone prolactin. It should be noted that mixed splenocyte cultures consisting of T lymphocytes, B lymphocytes, and splenic macrophages were used for the in vitro experiments. Although stimulation with Con A was carried out, which is a specific T lymphocyte stimulant, the results presented here do not allow us to absolutely rule out indirect effects on macrophages that may be present during in vitro prolactin treatment. Of interest is also the question whether prolactin has direct effects on T lymphocyte function or whether interactions mediated by macrophages contribute to the salutary effects seen after trauma-hemorrhage. In this regard, the present findings that metoclopramide treatment in vivo after trauma-hemorrhage restored the peritoneal macrophage proinflammatory cytokine release capacity suggest that, in addition to potential effects on T lymphocytes, direct or indirect effects of prolactin on macrophages might also occur.

The available information indicates that a close interaction between the immune and the endocrine system exists under physiological as well as pathological conditions. Stress, whether induced by disease, emotional, or physical condition, has been shown to activate endocrine responses that modulate the immune system (28). In this regard, prolactin is one of the major hormones involved in the endocrine stress response, and its effects on the immune system have been examined extensively. Several studies indicate that various immune cell populations express receptors for prolactin (8, 9, 22, 23). Russel et al. (22) reported the presence of high-affinity prolactin binding sites on human T and B lymphocytes isolated from human spleen as well as on peripheral human monocytes (22). In rodents, high-affinity prolactin receptors have been demonstrated in a tumor lymphoid cell line, Nb2, which was cloned from a rat lymphoma (10). Generally, stimulatory effects of prolactin in vivo or in vitro on these immune cells have been reported (20, 25). Spangelo et al. (25) demonstrated significantly potentiated proliferative responses of mouse spleen cells cultured in the presence of Con A and 0.2–200 ng/ml bovine prolactin. Furthermore, although stimulation with Con A, which is a
specific T lymphocyte stimulant, was carried out, the results presented here do not allow us to absolutely rule out indirect effects on macrophage that may be present during in vitro prolactin treatment. Thus, despite the available information suggesting receptor-mediated effects of prolactin on immune cells, additional studies are necessary to identify the pathway by which prolactin, either exogenously administered or increased due to metoclopramide treatment, restores cell-mediated immune functions after trauma-hemorrhage.

In this regard, it is of interest to note that intravenous dopamine administration, frequently used in the treatment of critically ill patients, is not typically reported to produce detrimental effects on immunity via such mechanisms, since dopamine does not readily cross the blood-brain barrier (18). However, there is evidence that prior shock can cause impairment of the blood-brain barrier function, which might in turn allow the translocation of peripherally administered dopamine (7). Therefore, the adjuvant use of metoclopramide to block dopamine’s central effects under those conditions might be beneficial to the immune response in those critically ill patients.

It should also be noted that metoclopramide is commonly used clinically for the treatment of nausea and emesis without any significant adverse effects during short-term administration. The dose used in the present study was chosen in agreement with studies by Brouwers et al. (3) who reported a 10-fold increase in circulating prolactin levels after metoclopramide administration. Moreover, this dose, which has marked effects on plasma prolactin levels, has been demonstrated to be pharmacologically safe, since no major side effects have been observed. Therefore, it appears that a metoclopramide could be used safely for immunomodulation in trauma victims.

In summary, the data presented here demonstrate that metoclopramide administration after trauma-hemorrhage restored the depressed cell-mediated immune functions. Therefore, our results suggest that the beneficial immunomodulatory effects of metoclopramide treatment after trauma-hemorrhage are mediated via upregulation of the immunoenhancing hormone prolactin. However, additional in vivo experiments, using either specific neutralizing antibodies, gene deficiency, or prolactin receptor antagonists, will be needed to obtain direct proof of this notion. Because metoclopramide increases plasma levels of the immunoenhancing hormone prolactin, metoclopramide should be considered as a safe and useful adjunct for the treatment of immunodepression in trauma victims.

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