Metoclopramide: a novel adjunct for improving cardiac and hepatocellular functions after trauma-hemorrhage

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J. Arrar, Doraid, Ping Wang, Grace Y. Song, Markus W. Knöferl, William G. Cioffi, Kirby I. Bland, and Irshad H. Chaudry. Metoclopramide: a novel adjunct for improving cardiac and hepatocellular functions after trauma-hemorrhage. Am. J. Physiol. Endocrinol. Metab. 278: E90–E95, 2000.—Although metoclopramide (MCP) administration after trauma-hemorrhage restores the depressed immune functions, it remains unknown whether this agent has any salutary effects on the depressed cardiovascular and hepatocellular functions under those conditions. Adult male Sprague-Dawley rats underwent a midline laparotomy (i.e., induction of soft-tissue trauma) and were then bled to and maintained at a mean arterial pressure (MAP) of 40 mmHg until 40% of the maximal shed blood volume was returned in the form of Ringer lactate (RL). The rats were then resuscitated with four times the shed blood volume in the form of RL over 60 min. MCP (2 mg/kg body wt) or vehicle was administered subcutaneously at the end of resuscitation. At 24 h after resuscitation, cardiac index and hepatocellular function (i.e., the maximum velocity and the overall efficiency of indocyanine green clearance) were determined. Plasma levels of interleukin (IL)-6 and prolactin were also assayed. The results indicate that treatment with MCP after trauma-hemorrhage and resuscitation significantly improved the depressed cardiac output and hepatocellular function. Furthermore, MCP administration significantly increased circulating levels of prolactin and decreased the plasma levels of the proinflammatory cytokine IL-6. Thus, administration of MCP, which increased prolactin secretion, appears to be a useful adjunct for restoring the depressed cardiovascular and hepatocellular functions and downregulating inflammatory cytokine release after trauma and hemorrhagic shock.

interleukin-6; prolactin; cardiac output; indocyanine green; liver function

DESPITE MAJOR ADVANCES in the prehospital care of trauma victims, a significant number of such patients succumb subsequently because of sepsis and multiple organ failure. Heckbert et al. (6) recently reported that hemorrhage-induced hypotension in trauma victims is predictive of high mortality and morbidity. Moreover, studies have shown that acute fluid resuscitation alone after trauma and hemorrhage does not restore or maintain hepatocellular function, microvascular blood flow, or cardiac output (17–19). In addition, nonspecific and specific immune functions are depressed after trauma-hemorrhage, which could explain the increased susceptibility to sepsis after such conditions (2, 3).

Recent studies from our laboratory have shown that administration of the anterior pituitary hormone prolactin after trauma-hemorrhage has salutary effects on the depressed immune functions (25). Moreover, administration of the central dopamine antagonist metoclopramide (MCP), which is known to increase prolactin secretion (1), restored the depressed macrophage immune function after hemorrhage (24). Further evidence for the importance of neuroendocrine factors in the response to trauma-hemorrhage came from studies with female rodents during different stages of the reproductive cycle. In this regard, studies have shown that females in the proestrus state, a state in which plasma prolactin levels are found to be the highest, have enhanced immune and cardiovascular responses after hemorrhage as opposed to decreased responses in males (8, 20). Because prolactin and MCP have been shown to have beneficial effects on immune responses after severe hemorrhage (24, 26), we hypothesized that administration of MCP after trauma-hemorrhage and resuscitation improves the depressed heart and liver functions under those conditions via upregulation of prolactin secretion. The aim of the present study, therefore, was to determine whether administration of MCP increases circulating levels of prolactin and improves the depressed cardiovascular and hepatocellular functions after trauma and severe hemorrhage.

MATERIALS AND METHODS

Experimental procedures. The previously described non-heparinized model of trauma-hemorrhage in the rat (17–19) was used with minor modifications. Briefly, male Sprague-Dawley rats (275–325 g, Charles River Laboratories, Wilmington, MA) were fasted overnight before the experiment but allowed water ad libitum. The rats were anesthetized by methoxyflurane (Mallinkrodt Veterinary, Mundelein, IL) inhalation before the induction of trauma (i.e., 5-cm midline laparotomy). The abdomen was then closed in layers, and catheters were placed in both femoral arteries and the right femoral vein (polyethylene [PE-50] tubing; Becton-Dickinson, Sparks, MD). The wounds were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, N.J.) throughout the surgical procedure to reduce postoperative pain. Rats were then bled to and maintained at a mean arterial pressure (MAP) of 40 mmHg until the animals could not maintain a MAP of 40 mmHg unless extra fluid in the form of Ringer lactate was given.

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This time was defined as maximum bleed-out, and the amount of withdrawn blood was noted. After this, the rats were maintained at MAP of 40 mmHg until 40% of the maximum bleed-out volume was returned in the form of Ringer lactate. The animals were then resuscitated with four times the volume of the withdrawn blood over 60 min (−45 ml/rat) with Ringer lactate. The shed blood was not used for resuscitation. At the end of the resuscitation period, the rats received 2 mg/kg body wt MCP (Sigma Diagnostics, St. Louis, MO) subcutaneously or an equal volume (−0.5 ml) of the vehicle phosphate-buffered saline. The catheters were then removed, the vessels were ligated, and the skin incisions were closed with sutures. Sham-operated animals underwent the same groin dissection, which included the ligation of the femoral artery and vein; however, neither hemorrhage nor resuscitation was carried out.

After the rats were returned to their cages, they were allowed food and water ad libitum. At 24 h after the completion of fluid resuscitation or sham operation, the animals were anesthetized with methoxyflurane and then catheterized via the right jugular vein. While rats were under continued general anesthesia with pentobarbital sodium (25–30 mg/kg body wt), cardiac output, hepatocellular function, and heart performance were measured in each animal. All animal experiments were performed according to the guidelines of the Animal Welfare Act and The Guide for Care and Use of Laboratory Animals from the National Institutes of Health. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital, Providence, RI.

Measurement of cardiac output. A 2.4 French fiberoptic catheter was placed into the right carotid artery, which was connected to an in vivo hemorefraction meter (Hospex Fiberoptics, Chestnut Hill, MA) as described previously (18). Indocyanine green (ICG, Cardio Green, Becton-Dickinson) solution was injected via the catheter into the jugular vein (1 mg/ml aqueous solution as a 50-µl bolus). Twenty ICG concentrations per second were recorded for 30 s with the aid of a data acquisition program (Asystant+, Asyst Software, Rochester, NY). The area under the ICG dilution curve was determined according to our previous publication (18) to calculate cardiac output. Cardiac output was then divided by the body weight to determine cardiac index.

Measurement of hepatocellular function. Hepatocellular function was measured by the in vivo ICG clearance technique (5). ICG was administered by bolus injection (50 µl) of 1, 2, and 5 mg/ml ICG in aqueous solvent. The arterial concentration of ICG was recorded each second for 5 min. After this, the initial velocity of ICG clearance for each dose was calculated after a nonlinear regression of the ICG clearance curves was performed according to an e-raised to second order polynomial function (5). The initial velocities of ICG clearance were then plotted against the ICG doses according to the methods of Lineweaver-Burk (4). This results in a straight line, allowing the determination of a maximum of ICG clearance (Vmax) and the Michaelis-Menten constant (Km). In this active hepatocellular membrane transport system, Vmax represents the functional capacity of the hepatocyte ICG carrier, whereas Km represents an index of efficiency of the active transport process (17).

Measurement of in vivo heart performance. After the determination of cardiac output and hepatocellular function, the fiberoptic catheter in the right carotid artery was replaced with PE-50 tubing, which was manually stretched to reduce the outer diameter by ≈50%. Under pressure control, this catheter was carefully advanced into the left ventricle. The position of the catheter was confirmed by recording the characteristic left ventricle pressure curve. Data were analyzed from an in vivo heart performance analyzer (Micro-Med., Louisville, KY) as described in our previous publication (13). Left ventricular performance parameters such as the maximal rate of pressure increase (+dP/dtmax) and decrease (−dP/dtmax) were documented with a data acquisition system (DMSI 200–8; Micro-Med.).

Determination of serum IL-6 levels. At 24 h after the completion of fluid resuscitation, blood was drawn and serum was separated and stored at −70°C until assayed. IL-6 levels were measured with an ELISA kit (BioSource International, Camarillo, CA) according to the instructions of the manufacturer. The sensitivity of the assay is <8 pg/ml, and the intra-assay coefficient of variation is 3.9% in the lower range and 4.9% in the higher range. The interassay coefficient of variation is 9.9 and 5.9% in the lower and higher range, respectively.

Determination of plasma prolactin levels. In addition, groups of animals, a femoral artery catheter was placed and blood samples were collected at site points as indicated in the results section. Blood was filled in prechilled microfuge tubes, and plasma was separated and stored at −70°C until assayed. Prolactin levels were measured with an Enzyme Immunoassay Kit (SPI Bio, Massy Cedex, France) according to the instructions of the manufacturer. Cross-reactivity with rat luteinizing hormone, growth hormone, and thyroid-stimulating hormone is below 1%. The sensitivity of the assay is 0.5 ng/ml, and the mean interassay variation is 14%. The intra-assay coefficient of variation is 9.4 and 8.6% in the lower and higher range, respectively.

Statistical analysis. Results are presented as means ± SE. One-way ANOVA and Tukey’s test were used, and the differences were considered significant at a p value < 0.05. There were 8–10 animals in each group. Comparison between sham-vehicle and sham MCP-treated animals were made by Student’s t-test.

RESULTS

Effects of MCP on cardiac index. The results in Fig. 1 indicate that cardiac index was 40.3 ± 1 ml·100 g−1·min−1 in sham-operated animals and decreased in the hemorrhaged animals to 29.3 ± 1.9 ml·100 g−1·min−1 (P < 0.05) at 24 h after the completion of hemorrhage and vehicle or MCP-treated group, respectively. *P < 0.05 vs. sham-operated group; #P < 0.05 vs. hemorrhaged and vehicle-treated group.
fluid resuscitation. Administration of MCP after hemorrhage, however, restored the depressed cardiac index to sham levels (35.5 ± 1.6 ml·100 g⁻¹·min⁻¹).

Effects of MCP on heart performance. The +dP/dt_max in the left ventricle was significantly decreased after trauma-hemorrhage (Fig. 2A). However, administration of MCP increased +dP/dt_max after trauma-hemorrhage and resuscitation, and the values were not different from sham-operated animals. The −dP/dt_max was also significantly decreased after hemorrhage compared with sham-operated animals (Fig. 2B). Administration of MCP significantly increased −dP/dt_max values compared with vehicle-treated animals after trauma-hemorrhage and resuscitation; however, these values were still lower than sham-operated animals (P < 0.05).

Effects of MCP on hepatocellular function. V_max was 1.1 ± 0.25 ml·kg⁻¹·min⁻¹ at 24 h after sham operation. In vehicle-treated rats subjected to trauma-hemorrhage and resuscitation, V_max decreased by 75% (P < 0.05). In contrast, administration of MCP during fluid resuscitation resulted in significant higher values for V_max (0.78 ± 0.07 ml·kg⁻¹·min⁻¹) at 24 h after trauma-hemorrhage, and the values were similar to sham-operated animals. As indicated in Fig. 3B, K_m was 2.8 ± 0.49 mg/kg in sham-operated animals and decreased by 63% (P < 0.05) after trauma-hemorrhage and resuscitation in vehicle-treated rats. MCP treatment significantly improved K_m (2.6 ± 0.92 mg/kg) at 24 h after the completion of resuscitation compared with vehicle-treated animals.

Effects of MCP on serum IL-6 levels. As indicated in Fig. 4, serum IL-6 was 34 ± 17 pg/ml in sham-operated animals. At 24 h after trauma-hemorrhage, IL-6 increased to 187 ± 29 pg/ml in vehicle-treated hemorrhaged animals (P < 0.05). Serum IL-6 levels in hemorrhaged and MCP-treated animals were significantly lower than in the vehicle group (36 ± 9 pg/ml) and were similar to sham-operated animals.

Effects of MCP on plasma prolactin levels. As indicated in Fig. 5, subcutaneous injection of MCP in a dose of 2 mg/kg body wt increased circulating levels of prolactin in sham and hemorrhaged animals from 6.3 ±
MATERIALS AND METHODS). There were 3 animals in each group at each time point.

Fig. 4. Effects of MCP on plasma interleukin (IL)-6 levels 24 h after completion of fluid resuscitation. Figure shows comparison of sham-operated, hemorrhaged and vehicle-treated, and hemorrhaged and MCP-treated animals. There were 8 animals in sham-operated group and 9–10 animals in hemorrhaged and vehicle- or MCP-treated group, respectively. * P < 0.05 vs. sham-operated group; # P < 0.05 vs. hemorrhaged and vehicle-treated group.

Fig. 5. Effects of subcutaneous injection of 2 mg/kg body wt MCP or vehicle on plasma prolactin levels over 24 h after sham operation or trauma-hemorrhage. Figure shows comparison of MCP-treated animals and vehicle (VEH)-treated hemorrhaged rats. (MB, maximal bleed-out; 0 Rx, end of resuscitation; for more details see MATERIALS AND METHODS). There were 3 animals in each group at each timepoint. * P < 0.05 vs. hemorrhaged and vehicle-treated group.

DISCUSSION

As more studies are forthcoming, it is becoming evident that the neuroendocrine axis plays an important role in regulating physiological and immunologic responses after injury. Recent studies from our laboratory have shown that administration of the anterior pituitary hormone prolactin enhances immune responses after severe hemorrhage (25, 27) and decreases mortality from subsequent sepsis (26). Moreover, treatment with the central dopamine antagonist MCP, which is known to increase prolactin secretion, had similar beneficial effects on the depressed immune responses after severe hemorrhage (24). Evidence for the importance of pituitary hormones in the maintenance of cardiovascular responses came from studies of Lanza et al. (9), who showed that dogs pretreated with a prolactin inhibitor (bromocriptine) had a significantly lower MAP than the control group in a model of volume-fixed hemorrhage. In view of the aforementioned studies, we hypothesized that administration of MCP after trauma-hemorrhage not only improves the depressed immunologic response but would also improve the physiological response (i.e., cardiovascular and hepatocellular functions) after trauma-hemorrhage and resuscitation.

The aim of the present study, therefore, was to determine whether MCP administration after trauma-hemorrhage and resuscitation increases circulating prolactin levels, improves cardiac output, left ventricular performance, and hepatocellular function and downregulates inflammatory cytokine release.

The results indicate that administration of MCP during resuscitation improved the depressed cardiovascular and hepatocellular functions after the completion of fluid administration. Cardiac output and heart performance parameters such as the + dP/dt max and − dP/dt max were significantly depressed at 24 h after trauma-hemorrhage and crystalloid resuscitation. Moreover, active hepatocellular function as assessed by the ICG clearance technique was significantly compromised at 24 h after the completion of fluid resuscitation. Treatment with the central dopamine antagonist MCP, which is known to increase prolactin secretion, had similar beneficial effects on the depressed immune responses after severe hemorrhage (24).

Table 1. Comparison between normal animals treated either with vehicle or metoclopramide

<table>
<thead>
<tr>
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<th>Sham + Vehicle</th>
<th>Sham + Metoclopramide</th>
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<tbody>
<tr>
<td>Cardiac index, ml·100 g⁻¹·min⁻¹</td>
<td>40.2 ± 1.05</td>
<td>41.7 ± 0.6</td>
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<tr>
<td>+dP/dt max, mmHg/g</td>
<td>10,858 ± 357</td>
<td>10,551 ± 267</td>
</tr>
<tr>
<td>−dP/dt max, mmHg/g</td>
<td>8,563 ± 417</td>
<td>7,645 ± 1126</td>
</tr>
<tr>
<td>V max, mg·kg⁻¹·min⁻¹</td>
<td>1.10 ± 0.25</td>
<td>1.24 ± 0.02</td>
</tr>
<tr>
<td>K m, mg/kg</td>
<td>2.47 ± 0.45</td>
<td>2.62 ± 0.9</td>
</tr>
<tr>
<td>n=</td>
<td>8 (from figures)</td>
<td>3</td>
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</table>

Values are means ± SE of 3–8 (n) rats in each group. There is no significant difference between sham-operated animals treated with vehicle and sham animals treated with metoclopramide. For further details see MATERIALS AND METHODS. Sham + vehicle group data are from figures. + dP/dt max and − dP/dt max, maximal rate of pressure increase and decrease, respectively; V max, maximum of indocyanine green clearance; K m, Michaelis-Menten constant.
met with MCP, however, restored the depressed cardiovascular and hepatocellular functions to sham levels. Moreover, administration of MCP attenuated the increased inflammatory cytokine release observed under such conditions. In addition, MCP administration increased plasma prolactin levels by 10-fold within 30 min in both sham and hemorrhaged animals, which remained significantly elevated for up to 4 h.

Taken together, our data clearly demonstrate that administration of MCP after trauma-hemorrhage and resuscitation increases endogenous prolactin secretion and improves the depressed cardiovascular and hepatocellular functions. It should be pointed out that we did not observe any adverse effects of MCP treatment in nonhemorrhaged animals. Administration of MCP increased circulating prolactin levels in sham-operated and hemorrhaged animals in the same fashion, with an increase from 6.3 ± 0.3 and 12.2 ± 1 ng/ml to 71.3 ± 11.2 and 69.3 ± 0.9 ng/ml within 30 min, respectively. MCP administration under normal conditions did not alter parameters such as cardiac index and ICG clearance to supranormal levels; however, under stress conditions such as severe hemorrhage, it facilitated the functional recovery of treated animals and the values were similar to sham-operated animals within 24 h after the insult. Whether or not administration of MCP also has beneficial effects at an earlier time point remains to be determined.

MCP, a synthetic substituted benzamide, is a dopamine-receptor antagonist, an antiemetic, and a stimulant of upper gastrointestinal motility. The increase in prolactin secretion is apparently the result of MCP’s antagonism of dopamine receptors in the anterior pituitary gland. Dopamine is produced in the arcuate and paraventricular nuclei of the hypothalamus and then released by nerve terminals in the median eminence, where it enters the portal circulation and reaches the anterior pituitary. Dopamine inhibits prolactin release via interaction with pituitary D2 receptors (16). Drugs that are dopamine antagonists, such as MCP, therefore increase prolactin levels by blocking the inhibitory effects of dopamine in the anterior pituitary. In addition to the classical effect on mammary gland, prolactin is involved in a broad spectrum of physiological processes. Prolactin effects are initiated by interaction with a specific receptor, which belongs to a large family of transmembrane proteins, including the receptor for growth hormone, erythropoietin, IL-2, IL-3, IL-4, IL-6, and granulocyte-macrophage colony-stimulating factor (7). A high concentration of prolactin receptors has been demonstrated in rat liver (12), and mRNA expression for it is also found in tissues such as skin, heart, skeletal muscle, lung, and adrenals (11). However, it remains unclear whether or not prolactin receptors are downregulated after hemorrhage and whether MCP via upregulation of prolactin secretion alters the functional capacity of these receptors.

The increased circulating levels of prolactin observed in the present study are consistent with studies by Brouwers et al. (1), who showed that acute administration of MCP in humans increased plasma prolactin levels by more than 10-fold, from an initial mean of 5.73 ng/ml to a mean of 60.2 ng/ml 90 min after ingestion. After 14 days of MCP therapy, the mean prolactin level increased 15-fold, from 5.4 to 83.3 ng/ml (1). In the present study, we used a single dose of 2 mg/kg MCP and observed improved organ functions 24 h later. Preliminary observations suggested that the 2-mg/kg dose was more effective in improving cardiac and hepatic functions than 1 mg/kg, a dose previously used in mice to improve immune functions 2 h after trauma-hemorrhage (24). Whether administration of MCP improves organ functions in the rat as early as 2 h after trauma-hemorrhage and resuscitation remains to be determined.

Several investigators have demonstrated that plasma levels of catecholamine such as norepinephrine, epinephrine, and dopamine are elevated early after the onset of hemorrhage and that sustained levels correlate with irreversibility of shock (15). Although we have not measured plasma levels of dopamine, a potential antagonist of prolactin secretion, the comparable baseline levels of prolactin in sham-operated and hemorrhaged animals suggest that early during hemorrhage, increased dopamine levels do not acutely suppress prolactin secretion. This is supported by the fact that the ability to secrete endogenous prolactin in response to MCP is not compromised in animals subjected to severe hemorrhagic shock. It is worth noting that long-term administration of dopamine, a natural catecholamine with hypophysiotropic properties, has been used for more than two decades as an inotropic and vasodilatory drug. Suppresses the circulating levels of all anterior pituitary hormones, except cortisol (16). The available evidence therefore suggests that the major effect of dopamine administration on the endocrine system is unlikely to have any beneficial effects on the depressed metabolic and immunologic homeostasis in the traumatized host.

Plasma levels of the inflammatory cytokine IL-6 are increased at 24 h after severe hemorrhage; however, administration of MCP attenuated this response. These findings support previous studies from our laboratory that showed that prolactin administration decreased cytokine gene expression in Kupffer cells after hemorrhage (27).

It has been documented that the proestrus state of the female rodent shows the highest plasma concentration of prolactin (10). In this regard, Wichmann et al. (20) have recently shown that female mice subjected to hemorrhage during the proestrus state of their cycle have enhanced immune responses as opposed to decreased responses in males (20). In addition, studies by Slimmer et al. (14) indicated that female rats hemorrhaged on the morning of proestrus exhibit a more vigorous restitution response than either metestrus females or males. Although a variety of different hormones account for the reported differences between males and females (21–23), it could be speculated that the increased prolactin levels do play an important role in the response to trauma-hemorrhage. We have also recently shown that females in the proestrus phase
subjected to hemorrhage not only have better immune functions, but also maintained their cardiovascular and hepatocellular responses as opposed to males (8).

In summary, our data indicate that administration of MCP after trauma-hemorrhage and resuscitation significantly improves cardiac output, left ventricular performance such as \( \frac{dP}{dt_{\text{max}}} \), and hepatocellular functions. Moreover, MCP decreased the plasma levels of the proinflammatory cytokine IL-6. We therefore conclude that MCP administration, which increases prolactin secretion, appears to be a useful adjunct for improving cardiovascular and hepatocellular responses and downregulating inflammatory cytokine release after trauma and hemorrhagic shock.

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REFERENCES


