

# Influence of Different Strategies of Volume Replacement on the Activity of Matrix Metalloproteinases

## An In Vitro and In Vivo Study

Carlo A. Volta, M.D.,\* Valentina Alvisi, M.D.,† Matilde Campi, M.D.,‡ Elisabetta Marangoni, M.D.,† Raffaele Alvisi, M.D.,§ Massimiliano Castellazzi, D.,|| Enrico Fainardi, M.D., Ph.D.,# Maria C. Manfrinato, Ph.D.,\*\* Franco Dallochio, D.,†† Tiziana Bellini, D.‡‡

**Background:** Excessive production of matrix metalloproteinase 9 (MMP-9) is linked to tissue damage and anastomotic leakage after large bowel surgery. Hence, the aim of this study was to verify whether different strategies of fluids administration can reduce MMP-9 expression.

**Methods:** In the *in vitro* experiment, the authors tested the hypothesis of a direct inhibition of MMP-9 by the fluids used perioperatively, *i.e.*, lactated Ringer's solution, 3.4% poligeline, and hydroxyethyl starch 130/0.4. In the *in vivo* experiment, 36 patients undergoing surgery for colon cancer were randomly assigned to three groups to receive lactated Ringer's solution, poligeline, or hydroxyethyl starch. MMP-9 and tissue inhibitor of metalloproteinases were measured from venous blood samples; the MMP-9/tissue inhibitor of metalloproteinases ratio was calculated as an index of equilibrium between the action of MMP-9 and its inhibition.

**Results:** In the *in vitro* experiment, the presence of hydroxyethyl starch 130/0.4 in the MMP-9 assay system showed a strong inhibition of the enzymatic activity compared with lactated Ringer's solution. In the *in vivo* experiment, MMP-9 and tissue inhibitor of metalloproteinases plasma levels did not differ among the three groups at baseline, whereas those levels increased significantly at the end of surgery. At that time, the MMP-9 plasma levels and the MMP-9/tissue inhibitor of metalloproteinases ratio were significantly higher in the lactated Ringer's solution and poligeline groups than in the hydroxyethyl starch group. These results were confirmed 72 h after surgery.

**Conclusions:** This study demonstrates that hydroxyethyl starch 130/0.4 decreases the circulating levels of MMP-9 in patients undergoing abdominal surgery.

NUMEROUS stimuli such as trauma, infection, burns, and major surgery may alter the physiologic immune balance and initiate systemic inflammatory process,<sup>1</sup> which is characterized by the release of potent inflammatory mediators into the circulation.

*Matrix metalloproteinases* (MMPs) are a family of zinc-containing enzymes with proteolytic activity against

extracellular matrix components such as elastin, proteoglycans, and collagen. More than 20 mammalian MMPs have been identified, all of which are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs).<sup>2</sup> MMPs and their inhibitors play a critical role in inflammatory diseases, tumor metastasis, and wound healing.

Matrix metalloproteinase 9 (MMP-9), also called gelatinase B, belongs to the subfamily of MMPs that play an important role in tissue remodeling in normal and pathologic inflammatory process. It is mainly produced by inflammatory cells, such as neutrophils, monocytes/macrophages, and eosinophils. During an acute inflammatory response, neutrophils are chemoattracted to the inflammatory site, and MMP-9 is degranulated to degrade type IV collagen, the major constituent of basement membrane.<sup>3</sup> Because the integrity of the tissue architecture is closely dependent on the delicate balance between MMPs and their inhibitors, excessive production of MMP-9 is linked to tissue damage and degenerative inflammatory disorders.<sup>4-7</sup>

A recent study has demonstrated that major abdominal surgery is responsible for an overexpression of MMP-9 as part of the inflammatory process triggered by the surgical procedure. In this connection, it should be pointed out that a recent study by Stumpf *et al.*<sup>8</sup> demonstrated a significantly greater expression of MMPs in mucosal and submucosal layers of patients with anastomotic leakage after large bowel surgery compared with patients with uncomplicated anastomotic healing.

It has previously been demonstrated that surgical patients are at risk of hypovolemia and, as a consequence, tissue underperfusion; optimization of the patient's intravascular volume status may have an important impact on immune response,<sup>9</sup> although the influence of resuscitation fluids on immune function, inflammatory response, and endothelial injury is not yet clear. Lang *et al.*<sup>10</sup> have assessed the effects of intravascular volume replacement therapy on the inflammatory response during major abdominal surgery. A significantly lower release of cytokines was detected in the patients treated with hydroxyethyl starch compared with those treated with lactated Ringer's solution (LR). The authors hypothesized that this was probably due to an improved microcirculation with reduced endothelial activation and less endothelial damage. Boldt *et al.*<sup>11</sup> found similar results in elderly patients undergoing major abdominal surgery.

\* Associate Professor, † Staff, ‡ Resident, § Professor and Chair, Section of Anesthesia and Intensive Care, || Biologist, # Staff, Multiple Sclerosis Center, Department of Neurology, \*\* Research Fellow, †† Chemical, Professor and Chair of Biochemistry, ‡‡ Biologist, Associate Professor of Biochemistry, Department of Biochemistry and Molecular Biology, University of Ferrara.

Received from the Department of Surgical, Anaesthesiological and Radiological Science, Section of Anesthesia and Intensive Care, S. Anna Hospital, University of Ferrara, Ferrara, Italy. Submitted for publication April 5, 2006. Accepted for publication September 22, 2006. Supported in part by a grant from Fondazione della Cassa di Risparmio di Ferrara, Ferrara, Italy, and by the Italian Ministry of the University and Research, Rome, Italy.

Address correspondence to Dr. Volta: Department of Surgical, Anaesthesiological and Radiological Science, Section of Anesthesia and Intensive Care, S. Anna Hospital, University of Ferrara, Corso Giovecca n. 203, 44100 Ferrara, Italy. vlc@dns.unife.it. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

They concluded that the differences in terms of inflammatory response and endothelial injury between patients treated with either hydroxyethyl starch or crystalloids could be explained by some direct, substance-specific beneficial effects of hydroxyethyl starch molecule on endothelial cells leading to less release of adhesion molecules, as shown by Collis *et al.*<sup>12</sup>

Because MMP-9 plays a pivotal role in inflammatory disease, the aim of current study was to verify the *in vitro* effect of colloids (hydroxyethyl starch 130/0.4 and 3.4% poligeline) and crystalloids (LR) on the MMP-9 activity. Moreover, we investigated the effect of intravascular volume replacement with colloid volume therapy *versus* colloid-free volume therapy on MMP-9 and TIMP expression in patients undergoing elective large bowel surgery. We decided to use hydroxyethyl starch 130/0.4, which is rapidly degradable, because of its low risk of hemostatic derangements when compared with hydroxyethyl starches that are slowly degradable.<sup>13</sup>

## Materials and Methods

### In Vitro Study

We tested the hypothesis of a direct inhibition of MMP-9 by the fluids used as volume replacement therapy *i.e.*, LR, 3.4% poligeline, and hydroxyethyl starch 130/0.4. MMP-9 activity was assayed by using the Biotrak activity assay system (Biotrak; Amersham Biosciences, Buckinghamshire, United Kingdom; cod. RPN2634). Recombinant pro-MMP-9 was adsorbed on antibody-coated wells and activated by p-aminophenylmercuric acetate (APMA). After activation, the APMA solution was removed, and 50  $\mu$ l of each fluid containing 10% vol/vol of 10 $\times$  assay buffer (Tris 0.5 M, pH 7.6, 15 mM NaCl, 5 mM CaCl<sub>2</sub>, 10  $\mu$ M ZnCl<sub>2</sub>, 0.1% BRIJ 35) and 50  $\mu$ l of detection reagent (the reporter enzyme, a modified prourokinase and the chromogenic peptide substrate of urokinase) were added to each well. The plate was read after 0, 2, and 5 h of incubation at 37°C. Control experiments were performed to verify the effects of fluids for volume replacement on APMA activation and on urokinase activity. To verify the effect on APMA activation, the experiments were performed on a serum sample already containing active MMP-9, and the inhibitions by fluids, observed with and without APMA activation, were compared. To verify the effect of fluids on urokinase, APMA-activated MMP-9 was first incubated 5 h with prourokinase, and then the solution containing activated urokinase was transferred to an empty well and the chromogenic substrate dissolved in fluids was added.

### In Vivo Study

**Patients and Grouping.** After approval by the ethics committee of the S. Anna Hospital of University of Ferrara, Ferrara, Italy, and patients' written informed con-

sent, we prospectively enrolled 36 consecutive eligible patients undergoing major abdominal surgery for colon cancer. Eligible patients were aged 18 yr or older. Exclusion criteria were cardiac insufficiency (New York Heart Association class III or IV), kidney dysfunction (serum creatinine > 1.4 mg/dl), altered liver function (alanine aminotransferase > 40 U/l), preoperative anemia (hemoglobin < 10 g/dl), preoperative coagulation abnormalities, and chronic use of corticosteroids or non-steroid antiinflammatory substances.

The enrolled patients were prospectively randomized by a computerized random number generator to receive LR (n = 12), 3.4% poligeline (mean molecular weight: 30 kd; Emagel<sup>®</sup>; Pierrel Medical Care [Tito Scalo, Italy]; 3.4% poligeline; n = 12), or a third-generation low-molecular hydroxyethyl starch (mean molecular weight: 130 kd) with a low degree of substitution (DS: 0.4; 6% hydroxyethyl starch 130/0.4; Voluven<sup>®</sup>; Fresenius Kabi, Bad Homburg, Germany; n = 12). Volume administration was started after induction of anesthesia and continued until the end of anesthesia. To compensate fluid loss by sweating, gastric tube and urine output fluids were administered at a dose of  $10 \pm 2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . The LR group was treated only with LR, whereas the latter was administered at the dose of  $7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  in the 3.4% poligeline and hydroxyethyl starch 130/0.4 groups. Hence, colloids were given at the dose of  $3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . In the case of suspected hypovolemia, defined by a central venous pressure less than 4 mmHg, patients were treated with additional volume of the same quality and percentage as previously described. Within the entire study period, packed erythrocytes were given when hemoglobin was less than 9 g/dl; fresh frozen plasma was administered when bleeding occurred and the activated partial thromboplastin time was greater than 70 s, fibrinogen was less than 2 g/dl, or antithrombin III was less than 40%.

Induction of anesthesia was performed with propofol (2 mg/kg), fentanyl (3  $\mu$ g/kg), and vecuronium (0.1 mg/kg) for neuromuscular blockade. Anesthesia was maintained with fentanyl (2–3  $\mu$ g  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>), sevoflurane (1.5–2%), and vecuronium, titrated according to the patients' needs. Postoperative analgesia was obtained by naropine (250–300 mg/day) administration through epidural lumbar catheter positioned before surgery. Non-steroid antiinflammatory drugs were not administered throughout the investigation period.

Mechanical ventilation was performed in all patients (40% air in oxygen) to keep the arterial oxygen saturation greater than 95% and end-expiratory carbon dioxide between 35 and 40 mmHg. A warming cover blanket system and fluid warmers were used to avoid hypothermia during surgery.

All patients were managed perioperatively by anesthesiologists who were not involved in the study and were masked to the aims of the study.

After surgery, patients were transferred to the surgical ward, where the three groups received a fixed amount of electrolyte solution (2,000 ml) during the first postoperative day, and 2,500 ml of solutions containing glucose, amino acids, and electrolytes were given in the next 2 postoperative days. In case of suspected hypovolemia, as previously defined, the LR group continued to receive LR while polygeline and hydroxyethyl starch were given to the other two groups. The percentage of colloids continued to represent 30% of the total amount.

**Measurements.** Perioperative hemodynamic monitoring included continuous measurement of the electrocardiogram, arterial blood pressure, and central venous pressure. MMP-9 and TIMP-1 were measured from venous blood samples; the MMP-9/TIMP-1 ratio was calculated as an index of equilibrium between the action of MMP-9 and its inhibition. Peripheral blood samples were collected in lithium-heparin 143 IU test tubes and kept in ice until centrifugation at 3,000 rpm for 10 min. Serum samples, also collected under sterile conditions, were stored in aliquots at  $-70^{\circ}\text{C}$  until assay.

Blood gas variables including lactate concentrations were measured from arterial blood samples. Measurements were performed after anesthesia induction but before volume administration (T0), within 1 h (T1), and 72 h after the end of surgery (T2).

**MMP-9 Detection Assay.** Serum concentrations of active and total (active + inactive) MMP-9 were measured by using a commercially available activity assay system kit (Biotrak), according to the manufacturer's instructions. All reagents, antibodies and standards were included in the kits. We applied 100  $\mu\text{l}$ /well of each sample in duplicate into a 96-well microplate precoated with anti-MMP-9 antibody. Serum samples were diluted at 1:50 to detect the active MMP-9 and at 1:100 to detect the total one. On each plate, we dispensed six serial dilutions of standard with a range of 8 to 0.125 ng/ml and dilution buffer in two wells instead of samples as negative control (blank).

After an overnight incubation at  $2^{\circ}$ – $8^{\circ}\text{C}$  and four washing cycles, total MMP-9 content was determined by activation with 50  $\mu\text{l}$  APMA, 1 mM, whereas active MMP-9 was determined by using dilution buffer instead of APMA.

After 5 h of incubation at  $37^{\circ}\text{C}$ , color development was read at 405 nm in a microtiter plate reader spectrophotometer (Microline Reader DV920; Poli Industria Chimica, Milan, Italy). The amount of active MMP-9 in any sample was determined by interpolation from a standard curve. Serum samples with concentrations outside the range of determination were reassayed at greater dilutions to obtain values falling into the standard curve. The detection limit of the assay was 0.125 ng/ml.

Pooled serum samples containing high titers of MMP-9 were used as references to calculate the intraassay and interassay variation, expressed as coefficient of variation.

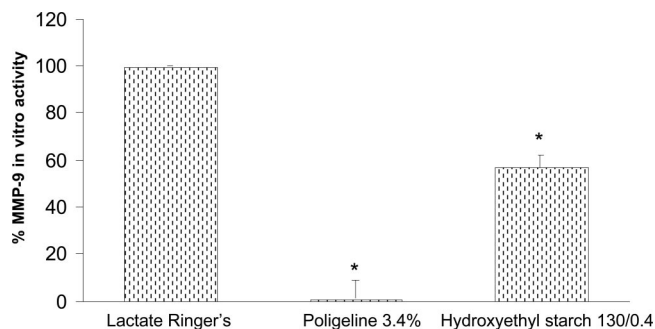
The intraassay variation was calculated for serum from the mean and SD of at least 20 consecutive analysis of control samples performed on the same microplate. The interassay variation was also determined from the mean and SD of at least 10 consecutive analyses performed in separate sessions. Both coefficient of variation values were less than 12% (data not shown).

**TIMP-1 Detection Assay.** Serum levels of TIMP-1 were measured by using a commercially available "sandwich" enzyme-linked immunosorbent assay kit (Biotrak) according to the manufacturer's instructions. All reagents, antibodies, and standards were included in the kits. In brief, 100  $\mu\text{l}$ /well of each sample was deposited, in duplicate, into microwells of microtiter plates precoated with anti-TIMP-1 antibody. Serum samples were diluted at 1:50 in the dilution buffer. On each plate, we added six serial dilutions of standard with a range of 50 to 0.7825 ng/ml and dilution buffer in two wells instead of samples as negative control (blank). After 2 h of incubation at room temperature and four washing cycles, any TIMP-1 present was bound to the wells, other components of the sample were removed by washing, and aspiration and 100  $\mu\text{l}$  of anti-TIMP-1 horseradish peroxidase were dispensed as detection antibody into each well. After 2 h of incubation at room temperature, detection antibody was removed by four further washing cycles, and 100  $\mu\text{l}$ /well of specific chromogen, 3,3',5,5'-tetramethylbenzidine/hydrogen peroxidase substrate, was pipetted into each well. Color development was obtained after 30 min at room temperature and then blocked by adding 100  $\mu\text{l}$ /well of stopping solution (1 M sulfuric acid solution). The absorbance was read at 405 nm with an automated reader (Microline Reader DV920). The amount of TIMP-1 in any sample was determined by interpolation from a reference curve. Serum samples with a concentration of TIMP-1 above the range of determination were reassayed at a higher dilution ratio. The sensitivity limit of the assay was 0.7825 ng/ml, which corresponds to the lowest standard concentration.

Pooled serum samples containing high titers of TIMP-1 were used as references to calculate the intraassay and interassay variation, expressed as coefficient of variation. The intraassay variation was calculated for serum from the mean and SD of at least 10 consecutive analysis of control samples performed on the same microplate. The interassay variation was also determined from the mean and SD of at least 10 consecutive analyses performed in separate sessions. Both coefficient of variation values were less than 10% (data not shown).

#### *Statistical Analysis*

Data are presented as mean  $\pm$  SD. To detect a difference in MMP-9/TIMP-1 ratio of 0.15 with an SD of 0.11, with a type I error of 0.05 and a power of 0.80, 36 patients had to be recruited, 12 in each group. The



**Fig. 1.** *In vitro* inhibition of matrix metalloproteinase 9 (MMP-9) activity. The enzymatic activity of MMP-9 in presence of hydroxyethyl starch 130/0.4 or 3.4% poligeline was compared with the activity observed in lactated Ringer's solution. Data are expressed as the mean of eight sets of experiments  $\pm$  SD. \* $P < 0.05$  compared with lactated Ringer's solution.

approximate degree of normal distribution was calculated for each parameter by the Kolmogorov-Smirnov test. In presence of normally distributed data, as was the case in the current study, differences from baseline and between groups were evaluated by using one-way repeated-measures analysis of variance, and the Tukey test was used for all pairwise multiple comparison procedures. All  $P$  values were two-tailed, and a  $P$  value of less than 0.05 was accepted as statistically significant.

## Results

### In Vitro Study

The presence of hydroxyethyl starch 130/0.4 in the MMP-9 assay system showed a strong inhibition of the enzymatic activity compared with LR (fig. 1), whereas no activity was found in the presence of 3.4% poligeline. However, the latter observation is misleading because poligeline is a perfect substrate for MMP-9 and, therefore, the absence of enzymatic activity was most likely due to a competition between poligeline and prourokinase, the reporter enzyme of the assay system. The hydroxyethyl starch showed a remarkable inhibition of the enzymatic activity, and control experiments showed that the decrease in enzymatic activity was not due to interference in the assay system, but to inhibition of MMP-9 activity.

The *in vitro* determination of enzymatic activity of MMP-9 was based on a coupled assay, where MMP-9 activated prourokinase and the extent of activation was determined by the enzymatic activity of urokinase on a synthetic substrate. Control experiments showed that hydroxyethyl starch 130/0.4 slightly increased urokinase activity on synthetic substrate, so that the small interference with the assay system may have caused an underestimate of MMP-9 inhibition.

### In Vivo Study

Patients did not differ with regard to characteristics and biometric data, type of surgery, and duration of anesthesia and surgery (table 1).

No differences in terms of quantity of fluids administered were detected within either 1 or 72 h after the end of surgery (tables 2 and 3). Only one patient (poligeline group) received 1 unit of packed erythrocytes.

Hemodynamic data, mean arterial blood pressure, central venous pressure, and heart rate did not differ among the three groups (table 2), and none of the patients required infusion of vasoactive agents aimed at increasing arterial blood pressure. Urine output was significantly greater in the hydroxyethyl starch and poligeline groups than in the LR group (table 2) at the end of surgery, but not after 72 h (tables 2 and 3). The results of blood gas analysis showed no changes in arterial lactate concentrations in any group. Although the oxygen arterial partial pressure/inspiratory oxygen fraction ratio ( $P_{aO_2}/F_{iO_2}$ ) was similar in the three groups at baseline, at the end of surgery, the Poligeline group showed an increased  $P_{aO_2}/F_{iO_2}$  ratio compared with baseline, whereas the group treated with hydroxyethyl starch exhibited a significantly higher  $P_{aO_2}/F_{iO_2}$  ratio compared not only with baseline, but also with the LR group and the poligeline group at T1 (table 2 and fig. 2C).

Matrix metalloproteinase 9 plasma levels did not differ in the LR, poligeline, and hydroxyethyl starch groups at baseline, whereas those levels increased significantly in all patients at the end of surgery. At this time, the MMP-9 plasma levels in LR and poligeline groups were significantly higher than in hydroxy-

**Table 1.** Clinical Characteristics and Data from Preoperative Period

	Lactated Ringer's Solution (n = 12)	3.4% Poligeline (n = 12)	Hydroxyethyl Starch 130/0.4 (n = 12)
Age, yr	66 $\pm$ 12	66 $\pm$ 11	65 $\pm$ 11
Men/women	8/3	3/7	5/5
Weight, kg	78 $\pm$ 14	72 $\pm$ 20	73 $\pm$ 27
Height, cm	173 $\pm$ 7	165 $\pm$ 9	169 $\pm$ 13
ASA physical status, II/III	8/3	6/4	5/5
Duration of surgery, min	229 $\pm$ 68	218 $\pm$ 51	221 $\pm$ 67
Duration of anesthesia, min	266 $\pm$ 34	254 $\pm$ 42	249 $\pm$ 51

Results are expressed as mean  $\pm$  SD. No significant difference was detected between groups.

ASA = American Society of Anesthesiologists.

**Table 2. Fluid Input and Output, Hemodynamic and Blood Gas Variables at the End of Surgery**

	Lactated Ringer's Solution (n = 12)	3.4% Poligeline (n = 12)	Hydroxyethyl Starch 130/0.4 (n = 12)
Infused crystalloid solution, ml	3,410 ± 915	2,670 ± 1,079	2,705 ± 1,272
Infused colloidal solution, ml	None	900 ± 358	940 ± 194
Total volume input, ml · kg <sup>-1</sup> · h <sup>-1</sup>	12.6 ± 2.2	12.0 ± 2.1	11.8 ± 3.9
Urine output, ml	198 ± 79	356 ± 145*	388 ± 244*
MAP, mmHg	87 ± 16	81 ± 13	84 ± 12
CVP, mmHg	12.5 ± 2.6	10.0 ± 2.9	13.5 ± 5.2
HR, beats/min	65 ± 14	68 ± 10	68 ± 13
Arterial lactate, mm	1.2 ± 0.45	1.22 ± 0.55	1.21 ± 0.51
Pao <sub>2</sub> /Fio <sub>2</sub>	274 ± 45	321 ± 37	406 ± 33†

Results are expressed as mean ± SD.

\*  $P < 0.05$  relative to lactated Ringer's solution. †  $P < 0.05$  relative to other groups.

CVP = central venous pressure; HR = heart rate; MAP = mean arterial blood pressure; Pao<sub>2</sub>/Fio<sub>2</sub> = oxygen arterial partial pressure/inspiratory oxygen fraction ratio.

ethyl starch group (fig. 2A). This result was confirmed 72 h after surgery (fig. 2A).

As for MMP-9, the plasma concentration of TIMP-1 did not differ among the three groups at baseline. However, at the end of surgery, the MMP-9/TIMP-1 ratio was significantly increased only in the LR and poligeline groups. Furthermore, the MMP-9/TIMP-1 ratio was significantly lower in the hydroxyethyl starch group both at the end and 72 h after the end of surgery (fig. 2B).

## Discussion

Our study shows that different volume replacement strategies can influence the serum levels of MMP-9 and its inhibitor TIMP-1 during and after major abdominal surgery. Furthermore, we observed that hydroxyethyl starch 130/0.4 was able to selectively inhibit the activity of MMP-9 *in vitro*, so that the effective activity of MMP-9 could be modulated by the volume replacement strategy in two ways, by modifying the levels of circulating enzyme and by acting on enzymatic activity itself.

These results are of clinical interest because an increased activity of matrix metalloproteinases such as MMP-9 has been implicated in numerous disease processes, including tumor growth and metastasis, asthma, idiopathic pulmonary fibrosis, chronic obstructive lung disease, adult respiratory distress syndrome, and cardiovascular disease.<sup>5-7</sup> Moreover, extracellular matrix degradation by MMPs is also involved in anastomotic leakage

after both large bowel surgery<sup>8</sup> and abdominal aneurysm repair suture.<sup>14</sup> Hence, procedures decreasing MMP-9 could play a fundamental role in the perioperative management of patients undergoing major abdominal surgery.

In our study, there were no significant differences among the three groups in terms of surgical procedures, biometric and hemodynamic data, blood loss, and duration of surgery or anesthesia. All patients had a high basal level of MMP-9, because tumor invasion is partially due to the MMP-9 activity,<sup>15,16</sup> and the increase in MMP-9 during surgery was likely due to surgery itself.<sup>17</sup> Therefore, we tested the hypothesis that different volume replacement therapies could exert some influences on MMP-9 and its inhibitor. In this connection, it should be noted that Boldt *et al.*<sup>11</sup> have reported that different volume replacement strategies have different influences on inflammation and endothelial activation, hydroxyethyl starch being able to significantly reduce plasma concentration of interleukins and circulating adhesion molecules when compared with LR or saline solution. The authors proposed several explanations for such a finding: a different action on leukocyte function, a direct beneficial effect on endothelial cells with less release of adhesion molecules, improved tissue oxygenation, or differences in coagulation process.<sup>11</sup> Lang *et al.*<sup>10</sup> found similar results and made the hypothesis that this was just due to the improved microcirculation obtained with colloidal solutions because of concomitant reduction in

**Table 3. Fluid Input/Output and Hemodynamic Variables 72 h after the End of Surgery**

	Lactated Ringer's Solution (n = 12)	3.4% Poligeline (n = 12)	Hydroxyethyl Starch 130/0.4 (n = 12)
Infused colloidal solution, ml	None	900 ± 358	940 ± 194
Total volume input, l	11.1 ± 6.4	10.7 ± 6.3	10.5 ± 6.4
Urine output, l	4.7 ± 1.6	5.1 ± 2.0	5.1 ± 1.5
MAP, mmHg	96 ± 11	97 ± 7	97 ± 9
CVP, mmHg	5.1 ± 1.5	5.5 ± 1.6	6.2 ± 1.9

Results are mean ± SD. No significant difference was detected between groups.

CVP = central venous pressure; MAP = mean arterial blood pressure.

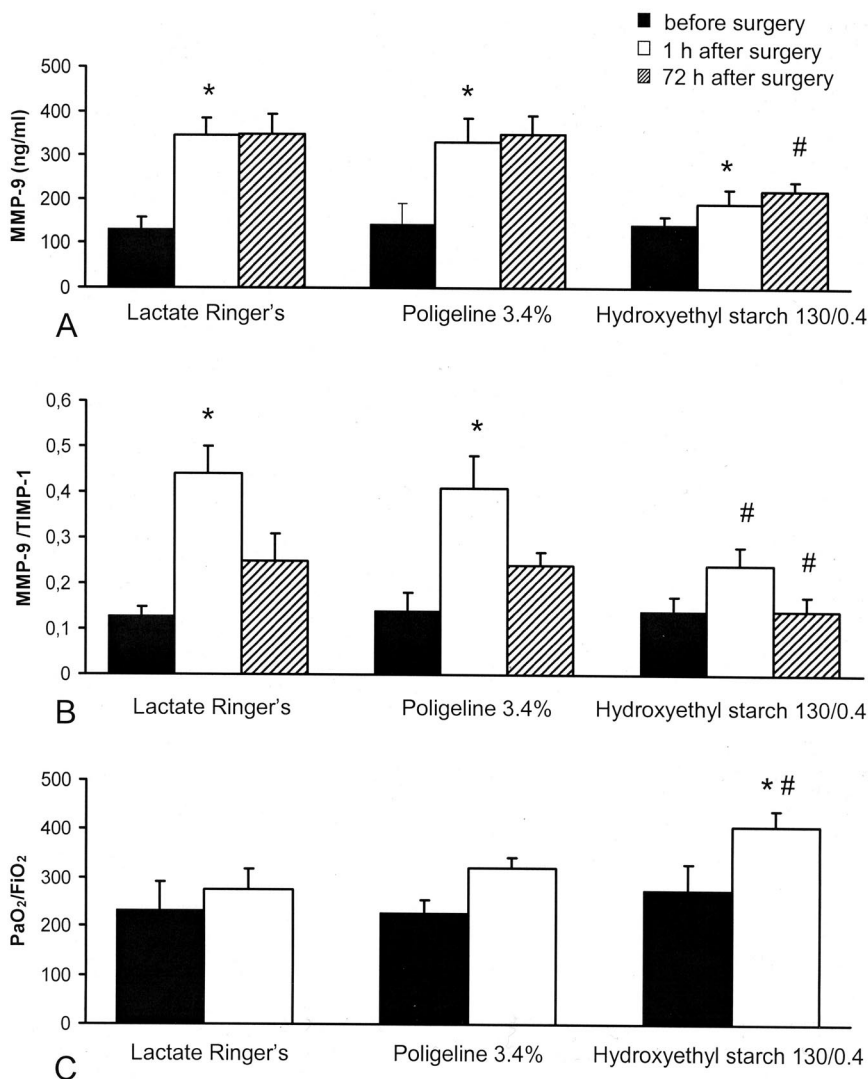


Fig. 2. Plasma levels of matrix metalloproteinase 9 (MMP-9) (A) and MMP-9/tissue inhibitor of metalloproteinases (TIMP-1) ratio (B) at different times in patients receiving lactated Ringer's solution, 3.4% poligeline, or hydroxyethyl starch 130/0.4 during major abdominal surgery. (C) Changes in oxygen arterial partial pressure/inspiratory oxygen fraction ratio ( $P_{aO_2}/F_{iO_2}$ ) in patients receiving lactated Ringer's solution, 3.4% poligeline, or hydroxyethyl starch 130/0.4 as volume replacement therapy during major abdominal surgery. Data are expressed as mean  $\pm$  SE (A and B) and mean  $\pm$  SD (C). \*  $P < 0.05$  relative to baseline. #  $P < 0.05$  relative to other groups.

endothelial activation. A recent article by Nohé *et al.*<sup>18</sup> underlines that almost all synthetic colloids inhibit neutrophil adhesion by inhibition of integrin function (a neutrophil-dependent mechanism) rather than interfering with endothelial cell activation. Although inhibition of adhesion molecules has been advocated to reduce organ dysfunction during shock and ischemia-reperfusion, on the contrary, inhibition of endothelial selectins or integrins could theoretically result in immunosuppression and worsened outcome during severe infection. However, the action of colloids on the inflammation cascade is variegated, as is demonstrated by our results. We found a significant increase in MMP-9 in each group at the end of surgery compared with baseline, but this growth was significantly lower in the hydroxyethyl starch group (fig. 2A). A higher plasma concentration of MMP-9 should mean an increased activity of collagen degradation in basement membrane, especially if there is no equivalent increase in MMP-9 inhibitor. At the end of surgery, we found a significantly lower MMP-9/TIMP-1 ratio in the hydroxyethyl starch group compared with

LR group (fig. 2B), with no significant differences in TIMP-1 concentration among the three groups.

The fact that the group treated with hydroxyethyl starch 130/0.4 exhibited the highest  $P_{aO_2}/F_{iO_2}$  ratio (fig. 2C) was probably due to a better  $V'/Q'$  ratio. It has previously been shown that colloids should enhance organ perfusion, and local oxygenation should, therefore, be greater in the group treated with colloids. However, the better  $P_{aO_2}/F_{iO_2}$  ratio observed with hydroxyethyl starch was not shared by poligeline. This could be due to different physical properties between the two colloids, although it may be hypothesized that the decrease in MMP-9 activity obtained by hydroxyethyl starch administration was responsible for such a difference.

The current study has several limitations. First, the number of patients studied does not allow certain conclusions on the clinical relevance of MMP-9 inhibition during large bowel surgery. Hence, the effects of hydroxyethyl starch 130/0.4 on outcome differences should be tested in a greater patients' population. Sec-

ond, our results were obtained by similar volume replacement regimens. That is, all patients received the same amount of fluids, the only difference being the quality and not the quantity of fluids. The replacement scheme of our department is based on a restrictive perioperative intravenous fluid regimen, which has been previously demonstrated to reduce complications after elective colorectal surgery.<sup>19</sup> However, the different redistribution pattern of colloids and crystalloids could have caused a relative less intravascular volume in the group treated with LR. This fact might have been responsible for a decrease urinary output, for a certain degree of hypoperfusion in the LR group, and hence for some inflammatory up-regulation. However, there is increasing evidence that the urinary output is probably a flawed monitor of the adequacy of volume administration because the infused fluid is not eliminated as urine, but is located peripherally rather than in the central volume.<sup>20</sup> Furthermore, the amount of fluids administered, approximately  $12 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (table 2), was responsible for a normal arterial lactate level and for a similar value of central venous pressure, mean arterial pressure, and heart rate. Finally, the differences in urinary output that were detected immediately after surgery can be also explained by the osmotic enhanced diuresis due to colloid renal elimination.<sup>21</sup> In this connection, it should be pointed out that the urinary output was equal 72 h after the end of surgery, but still, the plasmatic levels of MMP-9 were different among the three groups. Third, the MMP-9 inhibition by hydroxyethyl starch 130/0.4 seems to be solution specific because this result was not shared by 3.4% poligeline (figs. 2A and B). Hence, further studies are required to test different colloids solution such as saline, hypertonic saline, or hydroxyethyl starch of different molecular weights.

In conclusion, our study demonstrates that hydroxyethyl starch 130/0.4 is able to inhibit the MMP-9 expression during and after major abdominal surgery.

## References

- Schlang G, Redl H: Mediators of injury and inflammation. *World J Surg* 1996; 20:406-10
- Szmitko PE, Wang CH, Weisel RD, Jeffries GA, Anderson TJ, Verma S:

- Biomarkers of vascular disease linking inflammation to endothelial activation: Part II. *Circulation* 2003; 108:2041-8
- Parsons SL, Watson SA, Brown PD, Collins HM, Steele RJ: Matrix metalloproteinases. *Br J Surg* 1997; 84:160-6
- Ahrens D, Koch AE, Pope RM, Stein-Picarella M, Niedbala MJ: Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. *Arthritis Rheum* 1996; 39:1576-87
- Bosse M, Chakir J, Rouabhia M, Boulet LP, Audette M, Laviolette M: Serum matrix metalloproteinase-9: Tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am J Respir Crit Care Med* 1999; 159:596-602
- Yassen KA, Galley HF, Webster NR: Matrix metalloproteinase-9 concentrations in critically ill patients. *Anaesthesia* 2001; 56:729-32
- Torii K, Iida K, Miyazaki Y, Saga S, Kondoh Y, Taniguchi H, Taki F, Takagi K, Matsuyama M, Suzuki R: Higher concentrations of metalloproteinases in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1997; 155:43-6
- Stumpf M, Klinge U, Wilms A, Zabrocki R, Rosch R, Junge K, Kroner C, Schumpelick V: Changes of the extracellular matrix as a risk factor for anastomotic leakage after large bowel surgery. *Surgery* 2005; 137:229-34
- Wilson MA, Chou MC, Spain DA, Downard PJ, Quian Q, Cheadle WG, Garrison RN: Fluid resuscitation attenuates early cytokine mRNA expression after peritonitis. *J Trauma* 1996; 41:622-7
- Lang K, Suttner S, Boldt J, Kumle B, Nagel D: Volume replacement with HES 130/0.4 may reduce the inflammatory response in patients undergoing major abdominal surgery. *Can J Anesth* 2003; 50:1009-16
- Boldt J, Ducke M, Kumle B, Papsdorf M, Zurmejer EL: Influence of different volume replacement strategies on inflammation and endothelia activation in the elderly undergoing major abdominal surgery. *Intensive Care Med* 2004; 30:416-42
- Collis RE, Collins PW, Gutteridge CN, Faul A, Newland AC, Williams DM, Webb AR: The effect of hydroxyethyl starch and other plasma volume substitutes on endothelia cell activation, an *in vitro* study. *Intensive Care Med* 1994; 20:37-41
- Kozek-Langenecker SA: Effects of hydroxyethyl starch solutions on hemostasis. *ANESTHESIOLOGY* 2005; 103:654-60
- Lorelli DR, Jean-Claude JM, Fox CJ, Clyne J, Cambria RA, Scabrook GR, Towne JB: Response of plasma matrix metalloproteinase-9 to conventional abdominal aortic aneurysm repair or endovascular exclusion: Implications for endoleak. *J Vasc Surg* 2002; 35:916-22
- De Hingh IH, Waas ET, Lomme RM, Wobbes T, Hendriks T: Circulating matrix metalloproteinase-9 is transiently elevated after colorectal surgery. *Int J Colorectal Dis* 2004; 19:446-50
- Baker EA, Leaper DJ: The plasminogen activator and matrix metalloproteinase systems in colorectal cancer: Relationship to tumor pathology. *Eur J Cancer* 2003; 86:981-8
- Heslin MJ, Yan J, Johnson MR, Weiss H, Diasio RB, Urist MM: Role of matrix metalloproteinases in colorectal carcinogenesis. *Ann Surg* 2001; 233:786-92
- Nohé B, Johannes T, Reutershan J, Rothmund A, Haeblerle HA, Ploppa A, Schroeder TH, Dieterich HJ: Synthetic colloids attenuate leukocyte-endothelial interactions by inhibition of integrin function. *ANESTHESIOLOGY* 2005; 103:759-67
- Brandstrup B, Tonnesen H, Beier-Holgersen R, Hjortso E, Ording H, Lindorff-Larsen K, Rasmussen MS, Lanng C, Wallin L, Iversen LH, Gramkow CS, Okholm M, Blemmer T, Svendsen PE, Rottensten HH, Thage B, Riis J, Jeppesen IS, Teilmann D, Christensen AM, Graungaard B, Pott F, Danish Study Group on Perioperative Fluid Therapy: Effects of intravenous fluid restriction on postoperative complications: Comparison of two perioperative fluid regimens: A randomized assessor-blinded multicenter trial. *Ann Surg* 2003; 238:641-8
- Norberg A, Brauer KI, Prough DS, Gabrielsson J, Hahn RG, Uchida T, Traber DL, Svensen CH: Volume turnover kinetics of fluid shifts after hemorrhage, fluid infusion, and the combination of hemorrhage and fluid infusion in sheep. *ANESTHESIOLOGY* 2005; 102:985-94
- Boldt J, Priebe HJ: Intravascular volume replacement therapy with synthetic colloids: Is there an influence on renal function? *Anesth Analg* 2003; 96:376-82