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SYNTHETIC OLIGOPEPTIDES RELATED TO THE β-SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN ATTENUATE INFLAMMATION AND LIVER DAMAGE AFTER (TRAUMA) HEMORRHAGIC SHOCK AND RESUSCITATION

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ABSTRACT—Severe hemorrhagic shock (HS) followed by resuscitation induces a massive inflammatory response, which may culminate into systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and, finally, death. Treatments that effectively prevent this inflammation are limited so far. In a previous study, we demonstrated that synthetic oligopeptides related to the primary structure of human chorionic gonadotropin (HCG) can inhibit the inflammatory response and mortality that follow high-dose LPS-induced inflammation. Considering this powerful anti-inflammatory effect, we investigated whether administration of similar synthetic HCG-related oligopeptides (LQGV, AQGV, LAGV) during HS were able to attenuate the inflammatory response associated with this condition. Hemorrhagic shock was induced in rats for 60 min by blood withdrawal until a MAP of 40 mmHg was reached. Rats received a single injection with one of the hCG-related oligopeptides (LQGV, AQGV or LAGV) or 0.9% NaCl solution as control 30 min after induction of HS. Treatment with LQGV, AQGV, or LAGV prevented systemic release of TNF-α and IL-6 and was associated with reduced TNF-α, IL-6, and E-selectin mRNA transcript levels in the liver. LQGV treatment prevented neutrophil infiltration into the liver and was associated with reduced liver damage. Our data suggest that HCG-related oligopeptides, in particular LQGV, have therapeutic potential by attenuating the life-threatening inflammation and organ damage that is associated with (trauma) HS and resuscitation.

KEYWORDS—Hemorrhagic shock, HCG-related oligopeptides, cytokines, adhesion molecules, organ damage

INTRODUCTION

Severe hemorrhagic shock (HS) is caused by massive blood loss that cannot be compensated for by the body without treatment. The primary treatment of HS is focused on controlling bleeding and restoring intravascular volume to improve tissue perfusion. Many patients with severe HS who are successfully resuscitated develop an inflammatory response, which may culminate into systemic inflammatory response syndrome (SIRS) and, finally, multiple organ dysfunction syndrome (MODS) (1). In addition, approximately 40% of patients with HS develop sepsis as a result of increased gut permeability and development of compensatory anti-inflammatory syndrome (1, 2). Sepsis and MODS are the leading causes of death in critically ill patients in intensive care units all over the world, with approximately 50% mortality (3).

The inflammatory response after HS and resuscitation is characterized by increased expression of adhesion molecules such as E-selectin and intracellular adhesion molecule 1 (ICAM-1) on endothelial cells and hepatocytes (4). Upregulation of these adhesion molecules facilitates tissue infiltration by neutrophils, resulting in cell-mediated organ injury (5). Furthermore, increased levels of cytokines such as TNF-α, IL-1β, IL-6, and IL-10 are found systemically and locally in liver, lungs, and intestine (6–8). These cytokines, mainly produced by immune cells, affect organ integrity directly or indirectly through induction of secondary mediators such as thromboxanes, leukotrienes, and complement (9, 10).

In the last decade, research has focused on reducing systemic and local inflammatory responses with therapeutic agents that neutralize cytokine activity or inhibit inflammatory mediator production. However, in the case of HS, such treatments require initiation before the onset of shock to achieve an effect (11–13). Clearly, this is impossible in most clinical settings. Therefore, therapies that efficiently inhibit the inflammatory response when initiated after hemorrhage-induced shock are more relevant. Studies on such treatments are limited (14) but are highly needed.

During pregnancy, the maternal immune system tolerates the fetus by reducing cell-mediated immune responses while retaining normal humoral immunity. In addition, clinical symptoms of cell-mediated autoimmune diseases regress in many patients during pregnancy (15). Most likely, a specific hormonal environment is responsible for modulating the immune system during pregnancy (15). The hormone human chorionic gonadotropin (HCG) is secreted by placental syncytiotrophoblasts during human pregnancy. Human chorionic gonadotropin preparations exhibit not only endocrine effects but also immunosuppressive activity (16). We found that HCG preparations inhibited the onset of autoimmune type I diabetes in nonobese diabetic mice (17). This antidiabetic/anti-inflammatory activity was not due to the

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heterodimeric HCG nor to its α− or β− subunits but resided in a peptide fraction of 400 to 2,000 d, which likely originates from proteolytic cleavage of loop 2 of the HCG β-subunit (17–19). Subsequently, we successfully demonstrated that synthetic oligopeptides, related to the primary sequence of loop 2 of the HCG β-subunit, inhibit inflammation, disease severity, and mortality in high-dose LPS-induced SIRS (18, 19). Considering this powerful effect of synthetic HCG-related oligopeptides on inflammation, we hypothesized that the administration of such oligopeptides after induction of HS can inhibit the inflammatory response associated with this condition. To this end, we used LQGV, which is part of the primary structure of loop 2 of the β-subunit of hCG, and two alanine replacement variants, namely, AQGV and LAGV.

Using a rat model of (trauma) HS and resuscitation, we demonstrate that LQGV, AQGV, or LAGV, administered after the induction of HS, significantly prevented TNF-α and IL-6 release into the plasma and attenuated the increase in TNF-α, IL-6, and E-selectin mRNA transcript levels in the liver. In addition, LQGV treatment significantly prevented neutrophil accumulation in the liver, which correlated with decreased organ damage as reflected by reduced lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) plasma levels.

MATERIALS AND METHODS

Animals

Adult male specific pathogen-free Wistar rats (Harlan CPB, Zeist, The Netherlands) weighing 350 to 400 g were used. Rats were housed under barrier conditions at 25°C with a 12-h light-dark cycle and were allowed food and water *ad libitum*. The experimental protocol was approved by the Animal Experiments Committee under the Dutch Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the Protection of Experimental Animals" by the Council of Europe (1986; directive 86/609/EC).

HCG-related synthetic oligopeptides

The HCG-related oligopeptides (LQGV, AQGV, and LAGV) were synthesized by Ansynth Service B.V. (Roosendaal, The Netherlands) and dissolved in 0.9% NaCl at a concentration of 5 mg/mL.

Surgical procedures

Rats were deprived of food overnight before the start of the experiment but were allowed water *ad libitum*. Rats were anesthetized using a mixture of N_2O/O_2/isoflurane (Pharmachemie B.V., Haarlem, The Netherlands). Body temperature was continuously maintained at 37.5°C by placing the rats on a thermo-controlled "half-pipe" (UNO, Rotterdam, The Netherlands). Endotracheal intubation was performed, and rats were ventilated at 60 breaths per minute with a mixture of N_2O/O_2/isoflurane. Polyethylene tubes (PE-50; Becton Dickinson, St. Michielsgestel, The Netherlands) were flushed with heparin and placed via the right carotid artery in the aorta and in the right internal jugular vein. A 5-cm midline laparotomy was performed, and a supra pubic catheter was inserted to monitor urine production.

Experimental procedures

After an acclimatization period of 15 min, the rats were randomized into five different groups (eight rats per group): 1) sham, 2) HS, 3) HS with LQGV treatment (HS/LQGV), 4) HS with AQGV treatment (HS/AQGV), and 5) HS with LAGV treatment (HS/LAGV). Hemorrhagic shock was induced by blood withdrawal, reducing the circulating blood volume until a MAP of 40 mmHg was reached. This level of hypotension was maintained for 60 min. Rats received a single intravenous bolus injection of 5 mg/kg body weight of either LQGV, AQGV, LAGV, or 0.9% NaCl solution 30 after the induction of HS. The peptides and dosage were based on previous studies in which we performed dose-escalation experiments (N.A. Khan et al., unpublished data). Sixty minutes after induction of HS, rats were resuscitated by four times their shed blood volume over a period of 30 min to normalize the MAP and monitored for another 120 min, after which they were killed (Fig. 1A). The rats received no heparin before or during the experiment. Sham animals underwent the same surgical procedure as the HS animals but without blood withdrawal and administration of oligopeptide.

Measurements of MAP

During the experiments, MAP was continuously measured using transducers (Becton Dickinson, Breda, The Netherlands) that were connected in line to an electronic recorder (78354-A; Hewlett Packard, Cheshire, UK).

Plasma collection and storage

Arterial blood was obtained 15 min before and 30, 60, 90, 120, 150, and 180 min after onset of hemorrhage (Fig. 1A). After blood withdrawal, leukocyte numbers were determined using a Coulter counter (Beckman Coulter, Mijdrecht, The Netherlands) and corrected for the hematocyte. Approximately, 0.3 mL of blood was placed into mini collect tubes (Greiner; Bio-one, Alphen aan den Rijn, The Netherlands); plasma was obtained by centrifugation (1,500 rpm; 5 min), immediately frozen, and stored at −80°C until assayed.

Tissue collection and storage

Liver, lungs, ileum, and sigmoid were surgically removed at 180 min after HS induction, snap-frozen, and stored at −80°C until assayed.

Evaluation of cytokines in plasma

TNF-α, IL-6, and IL-10 plasma levels were determined by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, UK) according to the manufacturer's instructions.

Evaluation of mRNA levels by real-time quantitative polymerase chain reaction

RNA was isolated using a Qiagen RNeasy kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. *TNFA* (encoding TNF-α), *IL6* (encoding IL-6), *IL10* (encoding IL-10), *SELE* (encoding E-selectin), and *ICAM1* (encoding ICAM-1) gene expression levels were determined by real-time quantitative-polymerase chain reaction using an Applied Biosystems 7700

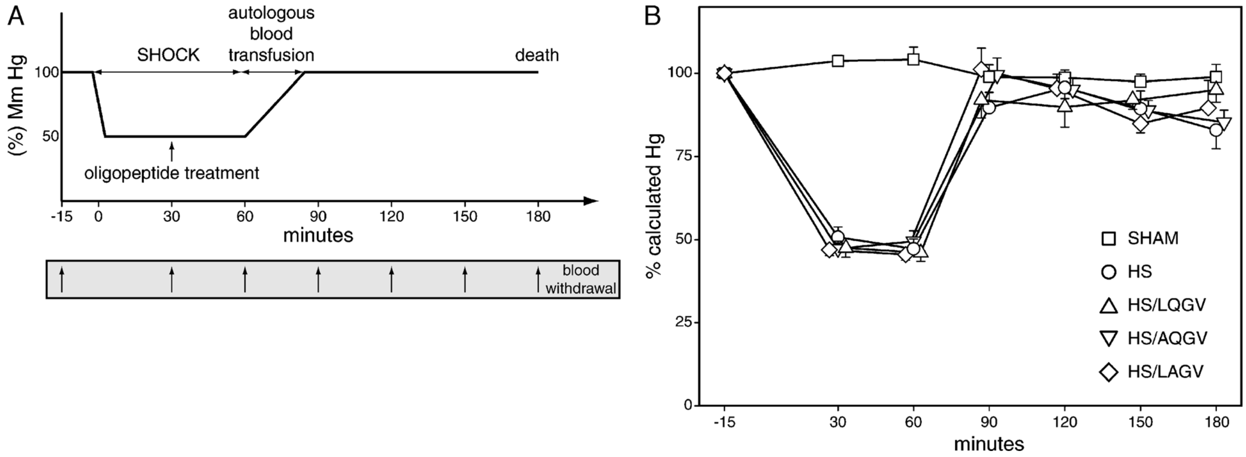

Fig. 1. **A, Schematic representation of the experimental design of induction of HS in rats**. B, The measured blood pressure in millimeters of mercury was recalculated in percentages to standardize the experiment and to compensate for animal differences.

polymerase chain reaction machine (Foster City, Calif). The expression levels of these genes were quantified by normalization against the mRNA levels of the household gene *GAPDH*. Primers and probes used are available upon request.

Immunohistochemical analysis

Cryosections (6 μm) were fixed in acetone/0.05%H_2O_2 for 5 min and subsequently air-dried for 10 min. Neutrophils were visualized by staining for myeloperoxidase (MPO). Hereto, sections were incubated overnight at 4°C with a mouse-antirat MPO monoclonal antibody (Hbt, Uden, The Netherlands). Subsequently, sections were incubated for 60 min at room temperature with a goat–antimouse–horseradish peroxidase–labeled monoclonal antibody (Dako B.V., Glostrup, Denmark). For visualization of horseradish peroxidase activity, 3-amino-9-ethylcarbazole substrate (Sigma Co., St. Quentin Fallavier, France) dissolved in 50 mM sodium acetate/0.02% hydroxyperoxide was used. Sections were embedded in Kaisers glycerol/gelatin (Boom B.V., Meppel, The Netherlands). Numbers of MPO-positive cells were counted per high-power field (HPF) at a magnification of ×200. Per section, 15 HPFs were analyzed. Per organ, three consecutive sections, each separated 18 μm from each other, were analyzed.

Blood biochemical analysis

Plasma alanine aminotransferase (ALT), AST, and LDH were determined at the Erasmus MC diagnostic facility according to standard procedures.

Statistical analysis

Data are presented as the mean values ± SD of the eight rats per group. Statistical analysis was performed using SPSS version 11 software (SPSS, Inc., Chicago, Ill). Intergroup differences were analyzed with Kruskal-Wallis statistical test. If Kruskal-Wallis statistical testing resulted in a $P < 0.05$, a Dunn multiple comparison test was performed, and $P < 0.05$ was considered statistically significant.

RESULTS

Induction of HS

Rats were rapidly bled, within 10 min, to a MAP of 40 mmHg, which was successfully maintained for 60 min in all four experimental groups (Fig. 1B). No change in MAP was observed in sham-treated rats (Fig. 1B). Rats were resuscitated to induce organ reperfusion 60 min after HS, which was associated with a normalization of urine production (data not shown). These data indicate that shock was induced equally in all four experimental groups and was followed by successful organ reperfusion. Heart rates in all four experimental groups increased immediately after induction of HS and returned to normal after resuscitation. Over time, heart rate slowly increased in all four experimental HS groups (data not shown).

Oligopeptide treatment prevents the release of proinflammatory cytokines into plasma

Before induction of HS, TNF-α plasma levels were comparable in all five groups (~15–24 pg/mL; Fig. 2). In the HS group, TNF-α levels started to increase 30 min after induction of HS. These levels were significantly increased after 60 min as compared with the sham group (331 vs. 29 pg/mL; $P < 0.01$). TNF-α levels reached a maximum of 384 pg/mL after 90 min in the HS group, after which levels declined again but continued to remain increased compared with the sham group (Fig. 2). In contrast, none of the oligopeptide-treated HS groups showed an increase in TNF-α plasma levels during the experiment (Fig. 2). In this model of HS, IL-6 levels are known to increase at a later time point than TNF-α (20). Therefore, we determined IL-6 levels in blood samples collected 120, 150, and 180 min after the onset of HS. In the HS group, IL-6 plasma levels were significantly increased as compared with the sham group at 120 (2,003 vs. 331 pg/mL; $P < 0.001$), 150 (2,444 vs. 333 pg/mL; $P < 0.001$) and 180 min (2,940 vs. 343 pg/mL; $P < 0.001$; Fig. 3). Although IL-6 levels tended to increase in the HS-/oligopeptide-treated rats as compared with sham-treated rats, this never reached significance. Treatment with oligopeptides significantly diminished the release of IL-6 into plasma as compared with the nontreated HS group ($P < 0.05$; Fig. 3). IL-10 was undetectable in plasma of all groups throughout the experiment (data not shown). These data demonstrate that treatment with a single dose of either LQGV, AQGV, or LAGV after induction of HS significantly attenuated the increase in TNF-α and IL-6 into plasma.

Oligopeptide treatment is associated with a decrease in TNF-α and IL-6 mRNA transcript levels in the liver

We also analyzed the TNF-α and IL-6 mRNA transcript levels in liver, lungs, ileum, and sigmoid tissues at 180 min after the onset of HS. In the liver, TNF-α transcript levels were significantly increased in the HS group as compared with the sham group ($P < 0.001$). Oligopeptide treatment was

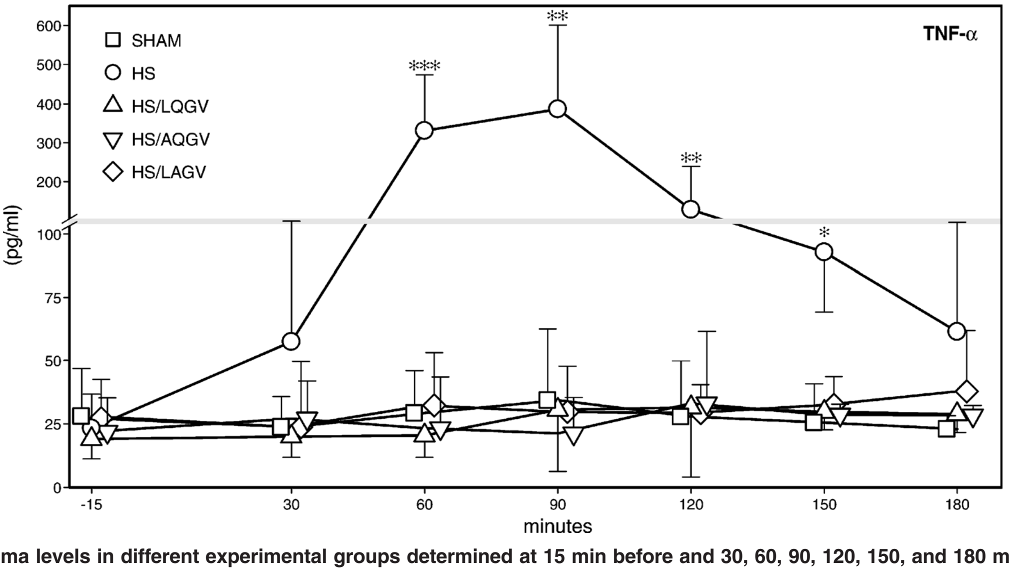

FIG. 2. **TNF-α plasma levels in different experimental groups determined at 15 min before and 30, 60, 90, 120, 150, and 180 min after the onset of HS**. Data are presented as the mean of eight rats per group ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

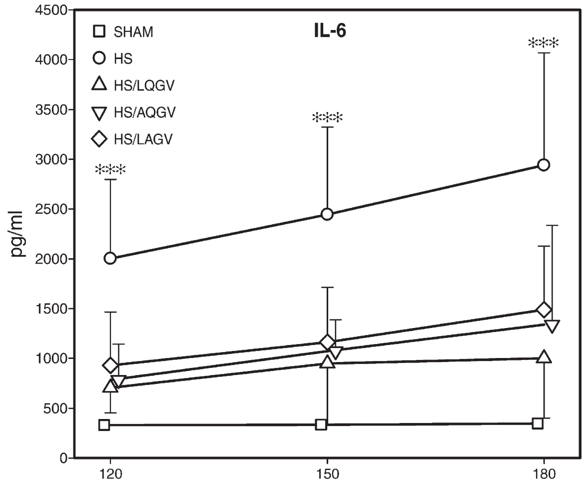

Fig. 3. **IL-6 plasma levels in different experimental groups determined at 120, 150, and 180 min after the onset of HS**. Data are presented as the mean of eight rats per group ± SD. ***$P < 0.001$.

associated with reduced TNF-α transcript levels in the liver as compared with nontreated HS rats, with only HS/LQGV showing a significant reduction ($P < 0.05$; Fig. 4A). In the HS group, IL-6 transcript levels in the liver were increased approximately 83 times as compared with the sham group ($P < 0.001$; Fig. 4B). None of the oligopeptide-treated groups showed a significant increase in IL-6 transcript levels as compared with the sham group. LQGV and AQGV treatments were associated with significantly lower IL-6 transcript levels as compared with the HS group ($P < 0.05$; Fig. 4B). Although IL-10 was undetectable in plasma, IL-10 transcript levels were increased in the livers of the HS group as compared with the sham group, which approached significance ($P = 0.08$). Although not significant, LQGV treatment was associated with decreased IL-10 transcript levels as compared with the nontreated HS group (data not shown). In lungs, ileum, and sigmoid tissues, no differences could be detected between the various groups for TNF-α, IL-6, and IL-10 transcript levels (data not shown). These data imply that oligopeptide treatment after shock induction significantly attenuated the increase in TNF-α and IL-6 transcript levels in the liver.

Oligopeptide treatment is associated with a decrease in E-selectin mRNA transcript levels in the liver

In the HS group, the ICAM-1 transcript level was significantly increased in the liver as compared with the sham group ($P < 0.001$; Fig. 5A). Intracellular adhesion molecule 1 transcript levels in the liver tended to decrease in the oligopeptide-treated groups as compared with the nontreated HS group. The E-selectin transcript level in the liver of the HS group was significantly increased as compared with the sham group ($P < 0.001$). LQGV and AQGV treatments were associated with significantly lower E-selectin transcript levels in the livers as compared with the nontreated HS group ($P < 0.05$; Fig. 5B). These data demonstrate that LQGV and AQGV treatment after shock induction significantly attenuated the increase in E-selectin transcript levels in the liver, whereas ICAM-1 transcript levels were down-regulated to a lesser extent.

LQGV treatment prevents neutrophil accumulation in the liver

In the HS group, the number of neutrophils in the liver was significantly increased as compared with the sham group ($P < 0.05$; Fig. 6A). LQGV treatment significantly ($P < 0.05$) prevented this neutrophil accumulation, whereas AQGV and LAGV treatments did not prevent neutrophil accumulation in the liver ($P < 0.05$; Fig. 6).

LQGV treatment attenuates organ damage

Alanine aminotransferase, AST, and LDH plasma levels were significantly increased in the HS group as compared with the sham group (ALT: $P < 0.01$, Fig. 7A; AST: $P < 0.01$, Fig. 7B; and LDH: $P < 0.01$, Fig. 7C), whereas LQGV treatment significantly ($P < 0.05$) attenuated this rise in AST and LDH. AQGV and LAGV treatments did not affect ALT, AST, and LDH plasma levels as compared with the untreated HS group.

DISCUSSION

In this study, we used a rat model of (trauma) HS to test the therapeutic capacity of three synthetic HCG-related oligopeptides (LQGV, AQGV, or LAGV). We demonstrate that a single

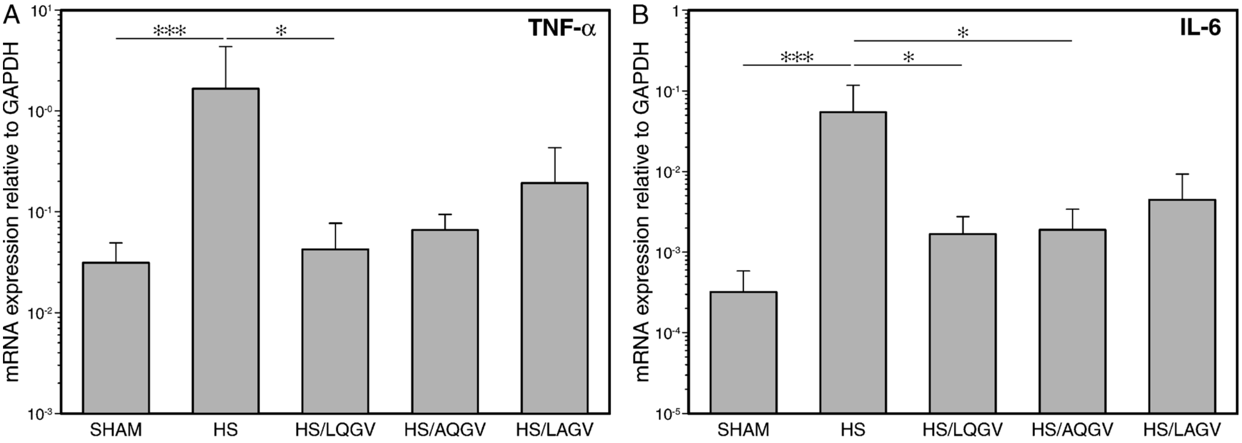

Fig. 4. **Relative expression of TNF-α (A) and IL-6 (B) mRNA transcripts in the liver 180 min after the onset of HS**. Transcript levels are normalized to the expression level of *GAPDH*. Data are presented as the mean of eight rats per group ± SD. *$P < 0.05$, ***$P < 0.001$.

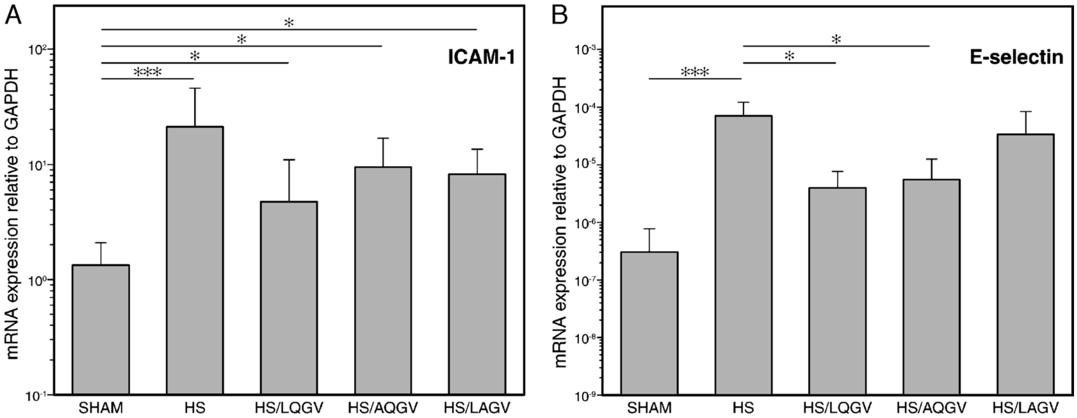

FIG. 5. **Relative expression of E-selectin (A) and ICAM-1 (B) mRNA transcripts in the liver 180 min after the onset of HS**. Transcript levels are normalized to the expression level of *GAPDH*. Data are presented as the mean of eight rats per group ± SD. *$P < 0.05$, ***$P < 0.001$.

administration of LQGV, AQGV, or LAGV 30 min after shock induction markedly prevents TNF-α and IL-6 release into plasma and diminishes the increase in TNF-α, IL-6, and E-selectin mRNA transcript levels in the liver. In addition, LQGV treatment significantly prevented neutrophil accumulation into the liver, which coincided with lower AST and LDH plasma levels.

Hemorrhagic shock followed by resuscitation is characterized by a massive production of proinflammatory cytokines such as TNF-α and IL-6 by immune cells (10). Despite improvement in treatment strategies, (trauma) hemorrhage patients may still develop an inflammatory response that can lead to sepsis, MODS, and, finally, death. In our model of HS and resuscitation, we observed an inflammatory response, as reflected by significantly increased levels of TNF-α and IL-6 in plasma. TNF-α is a key mediator of the innate immune system that is crucial for the generation of a local protective immune response against infectious or noninfectious agents (21). However, uncontrolled TNF-α production is lethal because it induces tissue damage and promotes the production of secondary proinflammatory mediators such as IL-6 (22).

Experimental treatment strategies aimed at neutralizing bioactive cytokines, especially monoclonal antibodies against TNF-α, have been successfully applied in several inflammatory disorders, including Crohn disease and rheumatoid arthritis (23, 24). However, clinical studies using monoclonal antibodies against TNF-α showed no improvement in trauma patients (25). IL-6 is a highly pluripotent cytokine, which facilitates neutrophil infiltration into organs, thereby contributing to cell-mediated organ damage (26). In our model of (trauma) HS and resuscitation, oligopeptide treatment was associated with significantly decreased levels of TNF-α and IL-6 in plasma. Reducing TNF-α and IL-6 plasma levels is of clinical importance because high systemic levels of TNF-α and IL-6 correlate with poor outcome and decreased survival in patients with severe trauma and infection (27). We found local TNF-α and IL-6 production in the liver after HS and resuscitation, which was reduced upon oligopeptide treatment, in particular with LQGV. In lungs, ileum, and sigmoid, we found no effect of HS on the transcript levels of TNF-α and IL-6. Trauma-hemorrhage has been recognized to induce acute lung injury/inflammation in humans and animals (28, 29). In our model, we detected no increase in TNF-α, IL-6, E-selectin, and ICAM-1 transcript levels in the lungs 3 h after HS, suggesting that a pulmonary inflammatory response was not (yet) evident. We have found that our oligopeptides efficiently inhibited SIRS and mortality that were induced upon LPS administration, which is an inflammatory model characterized by involvement of several organ systems, including the lungs (18, 19). Therefore, although we cannot conclude it from the current study, we expect that our oligopeptides do prevent HS-induced pulmonary inflammation.

IL-10 is an anti-inflammatory cytokine that reduces cell-mediated immune responses and proinflammatory cytokine production after HS (8). We were unable to detect IL-10 in plasma during the time frame of the experiments. However, local IL-10 production in the liver was detected because IL-10 transcripts increased in the HS group. LQGV treatment was associated with decreased IL-10 mRNA levels as compared with the nontreated HS group. Although this did not reach

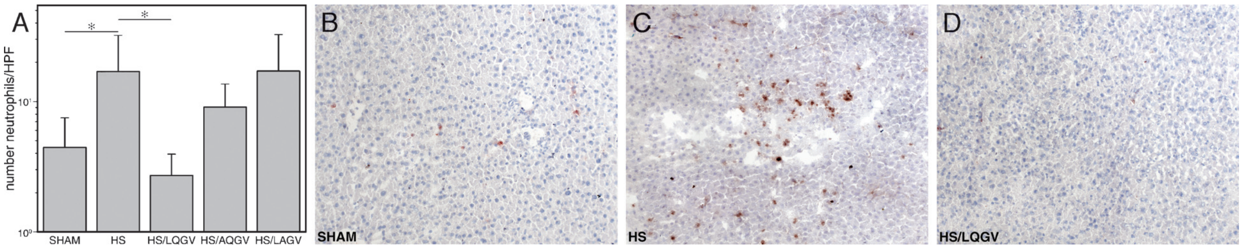

FIG. 6. **A, Number of neutrophils per HPF (magnification ×200) in the liver 180 min after the onset of HS**. Data are presented as the mean of eight rats per group ± SD. Representative examples of livers from sham (B), HS (C), and HS/LQGV (D) 180 min after the onset of HS. *$P < 0.05$.

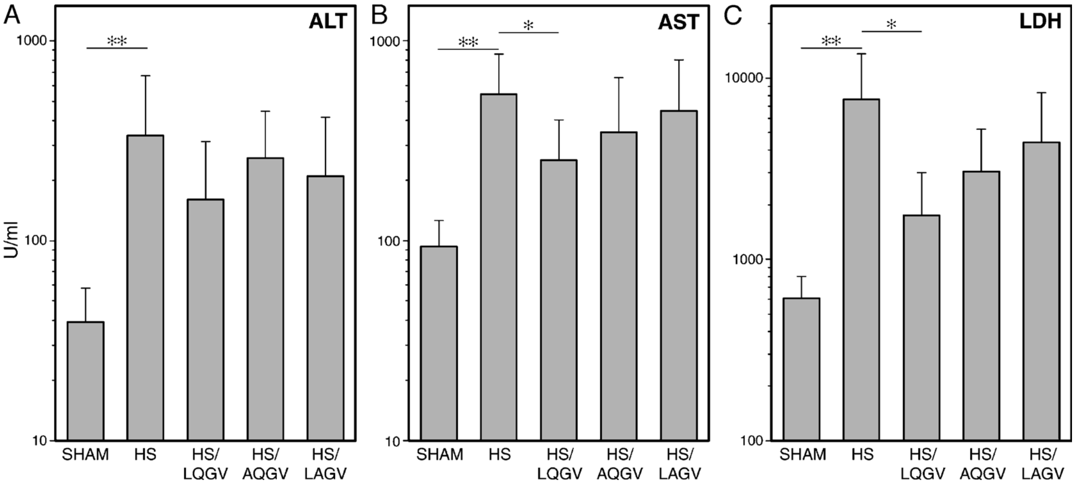

Fig. 7. **Plasma levels of ALT (A), AST (B), and LDH (C) 180 min after the onset of HS**. Data are presented as the mean of eight rats per group ± SD. $^*P < 0.05$, $^{**}P < 0.01$.

statistical significance, we propose that this may be of biological relevance because high IL-10 levels are associated with a high incidence of infection, MODS, and increased mortality in (trauma) hemorrhage patients (30).

Neutrophils induce organ damage and enhance inflammation by the release of oxygen radicals, proteolytic enzymes, and cytokines (31, 32). Neutrophil infiltration into organs is an early event of HS and resuscitation, and neutrophil depletion has been shown to prevent HS-induced inflammation and organ damage (5, 33). These data indicate a central role for neutrophils in the pathophysiology of HS and resuscitation. Leukocyte migration from blood into organs requires the consecutive events of rolling and sticking to activated endothelial cells, followed by diapedesis and chemotaxis (34). Among these processes, selectin-mediated rolling is indispensable for initiation of leukocyte transmigration and inflammation (35). In line with this, L- or E-selectin blockage, using monoclonal antibodies, reduced liver infiltration by neutrophils and inflammation and organ damage after HS (36). In our experiments, treatment with LQGV or AQGV significantly decreased E-selectin transcript levels in the liver. Furthermore, LQGV treatment prevented neutrophil accumulation in the liver and was associated with lower AST and LDH plasma levels after HS and resuscitation. These data suggest that oligopeptide treatment, in particular LQGV, after HS and resuscitation diminishes the expression of adhesion molecules, thereby inhibiting tissue infiltration by neutrophils and subsequent organ damage and systemic inflammation.

In this model, LQGV, originating from the primary sequence of loop 2 of the β-subunit of HCG, was the most effective oligopeptide for every parameter determined. Alanine replacement in this sequence reduced the biological activity. We cannot exclude that the decreased TNF-α and IL-6 mRNA levels (upon oligopeptide treatment) that we observed in the liver are the result of a diminished cellular infiltrate. However, the decrease in E-selectin mRNA, which is only expressed by endothelial cells, indicates that the tested oligopeptides also interfere with mechanisms that regulate expression/activation of genes involved in inflammation and immunity. So far, it is unclear what the underlying mechanism is by which these oligopeptides exert their effects. It is possible that they use yet unidentified receptors. However, we cannot exclude the possibility that, due to their small size and molecular weight, they penetrate the cell membrane (37) and exert their action either by interfering with signaling cascades or the transcriptional machinery. We do not exclude that different oligopeptides have different modes of action. Studies are in progress to reveal how these HCG-related oligopeptides exert their action.

In summary, we demonstrated that administration of a synthetic HCG-related oligopeptide (LQGV, AQGV, or LAGV) after the induction of severe HS significantly attenuated the proinflammatory response both systemically and locally in the liver. Treatment with LQGV prevented neutrophil infiltration into the liver and subsequent liver damage. These data suggest that these oligopeptides, in particular LQGV, have therapeutic potential and may reduce the morbidity and mortality associated with HS and resuscitation.

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