Enteral Administration of a Synthetic Monoacetyldiglyceride Improves Survival in a Murine Model of Abdominal Sepsis

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Background: A monoacetyldiglyceride (MADG) markedly improves survival in a murine model of abdominal sepsis. MADGs have been shown to stimulate hematopoiesis in vitro. We examined effects of MADG administration in setting of cecal ligation and puncture (CLP) and hypothesized that oral (p.o.) administration of MADG would result in alterations of cytokine and chemokine expression after CLP.

Methods: Four groups of 20 mice: sham group underwent celiotomy but not CLP; control group underwent CLP and administration of phosphate buffer solution; simultaneous treatment group had administration of 50 mg/kg MADG p.o. Immediately, before CLP and at 24, 48, and 72-hour post-CLP, posttreatment group had initial administration of MADG at 1-hour post-CLP, and at 24, 48, and 72-hour postoperative. We followed survival to 10-day postoperative. Serum and tissue levels of pro- and anti-inflammatory cytokines were measured. Serum levels of chemokines stromal cell-derived factor (SDF-1) and stem cell factor (SCF) were measured to ascertain if effects of MADG involve stimulation of bone marrow stromal and stem cells. Polymerase chain reaction was used to measure SDF and SCF mRNA expression in liver and lung.

Results: Administration of MADG (p.o.) significantly improved survival in mice after CLP with associated systemic alterations of a variety of cytokines. Increased levels of mRNA coding for SCF and SDF in lung and liver were found after CLP.

Conclusions: Administration of MADG (p.o.) after CLP results in marked improvement in survival. Cytokine level changes demonstrate associated immunomodulatory effects. These effects may be mediated by bone marrow stromal and stem cell activation, evidenced by increases in SDF and SCF. Further study of behavior of bone marrow-derived stem cells in setting of sepsis is warranted. MADG may hold promise for use in treatment of sepsis.

Key Words: monoacetyldiglyceride (MADG), sepsis, cecal ligation and puncture (CLP), bone marrow stem cells, stem cell factor (SCF), stromal cell-derived factor (SDF-1).

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Sepsis is a major cause of morbidity and mortality in hospitalized patients and remains the most common cause of death in non-cardiac critical care units in the United States. Mortality rates have been essentially unchanged since the 1980s.[1] If the pathogenesis of sepsis is defined as the development of organ dysfunction resulting from the body's own inflammatory response to systemic infection (rather than the result of the specific virulence factors of microbes), there are limited therapies that can be specifically directed toward the prevention or treatment of sepsis per se.[2] Currently, the care of the critically ill septic patient is comprised of primary source control if possible, administration of antimicrobial therapy, and support of organ systems until clinical resolution. Despite years of research into therapies directed at the modulation of the host inflammatory response, with the single, notable exception of recombinant human-activated protein C,[3] no clinically efficacious agents have been shown in prospective, randomized trials to reduce sepsis-related mortality.[4] We describe the use of a novel agent, a monoacetyldiglyceride (MADG-3), specifically, 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol, that markedly improves survival in a murine model of abdominal sepsis.

MADGs occur naturally in a variety of seed oils,[5] in bovine udder and milk fat,[6,7] and have been isolated from the antlers of Sika deer (*Cervus nippon* Temminck).[8] Various preparations of deer antler "extracts" have historically been used in traditional Chinese medicine to treat a wide variety of medical conditions and possess a long list of putative clinical benefits. MADGs isolated from deer antler appear to demonstrate measurable biological activity, specifically stimulation of hematopoiesis in vitro.[8] Furthermore, MADG-3 synthesized from glycerol, palmitic acid, and linoleic acid, chemically identical to naturally derived MADG, has been shown to stimulate proliferation of hematopoietic stem cells and bone marrow stromal cells in vitro and in vivo. These effects seemed to be mediated at least in part by the chemokine stromal cell-derived factor (SDF-1).[9] MADG-3 was also shown to stimulate proliferation of immature and mature immune cells, associated with increases in levels of IL-2, IL-4, and SDF-1 (data not published).

Based on these prior demonstrations of biological activity, we sought to examine the effects of MADG-3 administration in a well-described murine model of abdominal sepsis. We hypothesized that enteral administration of MADG-3 would result in alterations in cytokine and chemokine expression in the setting of the polymicrobial challenge of cecal ligation

and puncture (CLP). We measured serum and tissue levels of various cytokines, as well as the chemokines SDF-1 and stem cell factor (SCF), to ascertain if the effects of MADG-3 may be mediated by effects on bone marrow stem cells and stromal cells.

METHODS

Mice

Male C3H HeN mice (8–9 weeks old) were purchased from Orient Animal Farm (Seoul, Korea) and allowed to acclimatize a minimum of 7 days before surgery. The mice were housed in a specific pathogen-free unit (temperature, 22 ± 2°C; humidity 60 ± 4%) with a 12-hour light or dark cycle at the Animal Resource Center of the Asan Institute for Life Science, Asan Medical Center, Seoul, Korea. Water was provided ad libitum. All animals used in this experiment were cared for and used humanely according to the NIH Principles of Laboratory Animal Care (U.S. NIH publication No. 86–23, Revised 1995) guidelines, and approved by the Institutional Animal Care and Use Committee of Asan Institute of Life Sciences, Asan Medical Center, Seoul, Korea.

Cecal Ligation and Punction Model

CLP was performed as previously described.[10] Briefly, mice were anesthetized by intraperitoneal injection with ketamine (70 mg/kg) and rompun (30 mg/kg). A 1-cm longitudinal incision was made in the right lower quadrant of the abdomen. The cecum was ligated and perforated twice with a 22-gauge needle. The cecum was then replaced in its original position within the abdomen, which was closed in two layers. Sham-operated mice were handled in the same manner, except that the cecum was not ligated or punctured. All mice were warmed and administered 1 mL of sterile saline SC after surgery. Survival was followed for 10 days after surgery at which time surviving animals were sacrificed.

In Vivo Experimental Protocols

The mice were divided into four groups of 25 animals: the sham group underwent laparotomy, and the cecum was exposed but not ligated or punctured; the control group underwent CLP, and phosphate buffer solution was administered orally (p.o.) at 1, 24, 48, and 72 hours after CLP; the simultaneous treatment group was administered MADG-3 50 mg/kg (EnzyChem, Seoul, Korea) immediately before CLP and at 24, 48, and 72 hours after CLP; the posttreatment group underwent CLP, and MADG-3 50 mg/kg was administered p.o. at 1, 24, 48, and 72 hours after CLP. Survival was followed to 10 days. Five animals in each group of 25 were randomly selected, excluding obviously moribund animals, and sacrificed at 6, 16, and 24 hours for tissue analysis as described below.

Cytokine Measurements in Serum

Blood samples were obtained from the ophthalmic plexus of each group of surviving mice at 6, 16, 24, and 72 hours after the CLP. Sera were obtained after centrifugation at 3000 rpm for 10 minutes in room temperature and stored at −70°C until assayed. Serum samples were diluted to 1:3 before cytokine (IL-2, IL-4, IL-6, IL-10, IL-12p70, TNF-α, and IFN-γ) measurement. Serum levels of IL-2, IL-4, IL-6, IL-10, IL-12p70, TNF-α, and IFN-γ were measured using BD CBA Flex set assay as described previously.[11]

SCF, SDF-1 Measurements in Serum After CLP

Blood samples were obtained from the ophthalmic plexus of each group of mice at 6, 16, 24, and 72 hours after the CLP. Sera were obtained after centrifugation at 3000 rpm for 10 minutes in room temperature and stored at −70°C until assayed. SCF, SDF-1a levels in serum were quantified using ELISA kit (R&D system, Minneapolis, MN) according to the manufacturer's instructions.

SCF, SDF-1 Real-Time Quantitative PCR Analysis in Lung and Liver Tissues

Mouse lung and liver tissues were snap-frozen in liquid nitrogen and stored at −80°C until assayed. Real-time quantitative polymerase chain reaction (PCR) was performed on RNA derived from lung and liver tissues treated with MADG-3 and untreated group tissues after CLP. The RT-PCR analysis was performed on an Applied Biosystems Prism 7900 Sequence Detection System (Applied Biosystems, Carlsbad, CA) according to manufacturer's instructions.

Statistical Analysis

Survival curves were analyzed by the log-rank test. The serum cytokine concentrations data were analyzed by SAS version 9.1 using mixed between-within group analysis of variance for repeated measures. The data on SCF, SDF-1 real-time quantitative PCR analysis were compared with the Mann-Whitney U test for two independent groups. $p \leq 0.05$ was considered statistically significant. All data are expressed as mean ± SEM.

RESULTS

Administration of MADG-3 Improves Survival After CLP

As shown in Figure 1, enteral administration of MADG-3 (50 mg/kg) significantly improved survival rate after CLP. Ten-day survival did not differ when MADG-3 was administered immediately before (85%) or 1 hour after CLP (90%). In both groups, survival rate was significantly better than in the control animals (20%) ($p < 0.001$).

Effect of MADG-3 on Serum Cytokine Levels After CLP

Table 1 demonstrates the effects of administration of MADG-3 on serum levels of the "pro" inflammatory cytokines TNF-alpha, IFN-gamma, and IL-6. The time changes of TNF-alpha, INF-gamma, and IL-6 levels were not different among the three groups. This result was not unexpected, because the dead mice in the control group did not provide sera for analysis. While all 5 mice in the MADG-3 treated group survived beyond 72 hours after CLP, the control group had 3 mice at 24 hours and only 2 mice at 72 hours available for sampling. Even in this situation, a few significant differ-

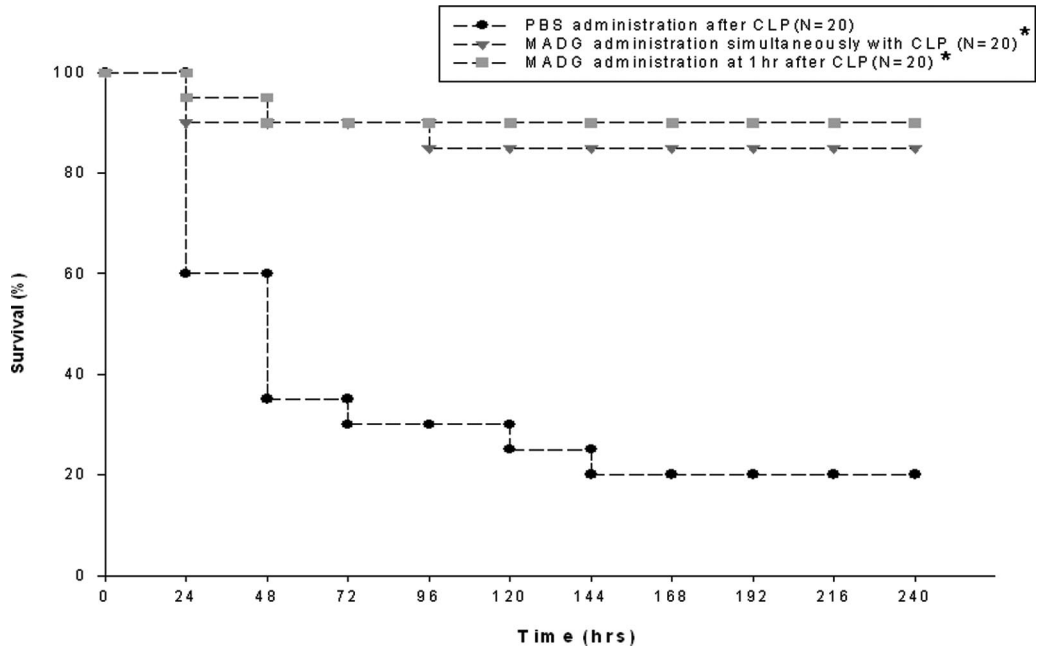

Figure 1. Survival rate in sepsis. *$p < 0.001$.

TABLE 1. Effects of MADG on "Pro" Inflammatory Cytokines in Serum After CLP (pg/ml)

	IL-6	IFN-γ	TNF-α
6 h after CLP			
Sham	67.72 ± 36.37	1.33 ± 0.12	21.46 ± 2.80
Control	10621.27 ± 3434.05	4.46 ± 1.36	59.14 ± 15.81
MADG	15153.07 ± 3570.21	3.54 ± 0.67	125.78 ± 36.33
16 h after CLP			
Sham	18.79 ± 3.28	3.85 ± 1.38	10.68 ± 3.08
Control	3978.61 ± 2848.39	6.34 ± 3.87	97.92 ± 49.45
MADG	564.46 ± 189.36	2.59 ± 0.80*	29.54 ± 9.49*
24 h after CLP			
Sham	9.51 ± 1.13	1.43 ± 0.29	12.48 ± 1.46
Control	4381.89 ± 3342.36	18.23 ± 15.79	231.55 ± 158.75
MADG	238.37 ± 58.72	4.16 ± 1.90*	26.46 ± 11.90*
72 h after CLP			
Sham	4.21 ± 1.23	1.59 ± 0.07	14.49 ± 3.50
Control	118.43 ± 26.47	3.26 ± 0.51	20.34 ± 10.40
MADG	52.53 ± 9.36	1.43 ± 0.18	14.81 ± 2.96

Control: phosphate buffer solution treated at 1, 24, 48, and 72 h. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 h. Data are expressed as mean ± SEM.
* $p < 0.05$ compared with the control group.

TABLE 2. Effects of MADG on Serum IL-2, IL-4, IL-10, and IL-12p70 After CLP (pg/ml)

	IL-2	IL-4	IL-10	IL-12p70
6 h after CLP				
Sham	1.85 ± 0.16	2.20 ± 0.65	31.16 ± 1.50	5.02 ± 2.42
Control	5.46 ± 1.72	5.43 ± 1.17	61.96 ± 15.33	3.80 ± 1.31
MADG	12.37 ± 1.00*	8.50 ± 0.41	111.568 ± 50.40	9.00 ± 2.25
16 h after CLP				
Sham	4 ± 1.03	2.15 ± 0.79	13.39 ± 5.54	5.61 ± 0.59
Control	4.24 ± 1.13	4.19 ± 1.85	56.74 ± 23.64	7.20 ± 0.28
MADG	3.70 ± 0.54	4.28 ± 1.06	33.35 ± 11.72	5.22 ± 1.24
24 h after CLP				
Sham	1.90 ± 0.11	2.20 ± 0.44	17.01 ± 2.05	4.90 ± 0.89
Control	2.64 ± 0.24	1.88 ± 0.30	152.48 ± 84.26	4.59 ± 0.54
MADG	3.39 ± 0.44	3.31 ± 0.55*	32.89 ± 11.94	7.07 ± 1.67*
72 h after CLP				
Sham	1.57 ± 0.20	2.58 ± 1.55	22.78 ± 0.33	6.25 ± 1.48
Control	1.60 ± 0.27	2.52 ± 0.31	20.33 ± 7.95	5.03 ± 1.44
MADG	1.98 ± 0.12*	1.71 ± 0.36	13.41 ± 1.42	5.83 ± 1.33

Control: phosphate buffer solution treated at 1 h, 24 h, 48 h, and 72 h. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 h. Data are expressed as mean ± SEM.
* $p < 0.05$ compared with the control group.

ences appear between the MADG-3 treated group and control. Overall levels of TNF-alpha and IFN-gamma were significantly lower in the MADG-3 treated group compared with control ($p < 0.001$). No such difference was observed with IL-6 ($p = 0.102$).

The effect of MADG-3 administration on serum levels of IL-2, IL-4, IL-10, and IL-12p70 is shown in Table 2. The time changes in IL-2 were significantly different among the three groups ($p < 0.01$), and IL-2 levels were significantly higher in MADG-3 treated mice than control at 6 hours and 72 hours post-CLP ($p < 0.01$). The time changes of IL-4, IL-10, and IL-12p70 were not different among the three groups. Also, overall levels of these cytokines were not significantly different among the groups.

Effect of MADG-3 on Serum SCF and SDF-1 Levels After CLP

As shown in Table 3, MADG-3 treated animals exhibited significantly higher serum levels of SCF at 6 and 24 hours ($p < 0.05$), when compared with controls. However, serum levels of SDF-1 were slightly lower in treated animals, when compared with controls at 16 and 24 hours ($p < 0.05$). Of note, when we compared with sham animals, serum levels of SCF in control were significantly lower at 6 hours ($p < 0.05$).

Effect of MADG-3 on SCF and SDF-1 RNA in the Liver and Lung After CLP

In addition to serum levels, we looked for evidence of changes in expression of SCF and SDF-1 in tissue by RT-PCR analysis on liver and lung specimens. As shown in Table 4 and Figure 2, A and B, administration of MADG-3 was associated with a significant increase in the liver tissue at 6 hours, then significant decrease at 24 and 72 hours of RNA expression for both Cxcl12 (SDF-1) and Kit (SCF), when compared with control. Table 5 and Figure 3, A and B show similar results of real-time PCR on lung tissue. Kit (SCF) RNA expression was significantly increased at 6 and 24 hours, when compared with control and decreased at 72 hours. Cxcl12 (SDF-1) RNA production was increased at 6 hours and decreased at 24 and 72 hours, when compared with control.

DISCUSSION

In this study, we demonstrate that enteral administration of MADG-3 to mice after CLP results in a significant improvement in survival. Differences in survival between treated and control animals are striking and warrant further elucidation of the biological effects of the substance. Measurement of serum levels of a variety of cytokines yields mixed data. Collectively, these alterations of systemic cytokine levels demonstrate an immunomodulatory effect, suggesting a degree of late (>24 hours) systemic immunosuppression in MADG-3 treated animals in response to CLP, when compared with controls. Further elaboration is made difficult for a number of reasons: first, there is the inherent complexity of the host innate immune response to the polymicrobial bacterial challenge of CLP; serum levels of cytokines are notoriously variable between species, and between individuals within a species.[12] Furthermore, because the injury was a highly lethal one in the control animals, later measurement of cytokines were, by necessity, only in the robust animals that managed to survive; the severity of the injury creates an inherent selection bias.[13] We attempted to address these issues, at least in part: in the course of experimental observation, we were able to identify moribund mice after CLP. Although it was outside of preset experimental protocol, we were curious about what their serum cytokines levels were and analyzed serum levels of certain cytokines (IL-6, TNF-α, and INF-γ) retrospectively, immediately postmortem. Dead mice were all from the control group, and when compared with treated mice at the nearest corresponding time, the levels of serum cytokines of moribund control animals were significantly higher (or significantly lower in treated group). These observations along with Table 1, which showed a trend toward increased levels of these cytokines in the control animals (albeit not statistically significant because of large standard errors), led us to speculate that treatment with MADG may have prevented late elevation of these proinflammatory cytokines in CLP mice. This prevention of cytokine storm may have benefited survival rates in the treated mice.

Based on previously documented effects,[14] one possible mechanism by which MADG-3 exerts some of its biological effects in the bone marrow may be mediated by SDF-1. SDF-1 is a CXC hematopoietic chemokine that binds with high affinity to its receptor CXCR4.[15] In the bone marrow, SDF-1 is constitutively secreted by bone marrow stromal cells and stimulates proliferation and specialization of myeloid progenitors. It is also thought to play a role in maintaining immature stem cells within the bone marrow niche.[16] SDF-1 secretion may also be induced in endothelial cells throughout the body, regulating migration of leukocytes from blood to tissue,[17] and lymphocyte trafficking and maturation as well.[18] SDF-1, therefore, may play an important role in the innate immune response to sepsis.

SCF is another hematopoietic cytokine that is constitutively secreted in the bone marrow. SCF also acts as a particularly potent chemokine for mast cells. Mast cells have previously been shown to have a role in the immune response to peritonitis.[19–25] The importance of the role of mast cells in the innate immune response to peritonitis has also been described.[24–28] To further investigate the role that these chemokines may play in sepsis, we measured serum levels of SDF-1, SCF, and corresponding mRNA gene expression (Cxcl12 and c-kit, respectively) in lung and liver.

Measurement of serum levels of SDF-1 and SCF yielded mixed results. MADG-3 treated animals exhibited significantly higher serum levels of SCF at all time points

TABLE 3. Effects of MADG in Serum SCF C-Kit$^+$ and SDF-1α After CLP

	SCF (pg/ml)	SDF-1α (ng/ml)
6 h after CLP		
Sham	97.76 ± 3.32	0.96 ± 0.05
Control	69.93 ± 3.6	1.04 ± 0.15
MADG	89.37 ± 5.03*	1.07 ± 0.13
16 h after CLP		
Sham	61.63 ± 3.27	1.25 ± 0.04
Control	93.80 ± 6.44	3.40 ± 0.10
MADG	109.82 ± 12.86	3.14 ± 0.23*
24 h after CLP		
Sham	74.75 ± 3.36	1.12 ± 0.17
Control	60.57 ± 3.24	6.49 ± 0.23
MADG	93.79 ± 3.75*	6.01 ± 0.21*
72 h after CLP		
Control	22.10 ± 2.94	1.09 ± 0.02
MADG	40.49 ± 4.56	1.38 ± 0.14

Control: phosphate buffer solution treated at 1, 24, 48, and 72 h. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 h. Data are expressed as mean ± SEM.
* $p < 0.05$ compared with the control (phosphate buffer solution) group.

TABLE 4. SCF, SDF-1α Real-Time Quantitative PCR Analysis in the Liver After CLP

	Cxcl12 (SDF-1α)	Kit (SCF C-Kit$^+$)	GAPDH	Cxcl12/GAPDH	Kit/GAPDH
6 h after CLP					
Control	1.0195 ± 0.0555	1.0529 ± 0.0998	1.0011 ± 0.0734	1.0184 ± 0.0554	1.0517 ± 0.0554
MADG	3.4173 ± 0.0358	8.1033 ± 0.5212	2.6243 ± 0.0736	1.3028 ± 0.0137*	3.0878 ± 0.0137*
24 h after CLP					
Control	0.9506 ± 0.0528	0.8547 ± 0.0294	0.9835 ± 0.0395	0.9665 ± 0.0537	0.8691 ± 0.0299
MADG	0.6838 ± 0.0277	0.6234 ± 0.0119	1.0573 ± 0.1043	0.6467 ± 0.0262*	0.5896 ± 0.0112*
72 h after CLP					
Control	0.9611 ± 0.0278	1.0133 ± 0.0463	0.8818 ± 0.0440	1.0899 ± 0.0315	1.1491 ± 0.0525
MADG	0.9457 ± 0.0428	0.6802 ± 0.0267	1.0540 ± 0.0180	0.8972 ± 0.0407*	0.6453 ± 0.0253*

Control: phosphate buffer solution treated at 1, 24, 48, and 72 h. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 h. Data are expressed as mean ± SD of triplicate reactions.
* $p < 0.05$ compared with the control group.

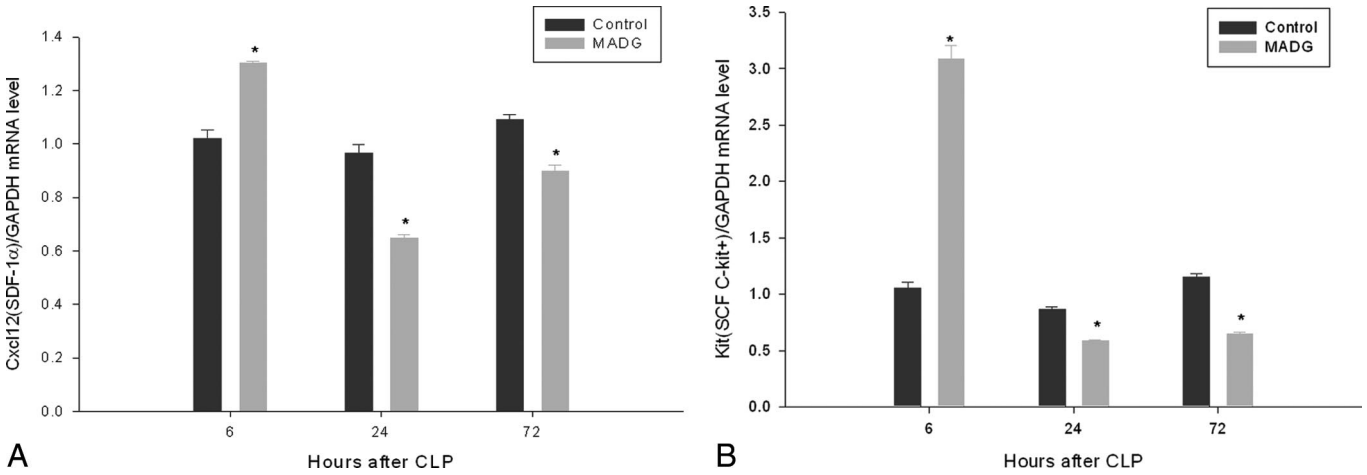

Figure 2. Effects of MADG on SCF, SDF-1α mRNA level in the liver after CLP: (*A*) Cxcl12 (SDF-1α)/GAPDH mRNA level; (*B*) Kit (SCF C-Kit$^+$)/GAPDH mRNA level in lung after CLP. Data are expressed as mean ± SD of triplicate reaction. All values *$p <$ 0.05 compared with control (phosphate buffer solution [PBS]) group. Control: PBS treated at 1, 24, 48, and 72 hours. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 hours.

TABLE 5. SCF, SDF-1α Real-Time Quantitative PCR Analysis in the Lung After CLP

	Cxcl12 (SDF-1α)	Kit (SCF C-Kit$^+$)	GAPDH	Cxcl12/GAPDH	Kit/GAPDH
6 h after CLP					
Control	1.0114 ± 0.0409	0.9573 ± 0.0346	0.9953 ± 0.0950	1.0162 ± 0.0410	0.9619 ± 0.0348
MADG	0.8335 ± 0.0446	0.6248 ± 0.0157	0.3950 ± 0.0106	2.1103 ± 0.1129*	1.5817 ± 0.0396*
24 h after CLP					
Control	0.9521 ± 0.0316	0.9259 ± 0.0426	0.9365 ± 0.0286	1.017 ± 0.0337	0.9888 ± 0.0455
MADG	0.9162 ± 0.0494	1.7351 ± 0.1296	1.5031 ± 0.1088	0.6100 ± 0.0328*	1.1544 ± 0.0863*
72 h after CLP					
Control	0.9597 ± 0.0332	0.8755 ± 0.0120	0.9473 ± 0.0467	1.0131 ± 0.0350	0.9242 ± 0.0127
MADG	0.8554 ± 0.0789	0.6718 ± 0.0360	1.1838 ± 0.0387	0.7226 ± 0.0667*	0.5675 ± 0.0305*

Control: phosphate buffer solution treated at 1, 24, 48, and 72 h. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 h. Data are expressed as mean ± SD of triplicate of reactions.
* $p < 0.05$ compared with the control group.

studied, when compared with controls. However, serum levels of SDF-1 were lower in treated animals, when compared with controls at 24 hours. Of note, when we examined the sham animals, serum levels of SCF were significantly higher in both the sham and MADG-3 animals, when compared with controls at 6 and 24 hours. SDF-1 levels in sham animals were significantly lower than control at 16 and 24 hours.

An interesting and previously unreported finding is the difference in serum levels of SDF-1 and SCF in sham-operated animals compared with animals that had CLP. At 6

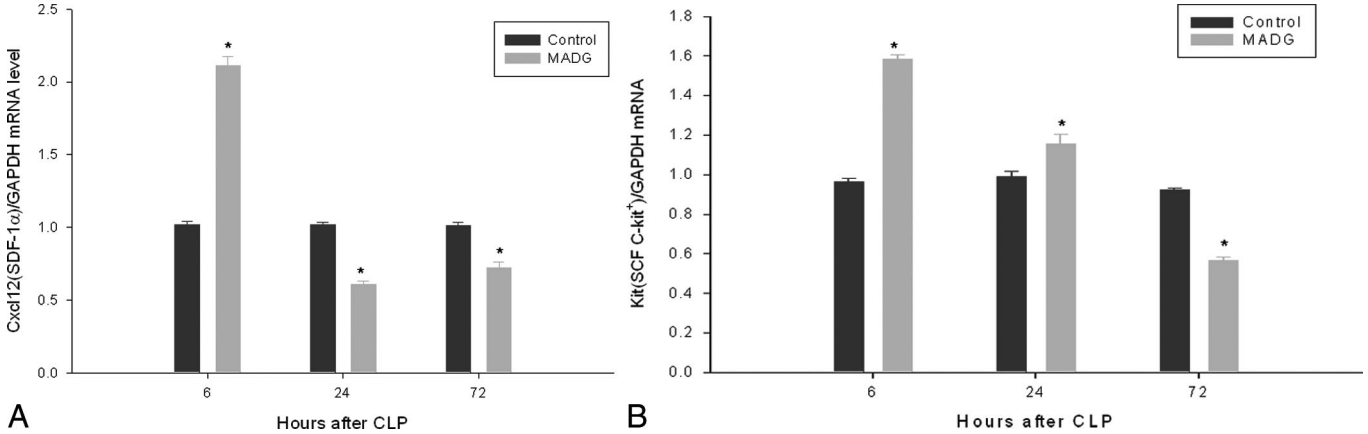

Figure 3. Effects of MADG on SCF, SDF-1α mRNA level in the lung after CLP: (*A*) Cxcl12 (SDF-1α)/GAPDH mRNA level; (*B*) Kit (SCF C-Kit$^+$)/GAPDH mRNA level in lung after CLP. Data are expressed as mean $\pm$ SD of triplicate reaction.*$p < 0.05$ compared with control. Control: phosphate buffer solution (PBS) treated at 1, 24, 48, and 72 hours. MADG: MADG (50 mg/kg) given at 1, 24, 48, and 72 hours.

hours, animals administered MADG-3 have SCF levels close to sham animals, and both groups are significantly higher than control animals who underwent CLP. Also previously unreported are the significantly increased serum levels of SDF-1 after CLP, when compared with sham-operated animals at 16 and 24 hours. As far as we know, this is the first report of the measurement of SDF-1 and SCF in the setting of sepsis in the literature.

From previous studies,[14] when RT-PCR was performed on bone marrow stromal cells after administration of MADG-3, there was a significant increase in mRNA specific for SDF-1 (Cxcl12). Based on these data, we chose to conduct real-time quantitative PCR in lung and liver tissue for Cxcl12 (SDF-1 mRNA) and c-kit (SCF mRNA). In the liver, SCF mRNA was higher than control at 6 hours, but lower than control at 24 and 72 hours. Similarly in the lung, SCF RNA was higher early, and lower later, in animals given MADG-3 compared with controls.

Taken as a whole, these data suggest one plausible mechanism of action for MADG-3 in a CLP model. We postulate that MADG-3 may stimulate SCF-related activation of mast cells, which results in an enhanced early proinflammatory response to bacterial peritonitis. This may in part account for the early improvement in survival that we demonstrate. SDF-1 and SCF may both play complementary roles in the early mobilization and maturation of immature pluripotent cells from the bone marrow to hematopoietic and immune (e.g., mast cells and neutrophils) or other cell lines.[15]

SDF-1 also appears to have a number of biological effects on mature immune effector cells, including but not limited to neutrophils[17] and CD4+ cells.[18,29] Intriguingly, SDF-1/CXCR4 complex has been shown to suppress toll-like receptor 4. Kishore et al.[30] have described a potential mechanism by which the interaction of SDF-1 and its receptor CXCR4 with TLR4 may "raise the threshold for activation of inflammatory cells." This may explain how SDF-1/CXCR4 complex could have a proinflammatory effect in the presence of large local levels of endotoxin, while having an anti-inflammatory effect by suppressing TLR4 in the absence of high systemic levels of endotoxin. Because MADG-3 administration results in increased early (at 6 hours after CLP) expression of SDF-1 mRNA in liver and lung tissues, this hypothetical mechanism may provide another plausible, complementary explanation for the dramatic improvement in survival that we see in our murine CLP model. This may also explain how there can be a late, systemic anti-inflammatory effect if, in the setting of low levels of endotoxin, SDF-1/CXCR4 suppresses TLR4.

Given the early increase in the lung and liver of SCF and SDF-1 mRNA in response to MADG-3, we postulate that the net effect is early mobilization and maturation of immune cells (e.g., mast cells and neutrophils) and stems cells from the bone marrow niche (and possibly circulating or end-organ based stem cells as well). This may result in an early, beneficial enhancement of the innate inflammatory response to the bacterial challenge of CLP. The later anti-inflammatory response that we document may be related either to a successful early host response to infection or, as we note above, to SDF-1/CXCR4 suppression of TLR4. Alternatively, the differences in serum cytokine levels and tissue levels of SCF and SDF-1 mRNA may be the result, not the cause, of the survival benefit that we see with MADG-3 administration.

Limitations of our study methodology include the somewhat arbitrary time points selected for sampling, which may allow for misinterpretation or ignorance of crucial data. Similarly, the somewhat arbitrary selection of the specific cytokines measured may miss potentially important biological effects that may be demonstrated with a more comprehensive sampling of cytokines and chemokines. These limitations may be addressed in future studies.

What is clear from our study is that enteral administration of MADG-3 was associated with a marked improvement in survival in this murine CLP model. On the basis of these results, we believe that MADG may hold promise as an immunomodulatory agent in the treatment of sepsis. Clearly, much work remains to be done to elaborate the mechanism of action,

to describe its effects in other models of infection and inflammation, and, ultimately, to test its potential clinical utility.

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