Sophorolipids Improve Sepsis Survival: Effects of Dosing and Derivatives


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Introduction. Sophorolipids, a family of natural and easily chemo-enzymatically modified microbial glycolipids, are promising modulators of the immune response. We have previously demonstrated that sophorolipids mediate anti-inflammatory effects, including decreasing sepsis-related mortality at 36 h in vivo in a rat model of septic peritonitis and in vitro by decreasing nitric oxide and inflammatory cytokine production. Here we assessed the effect of sophorolipids on sepsis-related mortality when administered as a (1) single bolus versus sequential dosing and (2) natural mixture versus individual derivatives compared with vehicle alone.

Methods. Intra-abdominal sepsis was induced in male, Sprague Dawley rats, 200 to 240 g, via cecal ligation and puncture. Sophorolipids (5–750 mg/kg) or vehicle (ethanol/sucrose/physiological saline) were injected intravenously (i.v.) via tail vein or inferior vena cava at the end of the operation either as a single dose or sequentially (q24 h/3 doses); natural mixture was compared with select sophorolipid derivatives (n = 10–15 per group). Sham-operated animals served as nonsepsis controls. Survival rates were compared at 1 through 6 d post sepsis induction and tissue was analyzed by histopathology. Significance was determined by Kruskal-Wallis analysis with Bonferroni adjustment and Student’s t-test.

Results. Sophorolipid treatment at 5 mg/kg body weight improved survival in rats with cecal ligation and puncture-induced septic shock by 28% at 24 h and 42% at 72 h for single dose, 39% at 24 h and 26% at 72 h for sequential doses, and 23% overall survival for select sophorolipid derivatives when compared with vehicle control (P < 0.05 for sequential dosing). Toxicity was evident and dose-dependent with very high doses of sophorolipid (375–750 mg/kg body weight) with histopathology demonstrating interstitial and intra-alveolar edema with areas of microhemorrhage in pulmonary tissue when compared with vehicle controls (P < 0.05). No mortality was observed in sham operated controls at all doses tested.

Conclusions. Administration of sophorolipids after induction of intra-abdominal sepsis improves survival. The demonstration that sophorolipids can reduce sepsis-related mortality with different dosing regimens and derivatives provides continuing evidence toward a promising new therapy. Toxicity is evident at 75 to 150× the therapeutic dose in septic animals. © 2007 Elsevier Inc. All rights reserved.

Key Words: sepsis; sophorolipids; glycolipids; derivatives; survival; mortality.

INTRODUCTION

Sepsis affects upwards of a half a million patients each year with mortality rates estimated at 35% [1]. Patients with sepsis require aggressive and intensive treatment. Nonetheless, approximately upwards of 90% of patients will succumb to hypotensive shock, and the economic toll of this disease approaches $10 billion annually [1]. Although there is one approved therapy for sepsis (Xigris) and a few more in development, there is a dire need for additional modalities.

We have previously demonstrated that sophorolipids, a novel class of glycolipids, were able to decrease...
sepsis-related mortality in experimental sepsis [2, 3]. Furthermore, recent studies in our laboratory and by our collaborators have demonstrated that sophorolipids possess antibacterial [4], antiviral [5], and anti-inflammatory properties [2, 5–7]. Sophorolipid production yields a natural mixture, which comprises many derivatives. Many of these natural and synthetically derived components have been shown to possess unique anti-inflammatory effects [5]. The mechanisms involved in sepsis-related anti-inflammatory effects include reduction of nitric oxide [2], regulation of inflammatory cytokines [2, 7], and modulation of cell surface adhesion molecules [6]. Other recent studies have demonstrated that sophorolipids are able to down-regulate expression of proinflammatory cytokines including Interleukin (IL)-1α, IL-1β, and IL-6 among others [7]. Sophorolipids represent a unique class of potential therapeutics in that they are easily chemo-enzymatically modifiable. This may well allow the production of organ- or cell-specific therapy by synthetically complexing targeting sequences (antibodies, ligands, etc.) with sophorolipids. However, the ideal dose, route, and derivative(s) for optimal antisepsis effect are poorly understood. Here we investigate the ability of single and sequentially administered sophorolipids, different dosing regimens, and select sophorolipid derivatives to decrease sepsis-related mortality in experimental sepsis.

MATERIALS AND METHODS

Natural Sophorolipids

Sophorolipids were synthesized as previously described by fermentation of C. bombicola [2]. The fermentation media contained glucose (100 g), yeast extract (10 g), urea (1 g), and oleic acid (40 g) per 1000 mL of water. After 7 d of fermentation, sophorolipids were extracted three times with ethyl acetate. The extracts were pooled and the solvent was removed. The obtained product was washed with hexane to remove residual fatty acids. Liquid chromatography/mass spectrometry and nuclear magnetic resonance analyses were carried out to verify the purity of the compounds. No residual fatty acids or media components were found in the sophorolipids.

Sophorolipid Derivatives

Select sophorolipid derivatives, ethyl ester and lactonic (Fig. 1), were studied for their effect in vivo, based on their ability to modulate IL-1 production in vitro [5]. Synthesis of these derivatives is described elsewhere [5]. Briefly, lactonic sophorolipids were purified from natural mixture using flash chromatography. Alcoholysis reaction of natural mixture was carried out using sodium ethoxide to synthesize ethyl ester sophorolipids.

Animals

All animals were housed singly in standard cages and had access to chow and water throughout the experiment. Animals were monitored over 6 d, and the survival rate was compared between the experimental and control groups. The study was approved by the Animal Care and Use Committee at SUNY Downstate Medical Center.

In Vivo Sepsis Induction

Cecal ligation and puncture (CLP) was used as an animal model of experimental sepsis as previously described [2, 8]. Briefly, 60 male adult Sprague Dawley rats (200–240 g; Taconic, Germantown, NY) were anesthetized with an i.p. injection of Nembutal (40 mg/kg; Abbott Laboratories, North Chicago, IL). The abdomen of each animal was shaved and scrubbed with Betadine. A midline laparotomy was performed and the cecum was ligated just below the ileocecal valve with a 3-0 silk ligature. For sepsis induction, the antimesenteric cecal surface was punctured with a 16-gauge needle proximal to the ligature. The abdominal incision was then closed in two layers with 2.0 silk [2, 8].

Sophorolipid Treatment

The CLP-treated animals (n = 15/group) were treated with sophorolipid natural mixture or select derivatives (ethyl ester, lactonic) at 5 to 750 mg/kg rat body weight (BW), in vehicle (ethanol/sucrose) following CLP, either as a single bolus or every 24 h for 3 d (three doses total) by i.v. (tail vein) injection. The two control groups (n = 15/group) consisted of CLP followed by a similar volume of saline or ethanol/sucrose vehicle i.v. (tail vein) at the end of the operation, or CLP with no treatment. Sham operated (laparotomy only) animals were given sophorolipids or vehicle IV (n = 3–4/group) as a nonsepsis control.

Histopathological Analysis

Tissues were removed from animals and placed in 10% buffered formalin. Tissues were processed according to standard procedures [9] and scored by light microscopy by a pathologist who was unaware.
of tissue designations. Lung tissue was assessed for edema, hemorrhage, and exudates as previously described [10]. The changes were scored according to their extent (score 0, 1, 2, and 3 for an extent of 0%, <25%, 25% to 50%, and >50%, respectively) and the severity of the injury (score 0 for no changes, score 1, 2, and 3 for more severe changes). The injury score represents the sum of the extent and the severity of injury.

**Statistical Analysis**

Survival scores were represented as a 4-point ordinal scale: 0 to 24 h, 24 to 48 h, 48 to 72 h, >72 h. An exact Kruskal-Wallis test, using unadjusted P-values with a Bonferroni adjustment for multiple tests, was used for the same test on pairs of the following groups: (1) CLP versus CLP + single bolus sophorolipid treatment [CLP + S(1)], (2) CLP versus CLP +3 d of sophorolipid treatment [CLP + S(3)], (3) CLP + vehicle versus CLP + S(1), (4) CLP + vehicle versus CLP + S(3). Histopathology scores were graded by an independent pathologist who was blinded to animal grouping. Significance was determined using student’s t-test (P < 0.05) (SPSS version 10.0, Chicago, IL).

**RESULTS**

When animals were induced with experimental sepsis, decreased animal survival was observed (31% ± 7% at 24–72 h) (Fig. 2). In contrast, CLP animals that were treated with sophorolipid mixture at 5 mg/kg BW trended toward improved survival by 28% at 24 h and 42% at 72 h for single dose (P > 0.05), and significantly increased survival by 39% at 24 h and 26% at 72 h for sequential doses (CLP + VC versus CLP + S(3): P = 0.011) (Fig. 2). In addition, select sophorolipid derivatives (ethyl ester) demonstrated a 23% increase in overall survival when compared with vehicle control (Fig. 3). Doses of sophorolipid mixtures or their derivatives (lactonic, ethyl ester) at 5 mg/kg BW did not result in any mortality in sham operated animals (data not shown).

To determine effective dosing profiles, animals were injected with very high doses of sophorolipid through the interior vena cava (IVC) after CLP. Interestingly, when doses of 375 to 750 mg/kg BW of sophorolipid natural mixture, representing 75 to 150 times our observed therapeutic dose, were administered, the animals immediately expired whereas the animals injected with vehicle control remained viable. Examination of lung tissue from these animals demonstrated interstitial and intra-alveolar edema with areas of microhemorrhage in pulmonary tissue, which were not seen in vehicle controls (Figs. 4 and 5). In contrast, no mortality was observed when sham operated animals were injected with high dose sophorolipids (data not shown).

**FIG. 2.** Survival rates after single and sequential sophorolipid treatment. Survival rates in IV natural sophorolipid mixture (SL) treated and vehicle control animals (15/group) induced with CLP (Kruskal-Wallis, Bonferroni); experiments ceased at 6 d. Data represent the differences in survival of (A) single (CLP + S1) and (B) sequential CLP + S(3) administration at 24 and 72 h and are reported as percent survival. CLP + S(1): CLP with single SL dose; CLP + S(3): CLP with sequential SL dose (Q24hr for three consecutive days). (Color version of figure is available online.)

**FIG. 3.** Effect of SL derivatives on mortality: animals (10/group) were subjected to CLP and injected i.v. with single dose SL derivates at 5 mg/kg BW and mortality was observed after 72 h. V: vehicle; SL: natural SL mixture; e-SL and L-SL: ethyl ester and lactonic SL derivatives; respectively. Data are reported as percent survival. P < 0.05 for natural mixture (SL), compared with controls.

**DISCUSSION**

Sophorolipids represent a unique class of microbial glycolipids. Our laboratory was the first to demonstrate a clinical utility for sophorolipids. Those studies demonstrated that sophorolipids were able to reduce sepsis-related mortality by 35% in the rat CLP model [2, 3]. The mechanisms involved in these studies included a decrease in nitric oxide production and proinflammatory cytokines [2, 5, 7]. Recent studies in our laboratory also demonstrated the utility of sophorolipids in atopic disease [11]; sophorolipids were able to...
others. In all cases, there were no deleterious related genes, including BSAP (Pax5) and IL-6 among and used mechanisms involving the reduction of IgE-decrease IgE production in a dose-dependent manner consistent with patchy hemorrhage.

FIG. 4. Effect of high doses of sophorolipids. Animals were given SL (375–750 mg/kg BW) through the IVC after CLP. Examination of lung tissue demonstrates central dusky/mottled appearance consistent with patchy hemorrhage.

decrease IgE production in a dose-dependent manner and used mechanisms involving the reduction of IgE-related genes, including BSAP (Pax5) and IL-6 among others [12]. In all cases, there were no deleterious effects to the host or somatic cell lines in vitro and in vivo at therapeutic doses.

In light of these findings, and in an effort to further characterize both sophorolipids natural mixture and select derivatives and their beneficial effect over time, the utility of single versus sequential dosing of sophorolipids was investigated as a treatment for sepsis. Sophorolipid mixture significantly decreased sepsis-related mortality when given sequentially (every 24 h for 3 d) when compared with vehicle controls. These data corroborate our earlier data demonstrating a similar reduction in sepsis-related mortality [2, 3]. Interestingly, and in contrast to our previous studies, single dose administration did not significantly differ from vehicle controls. This could be due to the fact that our earlier studies used dimethyl sulfoxide (DMSO) as a sophorolipid vehicle. It could be that DMSO provides different pharmacodynamics (PD) and pharmacokinetics (PK) than sucrose and ethanol, effects which were overcome with sequential dose sophorolipid administration. Indeed, DMSO has been shown to have innate immunomodulatory effects [13], which may synergize with sophorolipid therapy and increase efficacy after a single dose. However, DMSO can be toxic and would not make a suitable vehicle for human use [14, 15], thereby necessitating the investigation with our current vehicles. Liposome-based sophorolipids and additional vehicles are currently under study to elucidate of an optimal sophorolipid carrier for therapeutic use.

Since the sepsis model used most likely induces bacteremia, it is possible that sophorolipids decrease sepsis by exerting antimicrobial properties. In fact, sophorolipids have been shown to mediate antibacterial effects through mechanisms involving membrane destabilization and increased permeability [16]. Recent studies have demonstrated an antimicrobial effect of sophorolipids that are microbe- and derivative-dependent [4, 6]. It could be that certain derivatives are more effective in eradicating different types of bacteria, providing an opportunity for individualized antimicrobial therapy.

Sophorolipids are unique in that they are able to be chemo-enzymatically modified [2–5], thereby providing a platform for complexing sophorolipids to targeting agents (antibodies, cell surface ligands etc.). In addition, the side chain can be modified to affect hydrophobicity, PK, and PD. Studies by our collaborators have shown that sophorolipid derivatives differ from the parent natural mixture in their ability to modulate inflammatory cytokine production (IL-1 and IL-8) [5], and in their antimicrobial activity [4]. Therefore, the utility of two derivatives, which are present in the natural mixture, as a treatment for sepsis was investigated to determine if there is a single effective therapeutic construct. Interestingly, administration of the ethyl-ester sophorolipid derivative approached a similar reduction of mortality when compared with the natural mixture, whereas the lactonic derivative may paradoxically increase mortality. It is interesting to note that similar reduction of IL-1 and IL-8 with ethyl ester derivative and nonreduction with lactonic derivative were observed in vitro [5]. This could be due to the requirement of a few select derivatives to act synergistically to increase survival whereas each alone may not be effective. Additional investigation with other derivatives may reveal the ideal mixture for increased survival in sepsis.

The optimal dose of sophorolipids in the treatment of disease is unknown. We have previously demonstrated that IV treatment with 5 mg/kg BW effectively decreased sepsis-related mortality in the CLP model of experimental sepsis. To this end, the administration of extremely high doses of sophorolipid (75–150× therapeutic dose) was evaluated in an effort to possibly increase the therapeutic effect. Animals that received these high doses died immediately. The only difference, on autopsy, was found in the lung tissue, which demonstrated interstitial and intra-alveolar edema with areas of microhemorrhage not seen in vehicle controls. This is in contrast to earlier reports, which show sophorolipid toxicity only with much higher (1000×) doses [17–19]. Conceivably, our observed mortality at one tenth the reported lethal level might be due to administration through a different route in conjunction with different vehicles. Although no death was observed with vehicle alone, it is possible that different vehicles with sophorolipids create deleterious complexes that are toxic. Nonetheless, administration of sophorolipids at our earlier reported therapeutic dose (5 mg/kg)
[2, 3] continued to increase survival in experimental sepsis and was not toxic in all vehicles analyzed thus far. Furthermore, high dose sophorolipid treatment had no effect on sham operated controls. This suggests that the septic insult itself, possibly in conjunction with the vehicle, likely affects the response to sophorolipid treatment. Future studies will focus on additional vehicles for optimal sophorolipid delivery.

In conclusion, administration of sophorolipids after induction of intra-abdominal sepsis improves survival in an experimental animal model. The demonstration that sophorolipids exhibit anti-inflammatory effects in other diseases and can continue to reduce sepsis-related mortality with different vehicles, dosing regimens, and derivatives provides continuing evidence toward a promising new therapy. Future studies will further investigate optimal dosing parameters of sophorolipids in an effort to minimize toxicity and maximize therapeutic efficacy in sepsis.

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REFERENCES


