Effects of SBP-101 in a Mouse Model of Cerulein-induced Acute Pancreatitis

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Abstract

Increased polyamine (PA) catabolism has been shown to induce acute pancreatitis (AP) in animals, and this effect can be prevented by blocking hyper-catabolism. We hypothesized that SBP-101 (diethyl-dihydroxyhomospermine), a synthetic analog of spermine that reduces intracellular PA pools, could attenuate AP. This study sought to characterize effects of SBP-101 in AP, including the biologic effects of chiral uniformity. SBP-101 has two chiral centers and can have either an S,S, R,S or R,R configuration. METHODS: AP was induced in mice with 10 hourly 50 µg/kg intraperitoneal injections of cerulein (Cer). SBP-101 was administered in relation to the Cer injections at +3 and +8h (treatment, T) or at -3, 0, 3 and 6h (prevention, P). Blood and tissues were collected 3h after the last Cer dose to evaluate histology, plasma lipase and amylase, and expression levels of IL6 and MCP1. Group 1 received one of 4 SBP-101 dose levels (5, 12.5, 25 or 50 mg/kg) according to the T schedule; Group 2 received 25 mg/kg of either 98% S,S SBP-101 (2A) or 60% S,S (2B). RESULTS: In Group 1, lipase was reduced only at the 5 mg/kg dose level. There were no significant changes in cytokine mRNA levels. Similarly, there was no change in AP severity by histology. In group 2, amylase was significantly reduced from 10,859±1,204 to 7,011±674 in 2A (p=.016) and to 5,607±700 in 2B (p=.001). Lipase reduced from 971±131 to 602±62 (2A, p=.036) and 489±87 (2B, p=.006). Histology showed diminished inflammation, edema, vacuolization, and necrosis. Histology score decreased from 8.16 to 5.26 (p=.039). IL6 and MCP1 mRNA levels were slightly reduced in 2B, but not 2A. CONCLUSION: SBP-101 reduces amylase and lipase levels when administered by the P, but not the T schedule in this model. Histology findings correlated with these changes. Cytokine mRNA levels trended in the same direction. Effects were similar for 2A and 2B.

Background

- Acute Pancreatitis has many causes such as gallstones and ethanol, but no specific treatment.
- Hypercatabolism of PAs is evident in several animal models of AP as well as in human disease, suggesting a common biochemical pathway.
- Increased activity of Spermine/Spermidine Acetyltransferase (SSAT), the rate-limiting PA catabolic enzyme, can induce AP. (Hyvönen, et al *Am J Path* 2006)
- Inhibition of SSAT by alpha-methylspermidine can prevent or ameliorate AP caused by induction of SSAT.
- SBP-101 (diethyl-dihydroxyhomospermine) is a synthetic PA analog of spermine that depletes intracellular PA pools. (Bergeron, et al *J Med Chem* 1996)
- We hypothesized that depleted PA pools would have a similar effect in AP to blocking hypercatabolism.
- SBP-101 has two chiral carbons. In some cases, chirality determines the biologic effect of the molecule (e.g. Lactate). We sought to determine if different chiral formations of SBP-101 had different effects in this model of AP.

Methods

- Acute pancreatitis was induced in C57BL/6 mice with 10 hourly intraperitoneal (IP) cerulein injections (50 µg/kg).
- SBP-101 was administered subcutaneously according to either a therapeutic (T) or a prevention (P) dosing schedule (Figure 1).
- Blood and tissues were collected 3 hours after the last Cer injection (hour 12).
- Plasma amylase and lipase levels were determined.
- mRNA levels for IL-6 and MCP-1 were determined by RT-PCR in pancreas tissue.
- Pancreas histology was evaluated in Hematoxylin and Eosin (H&E) stained tissue sections.
- AP severity was determined by grading inflammation, cell death, vacuolization and edema on a scale of 0-4.

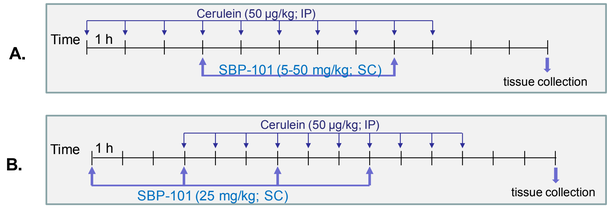

Figure 1. Administration schedules. A. Therapeutic schedule. SBP-101 was administered 3 and 8 hours after the first Cer injection. Dose range was from 5 to 50 mg/kg B. Prevention schedule. SBP-101 (25 mg/kg) was administered in relation to Cer at -3, 0, +3, and +6 hours.

Group 1

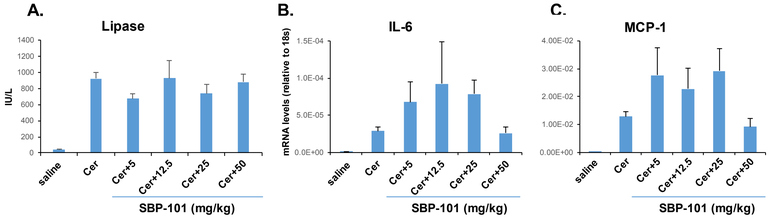

Figure 2. Group 1 Lipase and cytokine levels. SBP-101 (98% S,S) was administered according to the T schedule. A. Plasma lipase levels across all doses at 12 hours. B and C. IL-6 and MCP-1 mRNA levels (respectively) relative to 18s ribosomal RNA levels in pancreas tissue. Changes compared to Cer alone were ns (p>0.05).

Group 2 Serum Enzymes

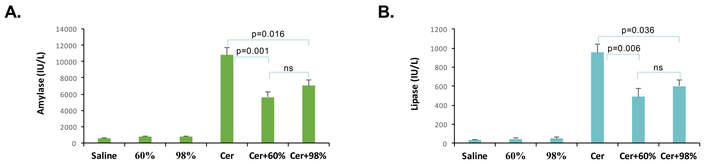

Figure 3. Plasma amylase and lipase levels following P schedule. Amylase (A) and lipase (B) levels were reduced with SBP-101 administration compared to Cer alone. Both formulations significantly reduced serum enzyme levels, and the effect of both formulations was similar.

Group 2 Tissue Cytokine mRNA

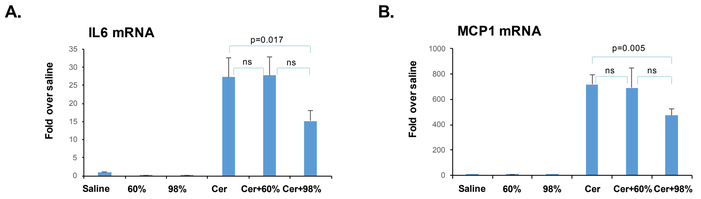

Figure 4. Tissue cytokine mRNA levels following P schedule. Interleukin-6 (A) and MCP1 (B) cytokine mRNA levels were reduced with the 98% S,S formulation, but not the 60% S,S formulation of SBP-101 compared to Cer alone when administered via the P schedule. However, when compared to each other, the effect of both formulations was not significantly different.

Group 2 Histology

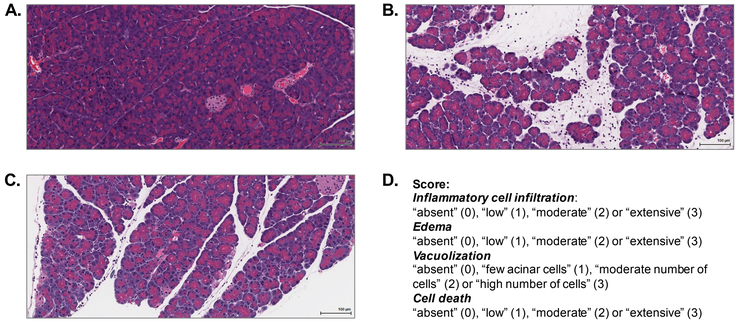

Figure 5. Representative pancreas histology and scoring criteria from Group 2. A. Saline control B. Cerulein control (no SBP-101) C. Cerulein and 25 mg/kg SBP-101 D. Scoring criteria as per Rakonczay et al (*Crit Care Med* 2008) Figure 3C is representative of the effect produced by administration of both formulations.

Group 2 Histology Scores

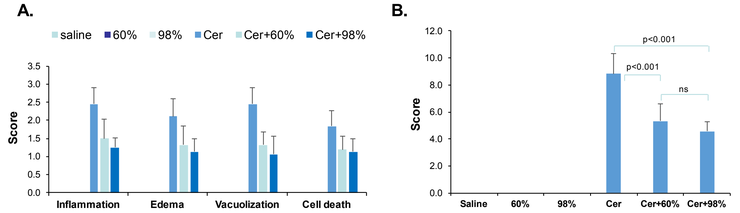

Figure 6. Histology scores for individual variables (A.) and total histology score (B.) in group 2. A. Scores for each of the four histologic variables. B. Total histologic score representing the sum of each of the four histologic criteria. Total histology score was significantly reduced compared to Cer control by both formulations (p<.001). All individual and total scores for control groups with Saline, 60% and 98% SBP-101 were 0 and therefore are not visible on the graphs.

CONCLUSIONS

- When administered according to a prevention, but not a treatment schedule, SBP-101 administration attenuates early manifestations of AP induced by cerulein including:
  - Plasma amylase and lipase.
  - tissue levels of inflammatory cytokine mRNA.
  - Individual and total histology scores.
- Both SBP-101 formulations produced similar effects, though the 60% formulation had less effect on tissue cytokines.
- Further evaluation is needed to evaluate effects at a later time point relative to SBP-101 administration or a model with more severe AP, such as taurocholic acid.