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# Association between remote organ injury and tissue polyamine homeostasis in acute experimental pancreatitis – treatment with a polyamine analogue bismethylspermine

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**Abstract:**
Experimental pancreatitis is associated with activation of polyamine catabolism. The polyamine analog bismethylspermine ($Me_2Spm$) can ameliorate pancreatic injury. We investigated the roles of polyamine catabolism in remote organs during pancreatitis and explored the mechanism of polyamine catabolism by administering $Me_2Spm$. Acute pancreatitis was induced by an infusion of 2 or 6% taurodeoxycholate before $Me_2Spm$ administration. Blood, urine and tissues were sampled at 24 and 72 h to assess multi-organ injury and polyamine catabolism. The effect of $Me_2Spm$ on mortality in experimental pancreatitis was tested separately. Liver putrescine levels were elevated following liver injury. $Me_2Spm$ increased the activity of spermidine/spermine $N^1$-acetyltransferase (SSAT) and depleted the spermidine, spermine or putrescine levels. Lung putrescine levels increased, and SSAT and spermine decreased following lung injury. $Me_2Spm$ enhanced the activity of SSAT and decreased the spermidine and spermine levels. Renal injury was manifested as an increase in creatinine or a decrease in urine output. Decreases in kidney SSAT, spermidine or spermine and an increase in putrescine were found during pancreatitis. In the 2% taurodeoxycholate model, $Me_2Spm$ decreased urine output and raised plasma creatinine levels. $Me_2Spm$ increased SSAT and decreased polyamines. Excessive $Me_2Spm$ accumulated in the kidney, and greater amounts were found in the 6% taurodeoxycholate model in which this mortality was not reduced by $Me_2Spm$. In the 2% taurodeoxycholate model, $Me_2Spm$ dose-dependently induced mortality at 72 h.
Like pancreatic injury, remote organ injury in pancreatitis is associated with increased putrescine levels. However, $Me_2Spm$ could not ameliorate multi-organ injury. $Me_2Spm$ administration was associated with significant renal toxicity and induced mortality, suggesting that the current dose is too high and needs to be modified.

**Key words:**
bismethylspermine, multi-organ injury, pancreatitis, polyamines, putrescine, spermidine, spermine

**Abbreviations:** ALAT – alanine transaminase, ASAT – aspartate transaminase, MeSpd – methylspermidine, $Me_2Spm$ – bismethylspermine, SSAT – spermidine/spermine $N^1$-acetyltransferase

## Introduction

Physiological polyamines, including spermidine, spermine and putrescine, are positively charged aliphatic amines, which act functionally as growth factors, antioxidants, second messengers, nutrients, metabolic regulators and stabilizers of DNA, RNA and membranes [26]. Cytosolic spermidine/spermine $N^1$-acetyltransferase (SSAT) is the rate-limiting enzyme regulating the catabolism of tissue polyamines [28]. Although all mammalian cells contain polyamines, general over-induction of SSAT activity in transgenic rats can induce acute pancreatitis [1, 25]. The reason is that the pancreas is exceptionally rich in polyamines. Interestingly, experimental pancreatitis induced by cerulein, L-arginine or taurodeoxycholate leads to pancreatic SSAT activation in wild-type rats (Fig. 1) [13, 18]. Depletion of spermidine and spermine in the pancreas is associated with the severity of pancreatitis in 2 and 6% taurodeoxycholate-induced models [18]. Polyamine changes disappear by 72 h in the surviving animals.

The inhibition of SSAT has not been studied due to the lack of specific inhibitors [27]. In SSAT knockout mice, the targeted disruption of SSAT did not lead to significant fluctuations in polyamine homeostasis [23]. However, it is the SSAT activation that is associated with pancreatitis (Fig. 1). The consequent decrease in polyamines may be partly compensated for by administering methylated polyamine analogues such as methylspermidine (MeSpd) and bismethylspermine ($Me_2Spm$). These compounds are able to ameliorate edema and histological injury of the pancreas in both transgenic and wild-type models [13, 18, 24]. Earlier reports have demonstrated that both MeSpd and $Me_2Spm$, when administered after the induction of pancreatitis, markedly reversed pancreatic damage in 24 h [13, 24]; this protective effect did not last up to 72 h in the wild-type animal model [17]. The reversal of polyamine homeostasis to normal by this time point explains the late ineffectiveness of the supplement therapy. These findings support the hypothesis that polyamine catabolism contributes to the evolution and development of pancreatitis, especially in the early stages of the disease.

As our earlier studies demonstrated [17, 18], the infusion of taurodeoxycholate into the pancreatic duct resulted in pancreatitis that manifested as edema, necrosis and leukocyte infiltrations, analyzed using histology, and showed that 6% taurodeoxycholate-induced pancreatitis was more severe than 2% taurodeoxycholate-induced pancreatitis [18]. In human pancreatitis, the severe necrotizing form of the disease may lead to multi-organ dysfunction, and most deaths are attributed to it [6, 10, 22]. Each organ has different metabolism and there are no data on the association between multi-organ dysfunction and polyamine changes [30]. In the present study, we explored whether the remote organs (liver, lung and kidney) that are often injured in severe acute pancreatitis develop changes in polyamine homeostasis, and whether the remote organ injury is influenced by treatment with $Me_2Spm$.

## Materials and Methods

### Animals

The present study was approved by the Institutional Animal Care and Use Committee of the relevant provincial government. The experiments were performed in accordance with the "Guidelines for the Care and Use of Laboratory Animals" (NIH publication No. 86-23, revised 1985). Adult male Sprague-Dawley rats (270–500 g) were fed a standard chow diet until 12 h before the experiment. Rats were anesthetized with an intraperitoneal injection of pentobarbital (Orion, Espoo, Finland, 60 mg/kg) before the operation and again for subsequent tissue, blood and urine sampling.

### Use of $Me_2Spm$

$Me_2Spm$ was donated by the Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio. Elemental analysis for $Me_2Spm$: $C_{12}H_{30}N_4$ (376.24 Da).

$Me_2Spm$ was synthesized as described previously [8] and dissolved in 0.9% NaCl solution at 25 mg/ml before use. $Me_2Spm$ was used as follows: the animals

received $Me_2Spm$ as a treatment, intraperitoneally (25 mg/kg) at 4 h and 8 h after the induction of pancreatitis.

### Taurodeoxycholate-induced pancreatitis models

Sodium taurodeoxycholate (Sigma-Aldrich, Buchs, Switzerland) at a concentration of 2 or 6% was infused into the pancreatic duct with 0.2 ml phosphate-buffered saline, pH 7.4 as described previously to induce moderate or severe lethal pancreatitis respectively [11, 18]. The infusion pressure was kept below 30 cm $H_2O$. There was a temporary clamping of the common bile duct at the liver hilum during the pancreatitis induction.

### Group design for pancreatitis models

The animals were divided randomly into 8 groups:

*Sham operation* (Groups 1, 2): Rats underwent a laparotomy only. They were anesthetized and euthanized for sampling at 24 h (Group 1, n = 6) and at 72 h (Group 2, n = 6) after this sham operation.

*Two percent taurodeoxycholate-induced pancreatitis* (Groups 3, 4): Rats were infused with 2% sodium taurodeoxycholate intraductally to induce pancreatitis, as described above. They were anesthetized and euthanized for sampling at 24 h (Group 3, n = 6) and at 72 h (Group 4, n = 6) after the infusion of taurodeoxycholate.

*Two percent taurodeoxycholate-induced pancreatitis, treatment with $Me_2Spm$* (Groups 5, 6): Rats were infused with 2% sodium taurodeoxycholate intraductally, as in Groups 3, 4, to induce pancreatitis. The animals received $Me_2Spm$ as described above. They were anesthetized and euthanized for sampling at 24 h (Group 5, n = 6) and 72 h (Group 6, n = 11) after the infusion of taurodeoxycholate. In Group 6, because of high mortality (5 rats died), we obtained samples from the 6 surviving rats.

*Six percent taurodeoxycholate-induced pancreatitis* (Group 7): Rats (n = 11) were infused with 6% sodium taurodeoxycholate intraductally as described above to induce pancreatitis. They were anesthetized and euthanized for sampling at 24 h after the infusion of taurodeoxycholate. Because of high mortality (5 rats died), we obtained samples from the 6 surviving rats.

*Six percent taurodeoxycholate-induced pancreatitis, treatment with $Me_2Spm$* (Group 8): Rats (n = 6) were infused with 6% sodium taurodeoxycholate intraductally to induce pancreatitis. The animals received $Me_2Spm$ as described above. They were anesthetized and euthanized for sampling at 24 h after the infusion of taurodeoxycholate. We used 9 rats to obtain samples from 6 rats because of high mortality. No valid samples were obtained from 6% taurodeoxycholate-induced pancreatitis at 72 h due to the death of the rats after the infusion, irrespective of $Me_2Spm$ treatment.

### Effect of $Me_2Spm$ on mortality in 6% taurodeoxycholate-induced pancreatitis

The animals were divided randomly into 2 groups:

*Six percent taurodeoxycholate-induced pancreatitis* (Group 9): Rats (n = 30) were infused with 6% sodium taurodeoxycholate intraductally as described above to induce pancreatitis. As treatment the animals received vehicle only (0.9% NaCl). The deaths of the animals were observed and recorded at 12 h intervals after the induction of pancreatitis.

*Six percent taurodeoxycholate-induced pancreatitis, treatment with $Me_2Spm$* (Group 10): Rats (n = 30) were infused with 6% sodium taurodeoxycholate intraductally to induce pancreatitis. The animals received $Me_2Spm$ as described above. The deaths of the animals were observed at 12 h intervals after the induction of pancreatitis.

### Assessment of multi-organ injury during pancreatitis

*Liver and renal functions:* Blood samples were collected and centrifuged for 10 min at 2,500 rpm, and the separated plasma was stored at –20°C for later measurements. The levels of alanine transaminase (ALAT), aspartate transaminase (ASAT), bilirubin and creatinine were measured with a Cobas Integra 700 analyzer (F. Hoffmann-La Roche Ltd. Diagnostics Division, Basel, Switzerland).

*Urine output:* Urine was collected, and urine volumes were measured during the experiment using metabolic and diuresis cage (Tecniplast, Italy).

*Water content of remote organs:* Tissue specimens were excised from the liver, lung and kidney and weighed before and after dehydrating at 110°C for 24 h in an electric oven (TAMRO-APTA 90-544011 Memmert, Germany). The water content of remote organs was expressed as the ratio of (wet weight-dry weight)/dry weight.

*Histology of remote organs:* The liver, lung and kidney specimens were fixed at room temperature in a pH-neutral, phosphate-buffered 10% formalin solution. The fixed tissue was embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and coded for blinded examination. The histological damage to liver, lung and kidney was graded using scoring criteria as previously described [7, 9, 20].

### Polyamine analysis of remote organs

The tissues (liver, lung and kidney) were frozen in liquid nitrogen and stored at –70°C for SSAT and polyamine measurements. SSAT activity was assayed according to Bernacki et al. [4]. The natural polyamines (spermidine, spermine and putrescine) and the polyamine analog ($Me_2$Spm) were measured by high-performance liquid chromatography according to the method of Hyvönen et al. [14].

### Statistical analysis

Data are expressed as the medians [range], and the groups were compared using the Mann-Whitney *U* test. The level of significance was set at $p < 0.05$.

## Results

### Remote organ injury in acute pancreatitis models

#### Liver injury

In 2% taurodeoxycholate-induced acute pancreatitis the liver injury manifested as increased plasma ALAT and ASAT levels (Tab. 1) and temporarily increased water content (Tab. 2), whereas the histological injury score did not change from that observed in the sham operation animals (Tab. 3). The livers of the animals in 2% taurodeoxycholate-induced pancreatitis showed a significant increase in putrescine levels at 24 h (Tab. 4), whereas the SSAT, spermine and spermidine levels remained unchanged.

In 6% taurodeoxycholate-induced acute pancreatitis the liver injury manifested as elevated plasma bilirubin levels (Tab. 1), increased water content (Tab. 2) and also increased histological injury scores (leukocyte infiltration and necrotic foci). The liver putrescine levels were highest in 6% taurodeoxycholate-induced pancreatitis at 24 h (Tab. 4), but similar to 2% taurodeoxycholate-induced pancreatitis, SSAT, spermine and spermidine levels remained unchanged.

Treatment with $Me_2$Spm did not have a significant effect on any of the observed liver injury manifesta-

**Tab. 1.** Blood tests of liver and renal functions in 2 and 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | ALAT (U/l) | ASAT (U/l) | Bilirubin (μmol/l) | Creatinine (μmol/l) | Urine (ml) |
|---|---|---|---|---|---|---|
| Sham | 24 | 94.5 [66.0–183.0] | 331 [255–453] | 1.9 [1.1–4.8] | 34.5 [28.0–41.0] | 21 [10–60] |
| | 72 | 40.5 [31.0–346.0] | 196.5 [112–367] | 1.75 [1.10–10.30] | 21 [16–30] | 37 [16–58] |
| 2% AP | 24 | 143 [135–237]* | 525 [399–835]* | 2.9 [2.2–4.4] | 43 [36–125]* | 23.0 [5.0–77.5] |
| | 72 | 64.5 [36.0–765.0] | 240.5 [124–1124] | 1.95 [1.20–9.10] | 31.5 [28–43]* | 39.5 [25.0–60.0] |
| 2% AP with $Me_2$Spm | 24 | 148.5 [82–258] | 685.5 [390–1291] | 3.25 [1.40–6.20] | 102 [42–349] | 5 [2.5– 7.0]† |
| | 72 | 74.5 [43.0–597.0] | 236.5 [134–1417] | 2.0 [1.4–12.7] | 100 [39–164] †† | 48.5 [32.0–87.5] |
| 6% AP | 24 | 112 [79–1370] | 316.5 [288–1424] | 4.7 [2.4–7.4]* | 96 [52–214]** | 3.9 [0–25.0]* |
| 6% AP with $Me_2$Spm | 24 | 191 [101–325] | 655 [558–951] | 3.8 [2.6–6.9] | 124 [34–158] | 4.7 [0–9.8] |

AP – acute pancreatitis; ALAT – alanine aminotransferase; ASAT – aspartate aminotransferase. All data are the medians [range]. * $p < 0.05$ *vs.* sham operation group at 24 or 72 h, respectively; ** $p < 0.01$ *vs.* sham operation group at 24 h; † $p < 0.05$ *vs.* 2% AP at 24 h; †† $p < 0.01$ *vs.* 2% AP at 72 h

**Tab. 2.** Ratio of (wet weight–dry weight)/dry weight of remote organs in 2 and 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | Liver | Lung | Kidney |
|---|---|---|---|---|
| Sham | 24 | 2.52 [2.39–2.65] | 4.35 [4.00–7.33] | 3.77 [3.00–4.13] |
| | 72 | 2.57 [2.34–3.00] | 4.59 [4.21–5.33] | 3.66 [3.09–4.12] |
| 2% AP | 24 | 2.77 [2.65–2.97]** | 4.73 [3.89–7.25] | 3.85 [3.30–4.41] |
| | 72 | 2.66 [2.49–2.91] | 4.66 [3.95–5.33] | 3.60 [3.24–3.86] |
| 2% AP with $Me_2Spm$ | 24 | 2.77 [2.46–3.60] | 4.70 [4.10–5.94] | 3.36 [2.04–4.12] |
| | 72 | 2.60 [2.37–2.92] | 4.72 [4.00–9.67] | 3.53 [3.30–4.13] |
| 6% AP | 24 | 2.87 [2.68–7.79]** | 4.57 [4.53–4.88] | 3.30 [2.62–3.38] |
| 6% AP with $Me_2Spm$ | 24 | 2.77 [1.96–6.96] | 4.29 [3.53–7.50] | 3.44 [3.19–3.67] |

AP – acute pancreatitis. All data are the medians [range]. ** $p < 0.01$ *vs.* sham operation group at 24 h

**Tab. 3.** Histological scores of remote organs in 2 and 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | Liver | Lung | Kidney |
|---|---|---|---|---|
| Sham | 24 | 0 [0–0] | 2 [0–4] | 1 [1–2] |
| | 72 | 0 [0–1] | 3 [1–4] | 1 [1–1] |
| 2% AP | 24 | 0 [0–0] | 1.5 [1–3] | 2 [1–2] |
| | 72 | 0 [0–3] | 2.5 [1–4] | 1.5 [1–2] |
| 2% AP with $Me_2Spm$ | 24 | 0 [0–4] | 3 [0–4] | 1.5 [1–2] |
| | 72 | 0 [0–3] | 3 [1–8] | 1 [1–1] |
| 6% AP | 24 | 1 [0–2]* | 3 [0–7]* | 1 [1–2] |
| 6% AP with $Me_2Spm$ | 24 | 1 [0–5] | 4.5 [3–6] | 1 [1–2] |

AP – acute pancreatitis. All data are the medians [range]. * $p < 0.05$ *vs.* sham operation group at 24 h

tions. $Me_2Spm$ seemed to accumulate in the liver (Tab. 4). $Me_2Spm$ reversed the increases in putrescine levels in both models, but this effect was statistically significant only in the 6% taurodeoxycholate-induced pancreatitis. There appeared to be some induction of SSAT in the $Me_2Spm$-treated groups with respective decreases in higher polyamines, especially in spermidine levels (Tab. 4).

### Lung injury

In 2% taurodeoxycholate-induced pancreatitis, no lung injury was observed. In 6% taurodeoxycholate-induced pancreatitis lung injury was observed using histological analysis (hemorrhage, leukocyte infiltration and increasing thickness of the alveolar wall).

**Tab. 4.** SSAT activity and polyamine levels in the liver in 2 and 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | Liver SSAT (pmol/mg protein/10 min) | Liver polyamines and $Me_2Spm$ (nmol/mg protein) | | | |
|---|---|---|---|---|---|---|
| | | | Spermidine | Spermine | Putrescine | $Me_2Spm$ |
| Sham | 24 | 2 [0–2.91] | 5.43 [4.51–7.21] | 3.72 [2.87–5.43] | 0 [0–0.1] | |
| | 72 | 0.51 [0–2.97] | 6.86 [4.18–9.61] | 4.25 [3.46–7.08] | 0 [0–0] | |
| 2% AP | 24 | 2.25 [1.42–3.62] | 6.34 [5.39–7.86] | 3.83 [3.06–4.66] | 1.3 [0.24–2.80]** | |
| | 72 | 1.19 [0–4.04] | 7.02 [3–9.91] | 3.81 [2.48–6.08] | 0 [0–0.2] | |
| 2% AP with $Me_2Spm$ | 24 | 6.64 [2.68–41.05] † | 1.74 [1.34–4.03]†† | 2.83 [2.2–3.4]† | 0.24 [0.04–2.25] | 5.37 [4.49–6.88] |
| | 72 | 1.20 [0.08–2.03] | 6.27 [2.26–9.26] | 3.26 [1.38–4.31] | 0.06 [0–0.15] | 4.62 [2.64–6.23] |
| 6% AP | 24 | 2 [0–13] | 6.18 [3.57–7.41] | 4.81 [4.34–5.24] | 3 [2.22–5.11]** | |
| 6% AP with $Me_2Spm$ | 24 | 4 [3–18] | 3 [1.97–4.11]†† | 3.82 [3.73–4.11] | 0.45 [0.04–2.19]†† | 6.74 [4.98–7.82] |

SSAT – spermidine/spermine $N^1$-acetyltransferase; $Me_2Spm$ – bismethylspermine; AP – acute pancreatitis. All data are the medians [range]. ** $p < 0.01$ *vs.* sham operation group at 24 h; † $p < 0.05$ *vs.* 2% AP at 24 h; †† $p < 0.01$ *vs.* AP without $Me_2Spm$ at 24 h

**Tab. 5.** SSAT activity and polyamine levels in the lung in 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | Lung SSAT (pmol/mg protein/10 min) | Lung polyamines and $Me_2Spm$ (nmol/mg protein) | | | |
|---|---|---|---|---|---|---|
| | | | Spermidine | Spermine | Putrescine | $Me_2Spm$ |
| Sham | 24 | 87.50 [34–113] | 4.90 [4.06–5.29] | 3.58 [2.56–4.27] | 0.18 [0–0.22] | |
| 6% AP | 24 | 34.50 [20–55]* | 4.25 [3.26–4.51] | 2.25 [2.01–2.44]** | 0.81 [0.43–1.42]** | |
| 6% AP with $Me_2Spm$ | 24 | 127 [59–363]†† | 2.31 [1.51–2.89]†† | 1.36 [0.84–1.59]†† | 0.71 [0.43–1.18] | 2.5 [2.3–3.7] |

SSAT – spermidine/spermine $N^1$-acetyltransferase; $Me_2Spm$ – bismethylspermine; AP – acute pancreatitis. All data are the medians [range]. * $p < 0.05$ *vs.* sham operation group at 24 h; ** $p < 0.01$ *vs.* sham operation group at 24 h; †† $p < 0.01$ *vs.* 6% AP at 24 h

This injury was associated with a decrease in lung SSAT activity. In addition, spermine levels were decreased, whereas the putrescine levels were increased at 24 h (Tab. 5).

The administration of $Me_2Spm$ had no effect on lung injury observed in 6% taurodeoxycholate-induced pancreatitis. $Me_2Spm$ appeared to also accumulate in the lung. $Me_2Spm$ reversed the SSAT depletion, which resulted in decreased lung spermidine and spermine levels (Tab. 5).

### Renal injury

In 2% taurodeoxycholate-induced acute pancreatitis plasma creatinine was increased at 24 and 72 h (Tab. 1). Compared with the sham operation group, urine output, kidney water content and histology remained unchanged (Tabs. 1–3). The kidneys demonstrated decreased SSAT activity at 24 h (Tab. 6). The spermidine and spermine levels also fell at 24 h, associated with increased putrescine levels at 72 h (Tab. 6).

In 6% taurodeoxycholate-induced acute pancreatitis, plasma creatinine increased two-fold compared to 2% taurodeoxycholate pancreatitis and by almost three-fold compared to the sham operation at 24 h. Urine output dropped significantly (Tab. 1). There was no difference in the kidney water content between the 6% taurodeoxycholate-induced acute pancreatitis group and the sham operation group. Microscopy revealed no histological changes. SSAT activity somewhat decreased whereas spermidine and spermine levels remained unchanged at 24 h (Tab. 6). Similar to the case of 2% taurodeoxycholate-induced pancreatitis, the putrescine levels were elevated (Tab. 6).

**Tab. 6.** SSAT activity and polyamine levels in the kidney in 2 and 6% taurodeoxycholate-induced pancreatitis models

| Groups | Time (h) | Kidney SSAT (pmol/mg protein/10 min) | Kidney polyamines and $Me_2Spm$ (nmol/mg protein) | | | |
|---|---|---|---|---|---|---|
| | | | Spermidine | Spermine | Putrescine | $Me_2Spm$ |
| Sham | 24 | 54.55 [34.50–71.60] | 3.11 [2.47–3.59] | 5.28 [5.05–5.99] | 0.15 [0–0.45] | |
| | 72 | 51.47 [41.60–73] | 3.59 [3.21–3.77] | 6.45 [6.08–6.94] | 0.11 [0–0.22] | |
| 2% AP | 24 | 30.30 [16.40–45.70] * | 2.33 [2.01–2.47]** | 4.62 [4.20–5.36]* | 0.41 [0.22–0.61] | |
| | 72 | 40.55 [30.70–53.70] | 3.42 [3.20–4.04] | 6.18 [5.85–6.66] | 0.32 [0.25–0.44] ** | |
| 2% AP with $Me_2Spm$ | 24 | 352.45 [97.60–641.30] †† | 1.49 [1.39–1.86] †† | 2.99 [2.73–3.38]†† | 0.42 [0.12–0.89] | 7.19 [5.81 –8.56] |
| | 72 | 52.9 [40.80–90.20] †† | 2.48 [2.18–3] †† | 3.53 [3.34–4.71]†† | 0.32 [0.21–0.54] | 8.37 [8.28–8.46] |
| 6% AP | 24 | 12 [3–19] ** | 2.30 [1.40–2.80] | 5.04 [3.77–5.51] | 0.94 [0.77–1.85] ** | |
| 6% AP with $Me_2Spm$ | 24 | 76 [67–209] ‡‡ | 1.26 [0.90–2.10] ‡ | 2.89 [2.81–3] ‡‡ | 0.23 [0.18–0.98] ‡ | 19.27 [9.66–24.35]§§ |

SSAT – spermidine/spermine $N^1$-acetyltransferase; $Me_2Spm$ – bismethylspermine; AP – acute pancreatitis. All data are the medians [range]. * $p < 0.05$ *vs.* sham operation group at 24 h; ** $p < 0.01$ *vs.* sham operation group at 24 or 72 h, respectively; †† $p < 0.01$ *vs.* 2% AP at 24 or 72 h, respectively;‡ $p < 0.05$ *vs.* 6% AP at 24 h; ‡‡ $p < 0.01$ *vs.* 6% AP at 24 h; §§ $p < 0.01$ *vs.* 2% AP with $Me_2Spm$ at 24 h

In 2% taurodeoxycholate-induced pancreatitis, $Me_2Spm$ accumulated in the kidneys, similar to that observed in the liver (Tab. 4 and 6). The treatment with $Me_2Spm$ decreased urine output considerably 24 h after the induction of acute pancreatitis with 2% taurodeoxycholate (Tab. 1). $Me_2Spm$ treatment also significantly increased the level of plasma creatinine at 72 h (Tab. 1). The water content and the histology was not ameliorated after $Me_2Spm$ was given. SSAT activity temporarily increased 10-fold with supplementation of $Me_2Spm$, but returned to baseline level by 72 h (Tab. 6). The induced SSAT activity resulted in lower spermidine and spermine levels.

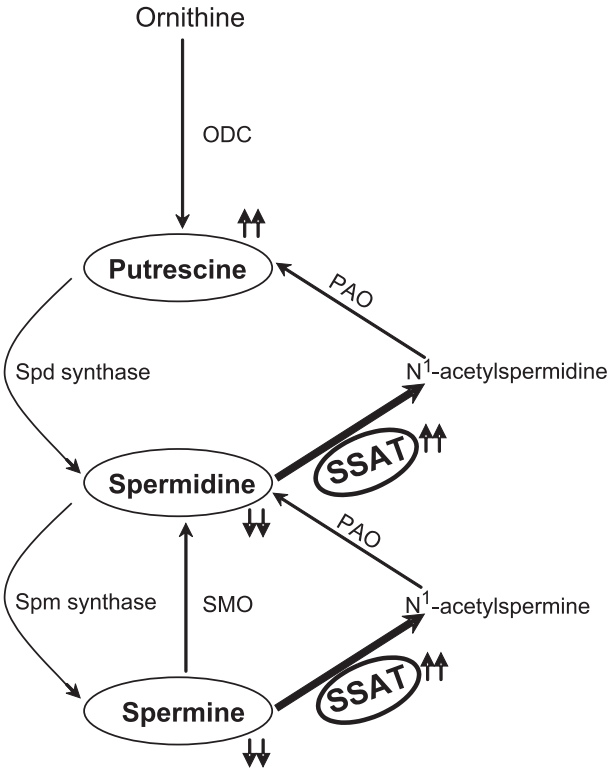


**Fig. 1.** Polyamine catabolism of the pancreas in an acute pancreatitis model. Polyamine synthesis includes the following: generating putrescine from ornithine by the catalysis of ornithine decarboxylase (ODC); yielding spermidine (spd) from putrescine by spd synthase; and yielding spermine (spm) from spermidine by spm synthase. Spermidine/spermine $N^1$-acetyltransferase (SSAT) catabolizes spermine to $N^1$-acetylspermine, which is catabolized by polyamine oxidase (PAO) to spermidine. SSAT and PAO also catabolize spermidine to putrescine, which is again the precursor of spermidine and spermine. Spermine is also partially converted back to spermidine by the catalysis of spermine oxidase (SMO). SSAT is the rate-limiting enzyme in polyamine catabolism. Increased SSAT and putrescine and decreased spermidine and spermine in the pancreas can be observed in the experimental pancreatitis model. "↑↑", increase; "↓↓", decrease

In 6% taurodeoexycholate-induced pancreatitis, treatment with the same dose of $Me_2Spm$ resulted in more accumulation in the kidneys than in the case of 2% taurodeoxycholate-induced pancreatitis (Tab. 6). $Me_2Spm$ did not ameliorate the plasma creatinine or urine output, which were already severely disturbed without any treatment at 24 h. As in 2% taurodeoxycholate-induced pancreatitis, SSAT activity increased after treatment with $Me_2Spm$, followed by a fall in spermidine and spermine levels (Tab. 6). In this case, the accumulated dose of renal $Me_2Spm$ was the highest, at 6.7 times the level of renal spermine.

### Mortality in acute pancreatitis models

Initially, we hypothesized that $Me_2Spm$ would perhaps reduce mortality, which was expected to be almost 100% in 6% taurodeoxycholate pancreatitis. Therefore, survival with and without $Me_2Spm$ treatment was investigated in an experiment with a large enough number of rats to assess the effect on mortality. In 6% taurodeoxycholate treated rats, half of the rats died within 24 h after the induction of acute pancreatitits and the mortality ratio increased up to 83% by 72 h (Fig. 2). The use of $Me_2Spm$ did not have any significant effect on the mortality in this 6% taurodeoxycholate-induced pancreatitis (Fig. 2).

The induction of acute pancreatitis by 2% taurodeoxycholate did not cause any mortality for 72 h. However, upon observing the groups studied to investigate

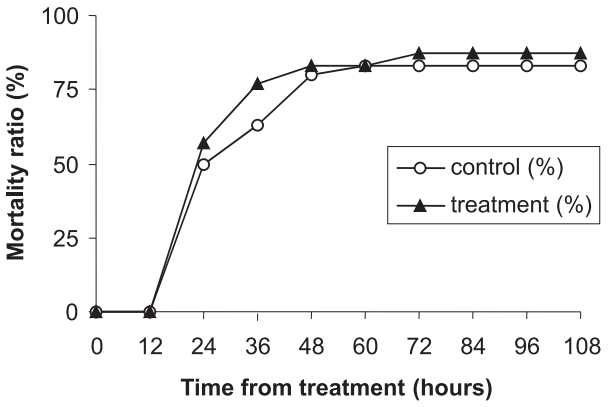


**Fig. 2.** Effect of $Me_2Spm$ on survival in 6% taurodeoxycholate-induced pancreatitis model. The animals began to die within 24 h in both the control and treatment groups. Mortalities increased with time. There was no significant difference in mortality between the two groups. Control, acute experimental pancreatitis treated with vehicle (0.9% NaCl); treatment, acute experimental pancreatitis treated with $Me_2Spm$ (25 mg/kg)

the remote organ injuries, we found that there were no deaths for 24 h after the administration of $Me_2Spm$, but 11 rats were needed to obtain 6 rats for observation at 72 h due to 45% mortality.

## Discussion

The infusion of taurodeoxycholate into the pancreatic duct was able to cause not only local pancreatic damage but also multi-organ damage, due to the presence of liver and kidney injuries [11]. However, lung injury in 2% taurodeoxycholate-induced pancreatitis was not found in an earlier [11] or in the present study. Whereas 4% taurodeoxycholate infusion induced transient pulmonary injury [21], more persistent injury was found with the 6% infusion treatment in this study, as it was shown that the higher the taurodeoxycholate concentration, the higher the mortality. Previously, 2% infusion of taurodeoxycholate yielded no association with mortality [11], 3% taurodeoxycholate resulted in 24% mortality within 72 h, whereas 5% solution led to 100% mortality in 31 h [19]. These data are in agreement with the present results, where 2% infusion of taurodeoxycholate was not associated with mortality and 6% taurodeoxycholate was associated with 50% mortality at 24 h.

No data have been reported on the polyamine changes in pancreatitis-associated remote organ injury nor have attempts been made to ameliorate such injury with polyamine supplementation. The rationale for this approach lies in the recent findings [13, 17] that remarkable changes in pancreatic polyamine homeostasis take place, and that supplementation by long-acting synthetic polyamine analogs partially reverses the injury. However, the changes in polyamine catabolism in remote organs were not similar to the polyamine changes in the pancreas during pancreatitis.

The stimulation of SSAT in the pancreas was higher in the less severe 2% taurodeoxycholate treatment than in the more severe 6% taurodeoxycholate-induced pancreatitis [18]. Similar stimulation was found in the liver and kidney in the less severe 2% taurodeoxycholate-induced pancreatitis, whereas more severe 6% taurodeoxycholate-induced pancreatitis was associated with unchanged (liver) or decreased (kidney and lung) rather than increased SSAT activity. The reason why SSAT activity seems to be inversely associated with the level of organ damage is not known.

In the experimental pancreatic injury, the increased SSAT activation was associated with lower spermidine and spermine levels together with elevated putrescine levels (Fig. 1) [1, 17]. In this study, it was observed that SSAT activation was absent and that putrescine levels were elevated in all the organs studied, whereas spermidine and spermine levels remained unchanged or decreased. Earlier studies have reported that liver injury was characterized by an accumulation of putrescine and depletion of spermine [16, 24]. SSAT activation was observed in a kidney ischemia-reperfusion injury model [29], and paralleling the increase of polyamine transport in lung tissue, polyamine content was enhanced by pulmonary hypoxic exposure [3, 12]. Thus, the polyamine changes differ according to the model studied. The present studies suggest that an increased putrescine level may be associated with remote organ injury.

Treatment with $Me_2Spm$ has been shown to ameliorate pancreatic injury during the first 24 h [13, 17], but in the present study, in the case of 2% taurodeoxycholate-induced pancreatitis, $Me_2Spm$ was found to exert severe renal toxicity. Furthermore, the administration of $Me_2Spm$ led to high mortality in the 6% taurodeoxycholate-induced model. The dose of $Me_2Spm$ in the present study was not beneficial to remote organs in the taurodeoxycholate-induced pancreatitis model. The same doses in other studies have been shown to be beneficial to the pancreas [13, 17]. Thus, in this experimental setting, the side effects of $Me_2Spm$ in the remote organs exceed its beneficial effects on the pancreas. However, smaller doses might also lack beneficial effects to the pancreas because of the lower accumulation of $Me_2Spm$ in the tissue [17].

The effect of polyamine analogues may be variable in different pancreatitis models. According to earlier studies [1, 13, 17], the protective effect of $Me_2Spm$ or MeSpd has been observed in SSAT-induced transgenic pancreatitis, L-arginine-induced pancreatitis and taurodeoxycholate-induced pancreatitis models. In a study by Biczo in 2010 [5], the benefit of MeSpd was not found in L-ornithine-induced acute pancreatitis model. The variation might be caused by the types of animal models, the timing of administering and the dose administered.

During the treatment of pancreatitis with $Me_2Spm$, the level of polyamine catabolizing enzyme SSAT was increased in the remote organs. This is consistent with what has been observed in the pancreas, where $Me_2Spm$ seemed to induce SSAT and decrease sper-

midine and spermine levels [17]. In the liver, spermidine and spermine are required for cell regeneration and are involved in cell growth after injury [2]. Similarly, the reductions of spermidine and spermine levels in the blood and increase in the level of renal SSAT were observed in renal failure patients and in the kidney ischemia-reperfusion model [15, 29]. The perturbations from accumulated $Me_2Spm$ are likely to contribute to the structural or functional injury of remote organs.

The amount of $Me_2Spm$ accumulated in the tissues varied in the different organs (Tabs. 4–6). In the liver, the levels of accumulated $Me_2Spm$ remained stable. However, the renal levels of $Me_2Spm$ were changed according to different time points or different severity of pancreatitis. The disturbances in the natural homeostasis of polyamines, as induced by exogenous administration of the substrate ($Me_2Spm$) for SSAT, may suggest a mechanism for the toxic effects of $Me_2Spm$.

In conclusion, although polyamines have been previously shown to be closely connected with the development of pancreatic injury in acute pancreatitis, the changes in polyamine homeostasis in the remote organs have some dissimilarities, while the increased putrescine level according to the severity of the injury was shown to be a common feature. Supplementation with a synthetic polyamine analog, $Me_2Spm$, does not ameliorate the remote organ injuries, but causes renal toxicity and is associated with mortality of the animals when tested at a dose that has an ameliorative effect on the pancreatic injury.

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