Octreotide prevents liver failure through upregulating 5′-methylthioadenosine in extended hepatectomized rats

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Keywords
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Abbreviations
GC-MS, gas chromatography-mass spectrometry; Met, methionine; MTA, 5′-methylthioadenosine; PH, partial hepatectomy; PVP, portal vein pressure; SAH, S-adenosylhomocystein; SAMe, S-adenosylmethionine.

Abstract

Background & Aims: Insufficient liver regeneration and hepatocyte injury caused by excessive portal perfusion are considered to be responsible for post-hepatectomy liver failure (PLF) or small-for-size syndrome in living-donor liver transplantation. Somatostatin can decrease portal vein pressure (PVP) but simultaneously inhibits liver regeneration. This interesting paradox motivated us to investigate the outcome of PLF in response to somatostatin treatment. Methods: Rats receiving extended partial hepatectomy (90% PH) were treated with octreotide, a somatostatin analogue, or placebo. Animal survival, serum parameters and hepatic histology were evaluated. Metabolomic analysis was performed to investigate the effect of octreotide on hepatocyte metabolism. Results: Despite significantly inhibiting early regeneration, octreotide application noticeably improved the hepatic histology, liver function and survival after PH but did not decrease the PVP level. Metabolomic analysis exhibited that octreotide profoundly and exclusively altered the levels of five metabolites that participate in or closely associate with the methionine cycle, a biochemical reaction that uniquely produces S-adenosylmethionine (SAMe), an active methyl residual donor for methyltransferase reactions. Among these metabolites, 5′-methylthioadenosine (MTA), a derivate of SAMe, increased three-fold and was found independently improve the hepatic histology and reduce inflammatory cytokines in hepatectomized rats. Conclusions: Octreotide exclusively regulates the methionine cycle reaction and augments the MTA level in hepatocytes. MTA prominently protects hepatocytes against shear stress injury and reduces the secondary inflammation, thereby protecting rats from PLF.

The liver is able to restore its mass and function after partial hepatectomy (PH) by activating the proliferation of matured hepatocytes (1–3). This phenomenon has been frequently exploited for hepatic resection, and has become the most effective treatment for selected patients with liver malignancies or certain benign diseases. With advances in surgical techniques, increasingly extended or complex hepatectomies have been carried out in clinical settings; however, such procedures often result in post-hepatectomy liver failure (PLF) (4–6). Living-donor liver transplantation (LDLT) is another procedure that is carried out based on the principle of rapid liver regeneration both in the donor and recipient (7, 8). However, a proportion of these recipients suffer from small-for-size syndrome (SFSS), a high-mortality complication after LDLT (9, 10).

Insufficient hepatocyte proliferation is considered the major cause of PLF (11, 12). However, excessive perfusion of the portal vein was recently reported to be an independent predictive factor of PLF after major liver resection in patients without cirrhosis (13). In addition, accumulating evidence shows that the progressive increase in portal vein pressure (PVP) and shear stress results in hepatocyte injury and liver failure, and plays a critical role in the occurrence of SFSS (14). Several studies have shown that when the full portal vein flow has to traverse through a liver severely reduced in size, the pressure building up in the portal vein shuts down the flow through the portal arterioles and the liver becomes “dearterialized”, which results in a defect in its regeneration ability and contributes to the occurrence of PLF or

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SFSS. Any strategy that decreases PVP, including splenectomy and portacaval shunt, has been found to prevent the development of SFSS (15, 16).

Somatostatin and its synthesized analogue peptide octreotide are widely used in clinics to treat upper digestive tract bleeding caused by cirrhosis, for its capability to decrease PVP (17, 18). Some animal and clinical studies have demonstrated that somatostatin treatment could help to prevent or reduce the incidence of SFSS (19, 20). Xu et al. (20) reported that in a mouse model, low-dose somatostatin could rescue small-for-size grafts from acute-phase injury in the liver transplantation by attenuation of acute-phase shear stress resulting from transient portal hypertension. However, it is still unclear whether somatostatin treatment reduces the physical injury induced by the tremendously increased levels of PVP. Somatostatin is also known to inhibit liver regeneration (21, 22), which might harm hepatic function restoration. These paradoxical effects of somatostatin treatment in PLF prompted us to investigate its precise action in rats receiving extended hepatectomy.

Materials and methods

Animals and treatments

Ten- to 12-week-old male Sprague–Dawley rats weighing 250–300 g were used for this study. The experiments were approved by the Animal Care and Use Committee of Sichuan University and all animals received humane care. For 90% PH surgery, the left, right and quadrate lobes were removed, leaving the caudate lobe, which represents 10–11% of the original liver mass (23). Rats were subcutaneously treated with 12.5 μg octreotide or equal volume of saline 1 h before and every 12 h following surgery. The rats receiving only laparotomy were used as the sham control. At least six rats in each group were killed at each indicated time point to harvest liver specimens and blood samples. A single dose of 50 mg/kg 5-bromo-2'-deoxyuridine (BrdU; Sigma-Aldrich; St. Louis, MO, USA) was administered intraperitoneally 1 h before sacrifice, as described before (24).

Measurement of PVP

A catheter connected to a pressure transducer (BL-420F biological experimental system; Chengdu Technology and Market Co. Ltd.; Chengdu, China) was placed in the portal vein to measure the free PVP (25).

Histology and Immunohistochemistry

Haematoxylin and eosin (H&E) staining and immunohistochemistry staining of Ki67 (Abcam; Cambridge, UK), BrdU (Thermo; Waltham, MA, USA) and CD68 (Abcam) were performed. The number of positive nuclei per 1000 cells was counted in consecutive high-power fields.

Western blot assay

Rat liver samples were harvested at indicated time points and were homogenized for western blot assay. The cell cycle related proteins (cyclins and cell cycle-dependent kinase, CDKs) were examined. An ECL reagent (Millipore; Boston, MA, USA) was used for chemiluminescence detection.

Metabolomic analysis

Rat liver tissue samples were subjected to gas chromatography-mass spectrometry (GC-MS) analysis to collect and derivatize the metabolites. The analyte was injected splitlessly into an Agilent 7890 GC system (Agilent; Santa Clara, CA, USA) coupled with a Pegasus 4D time-of-flight mass spectrometer (LECO; St. Joseph, MI, USA). Chromatographic separation was performed on a DB-5MS capillary column (30 m × 250-μm ID, 0.25-μm film thickness; J&W Scientific; Folsom, CA, USA). Helium was used as the carrier gas. The MS data were acquired in full-scan mode. High-performance liquid chromatography (HPLC) was performed for the metabolites that could not be detected by GC-MS, as previously described (26).

Cytokine analysis

A MILLIPLEX MAP Rat Cytokine/Chemokine Magnetic Bead Panel (Millipore) was used to analyse the levels of serum cytokines, chemokines and growth factors on a Luminex 200 System (Millipore) according to the manufacturer’s instructions. Plasma C reactive protein (CRP) was measured on a Modular Analytics Cobacs 6000 (Roche Diagnostics; Basel, Switzerland) by an immunoturbidimetric technique. Hepatocyte growth factor (HGF) was measured using an ELISA kit (TSZ ELISA; Waltham, MA, USA).

Statistical analysis

All data were expressed as means ± standard deviations (SD). After testing for normality and equal variance
across groups, intergroup differences were assessed using the appropriate pairwise comparison test (Student’s t-test or Mann–Whitney rank sum test). Survival curves were computed using Kaplan–Meier methods and compared using the log-rank test. Results with P-values <0.05 were considered statistically significant.

Results

Octreotide improves animal survival and liver function after extended hepatectomy

The 90% hepatectomy caused notable animal death during 24–48 h after PH regardless of octreotide injection; however, octreotide significantly decreased mortality from 63% (19/30) to 33% (10/30) (P = 0.017) (Fig. 1A).

The serum biomarkers of liver injury and parameters of liver function exhibited large fluctuations from 12 h to 48 h after PH, and the levels were mostly recovered at 72 h post-operation if the rats survived. Nonetheless, the rats with octreotide injection exhibited much lower levels of total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR) and serum ammonia, but higher albumin (ALB) at most time points before 48 h (Fig. 1B).

Octreotide inhibits early hepatocyte proliferation

Liver regeneration was assessed by immunohistochemistry of Ki67 and BrdU incorporation. Both markers indicated that the mitosis of hepatocytes

![Fig. 1. Octreotide administration improves animal survival and liver function after extended hepatectomy. (A) Cumulative survival curves of rats in the saline-treated group and the octreotide-treated group. (B) The dynamic changes of laboratory parameters, including total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR), ammonia and albumin (ALB), between saline-treated and octreotide-treated rats following extended hepatectomy. The data represent the mean ± SD; n = 6. *P < 0.05.](image-url)
reached a peak at 36 h after PH (Fig. 2A). Notably, octreotide significantly decreased early liver regeneration at 12, 24 and 36 h after PH. Even at 48 h, the mitotic index was lower in octreotide-treated mice, although the difference was not statistically significant (Fig. 2B). Hepatocyte proliferation in the octreotide-treated rats moderately exceeded that of their counterparts only at 72 h. Similarly, the liver weight/body weight ratios displayed slower recovery in the octreotide-treated rats (Fig. S1A). Furthermore, all the expression levels of the included cell cycle markers were significantly reduced in response to octreotide injection at each indicated time point (Fig. 2C).

Octreotide improves parenchymal necrosis but does not reduce PVP

We examined the histological changes in the liver. In rats without octreotide injection, severe sinusoidal narrowing was observed at 12 h; prominent focal parenchymal necrosis was detected at 24 and 36 h, especially in the necropsy of the dead animals. Necrosis disappeared at 48 h in the survivors, although notable cell degeneration was present, including swelling, fat change and nuclear condensation. In octreotide-treated rats, the tissue injury was substantially improved and much less necrosis was observed (Fig. 2D).

We next investigated whether octreotide could decrease the PVP. A sharp PVP increase was observed in all rats, and octreotide treatment did not effectively decrease the PVP levels during the first 72 h after PH (Fig. S1B).

Octreotide changes the metabolome of the remnant liver

Since octreotide prominently protected the tissue from necrosis, we speculated that dramatic pathophysiological changes occurred in the hepatocytes in response to octreotide exposure. Metabolomic analysis presents a profile of all the metabolites, the end-products of cellu-
lar processes; therefore, the metabolic disarrangement might reflect the pathophysiological disorders in a cell, tissue, organ or organism. We therefore conducted metabolomic analysis to reveal the metabolic disorders occurring in the remnant liver. Tissue samples were taken at 24 h, because most of the mortality occurred after 24 h, indicating that a dramatic metabolic disarrangement had developed within this period. Representative GC-MS total ion chromatograms of the liver samples were obtained and a total 509 peaks were observed (Fig. 3A). Among these compounds, the majority were common metabolites such as amino acids, organic acids and carbohydrates.

Principal component analysis (PCA), an unsupervised projection method that is the most commonly used algorithm in metabolomic studies (27, 28), was employed to visualize the GC-MS dataset and display the similarities and differences among the samples analysed in this study. The PCA scores plot (PC1 vs. PC2) showed that different samples were approximately clustered with respect to experimental treatments (Fig. 3B upper panel). Spots of the sham-operated rats were sig-

Fig. 3. Metabolomic analysis of the liver tissues. (A) Representative GC-MS total ion chromatograms of the liver samples from differently treated mice. (B) Score plots derived from the GC-MS data of the liver tissues at 24 h post-operation. PCA and PLS-DA plots derived from GC-MS spectra of liver tissues from all three groups; sham vs. saline, and saline vs. octreotide are respectively presented. (C) Schematic diagram of the methionine cycle reaction. (D) The relative levels of the critical metabolites in octreotide-treated rats are presented as the average fold increase or decrease over the counterparts that were treated with saline, which were set to 1. The data represent the means of six samples per group. (Met, methionine; SAMe, S-adenosylmethionine; MTA, S'-methylthioadenosine; SAH, S-adenosylhomocysteine; Hcy, homocysteine.)
nificantly discriminated from those of rats receiving PH without octreotide, indicating that liver resection contributed to a major portion of the metabolic variation in the remnant liver (Fig. 3B). Furthermore, although the discriminability was not very clear between the hepatectomized rats with or without octreotide, 57% (see Fig. 3B, saline vs. octreotide, PC1 (37.9%) + PC2 (19.1%) = 57%) of the variation between these groups was nevertheless detected, which indicated that octreotide notably altered the metabolome in hepatectomized rats.

Partial least squares-discriminate analysis (PLS-DA) permits evaluation as to whether the metabolic state could be used to predict the experimental group that the sample originated from (28). Figure 3B also shows the three-group separation established by the PLS-DA model. Clear discrimination was observed among the groups, which confirmed the PH-associated and octreotide-associated metabolic variations. The goodness-of-fit and predictability of the pattern analysis models were calculated as $R^2$ and $Q^2$, respectively, and the results are shown in Table S1. The high $R^2$ and $Q^2$ values demonstrate that this model could clearly and predictably classify the groups.

Octreotide regulates the methionine cycle reaction in the remnant liver

We next identified the metabolites that showed remarkable alterations upon various treatments. Fourteen metabolites were significantly changed between PH/saline group and sham-operated group, and 10 between PH/octreotide group and sham group. In hepatectomized rats, only five metabolites were significantly altered in response to octreotide administration (see Table S2). Three of these metabolites, including threonine, creatine and threitol, are universally involved in various biochemical reactions. Intriguingly, the remaining two metabolites, methionine (Met) and homocysteine (Hcy), are exclusively involved in the methionine cycle reaction (see Table S3). As one of the most important biochemical reactions, the methionine cycle generates S-adenosylmethionine (SAMe), a methyl donor for approximately 70% of methyltransferase reactions (29). It has long been recognized that the liver plays a central role in methionine metabolism, and marked impairment of methionine metabolism is observed in patients with liver diseases (29, 30). We next performed HPLC to examine the levels of these five metabolites following octreotide injection in rats receiving sham operation or classic two-thirds PH. Consistent with the findings in the 90% PH model, octreotide significantly increased the levels of Met and MTA, while decreased SAMe, SAH and Hcy (Fig. 3D).

MTA improves remnant liver function and reduces systemic inflammation

Besides serving as a methyl donor, SAMe has also been found to protect the liver against various injuries (29–31). MTA is derived from SAMe both enzymatically and non-enzymatically, and might play the same role as SAMe in liver protection (30). Our findings of the decrease in SAMe together with the increase in MTA indicated that SAMe was converted to MTA, which might have mediated the liver protection effect of octreotide. To test this hypothesis, we treated another 40 extended hepatectomized rats with MTA (0.1 μmol/kg body weight) subcutaneously every 12 h post-operation. Survival analysis showed that MTA administration significantly improved animal survival (Fig. 4A). The histological and biochemical examinations also demonstrated that MTA could notably improve the histology and liver function without significant regeneration inhibition (Fig. 4B–C and Fig. S2).

SAMe has been shown to regulate systemic inflammation, which plays a crucial role in the promotion of liver failure after extended resection or massive necrosis (30, 31). However, it remains unknown whether MTA plays the same role in inflammatory inhibition. Kupffer cells (KCs) are specialized macrophages located in the liver that can be activated in response to diverse liver injuries. The activation of KCs is a double-edged sword. Moderate activation of KCs helps to secrete certain cytokines to promote liver repair; however, over-activation of these macrophages and the secondary uncontrolled cytokine production has been shown to exacerbate local tissue damage and systemic inflammatory reaction. We examined the activation of KCs in the rat livers. Enlarged KCs, as marked by CD68 immunoreactivity, with rich cytoplasm were clearly observed in the liver tissue without octreotide or MTA exposure (Fig. 5A).

We next measured the systemic inflammatory markers at 24 h post-operation in 90% hepatectomized rats. Our results showed that serum levels of IL-1α, IL-1β, IL-6 and TNFα were significantly lower following MTA administration. Cytokines, including IL-2, CRP and IFNγ, were not statistically changed. IL-10, a cytokine that inhibits inflammation, increased although without statistical significance. In addition, MTA did not remarkably change the production of growth factors, including HGF, EGF and VEGF (Fig. 5B). Finally, we treated rats with intraperitoneal injection of carbon tetrachloride (CCL4) and investigated whether MTA protects the liver from chemical
Fig. 4. MTA administration markedly improves animal survival, histology and function of the remnant liver. (A) Cumulative survival curves of rats in the saline group and the MTA group. (B) H&E staining of liver sections shows better liver histology in MTA-treated rats after 90% PH. The necrotic zones are circled with white-dotted lines. Scale bar = 100 μm. (C) The dynamic changes of laboratory parameters, including total bilirubin (TBIL), aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR), ammonia and albumin (ALB) following extended hepatectomy. The data represent the mean ± SD; n = 6. *P < 0.05. MTA, 5′-methylthioadenosine.
injury. Twenty-four hours after CCl4 challenge, MTA notably improved the histology and inhibited the activation of KCs (Fig. S3A–B). Similarly, MTA treatment statistically decreased the levels of IL-1β and TNF-α, but increased IL-10 (Fig. S3C).

Discussion
In this study, we observed marked parenchymal necrosis in the extended hepatectomized rats, which might lead to acute liver failure. Octreotide administration promi-
nently improved the histology, liver function and survival of the 90% hepatectomized rats although it inhibited early liver regeneration and did not notably decrease PVP. Using metabolomic analysis, we identified significant alterations of five metabolites following octreotide injection, which are exclusively involved in the methionine cycle reaction. Among these metabolites, MTA was robustly increased and was found to independently protect the remnant hepatocytes against shear stress injury and to reduce the consequent systemic inflammation, thereby improving the outcome of the hepatectomized animal.

Regeneration deficiency is likely to account for acute liver failure after partial hepatectomy. It is interesting that rats or mice receiving a two-thirds PH could survive even when their liver regeneration is severely deprived by chemical agents (32). Active regeneration was found in our 90% hepatectomized rats that lacked octreotide, on the contrary, octreotide injection notably delayed liver repair but led to a much better outcome, suggesting that regeneration deficiency should not be regarded as the major cause of liver failure.

The increase in the sinusoidal shear stress after hepatectomy is a critical factor to initiate regeneration. However, shear stress exceeding the capacity of the remnant liver causes flow injury to sinusoidal endothelial cells and loss of microvilli of hepatocytes, resulting in collapse of the space of Disse and disruption of hepatic microcirculation. In a two-thirds PH, the resected tissues are amenable to "clean" removal, and the resection is not associated with massive necrosis and inflammation, indicating that the increased pressure is tolerable (33). On the contrary, in agreement with previous reports (33), severe necrosis was observed in the 90% hepatectomized rats, suggesting that the portal pressure has exceeded the tolerance limit of the remnant cells. Octreotide has long been used in clinics to decrease PVP caused by chronic cirrhosis, however, in this study, octreotide failed to decrease the overwhelmingly elevated PVP caused by the extreme sudden loss of capillary beds and outflow obstruction. Nonetheless, our data demonstrated that excessive portal perfusion leads to massive necrosis and subsequent liver failure, whereas octreotide protected the residual hepatocytes against the physical injury independent of PVP reduction.

Metabolomic analysis has become a popular strategy to reveal processes occurring in cells. We unexpectedly found that several components exclusively involved in the methionine cycle reaction were significantly altered upon octreotide administration. The most important function of these sequential biochemical reactions is to generate SAMe. Although mainly functioning as a methyl donor in methylation reactions, preliminary studies have reported that SAMe has the capacity to protect the liver against various injuries through several mechanisms, including increasing glutathione levels, regulating the hepatocyte apoptotic response, improving membrane fluidity and decreasing the expression of TNFα (29–31, 34). MTA, a downstream derivative of SAMe, obtains the same function in liver protection but with much greater stability than SAMe both in vivo and in vitro (29, 30). In fact, the role of SAMe in liver protection might actually be mediated by MTA, because oral administration of MTA but not SAMe can easily transport across the plasma membrane (35). We also confirmed that MTA administration alone could improve the histology and function of the remnant liver.

Dying hepatocytes activate inflammatory cells and release numerous proinflammatory cytokines such as TNF-α and IL-1β, which recruit neutrophils and lymphocytes to the liver and aggravate liver injury in a feedback–inflammatory loop. In addition, necrotic hepatocytes increase IL-1α release in parallel with the activation of KCs, leading to the production of IL-6, which promotes hepatocyte proliferation and simultaneously, exacerbates inflammation (36, 37). Therefore, any treatment that disrupts this loop would ameliorate liver injury. A previous study showed that MTA inhibits T-cell proliferation and the secretion of Th1/Th2 cytokines, including IL-2, IL-4, IL-5, IL-6, IL-10, TNFα, INFγ and GM-CSF, and thus acts as an inhibitor in lupus autoimmunity (38). In this study, MTA did not significantly inhibit the proliferation and activation of T cells (Fig. S4), and the altered cytokines were not the same as those involved in immunomodulation; nevertheless, we found that MTA could suppress the production of inflammatory factors such as IL-1α, IL-1β, TNFα and IL-6, and effectively inhibited the activation of Kupffer cells. Our data did not support that MTA suppressed systemic inflammation, but it did prevent the liver damage, therefore, decreased the initiation and exacerbation of the consequent inflammation. The mechanism of the exclusive relationship between octreotide administration and MTA induction remains unresolved. Somatostatin is chiefly produced by the hypothalamus and can inhibit the secretion of various other hormones, including somatotropin, glucagon, insulin and thyrotropin (39). In general, somatostatin reduces metabolism through its action on digestive enzyme inhibition, but its role in the metabolism of a given substance or in a biochemical reaction is far from clear. Octreotide has been shown to exhibit similar pharmacological action to its synthetic analogue somatostatin (18). We confirmed that octreotide application can regulate the methionine cycle in hepatocytes even in an intact liver, but the underlying mechanism requires further investigation.

In summary, our study provides the first evidence that octreotide administration exclusively upregulates the level of MTA, a derivative of the methionine cycle, which in turn protects the remnant hepatocytes against physical and chemical injuries, and therefore improves the histology and function of the remnant liver after extended hepatectomy. Unlike octreotide, MTA is a
natural metabolite in hepatocytes and does not significantly disturb liver regeneration. Considering its validity, stability, availability and could be orally administered, MTA is a promising agent for liver protection in clinical settings.

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Conflict of interest: The authors do not have any disclosures to report.

References


**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Goodness-of-fit ($R^2$) and predictability ($Q^2$) of pattern analysis models.

**Table S2.** Identification results of VIP metabolites based on PLS-DA.

**Table S3.** Altered metabolites in liver samples compared saline-treated group with octreotide-treated group.

**Fig. S1.** (A) The liver weight/body weight (LW/BW) ratios was calculated at different time points after PH. The data represent the mean ± SD; n = 3. *P < 0.05. (B) Octreotide treatment does not significantly decrease the PVP. The data represent the mean ± SD; n = 6.

**Fig. S2.** MTA does not impair liver regeneration after 90% PH.

**Fig. S3.** MTA improves histology and inhibits systemic inflammation in CCl4 treated rats.

**Fig. S4.** MTA does not inhibit the proliferation and activation of T lymphocytes.