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ORIGINAL ARTICLE

# Metabolomic analysis of sulfur-containing substances and polyamines in regenerating rat liver

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**Abstract** We studied the significance of alterations in the metabolomics of sulfur-containing substances in rapidly regenerating rat livers. Male rats were subjected to two-thirds partial hepatectomy (PHx), and the changes in hepatic levels of major sulfur-containing amino acids and related substances were monitored for 2 weeks. Liver weight began to increase from 24 h after the surgery, and appeared to recover fully in 2 weeks. Serum alanine aminotransferase and aspartate aminotransferase activities were elevated immediately after the surgery and returned slowly to normal levels in 2 weeks. Methionine, *S*-adenosylmethionine (SAM), cystathionine and cysteine were increased rapidly and remained elevated for longer than 1 week. Hepatic glutathione concentration was increased gradually for 24 h, and then decreased thereafter, whereas hypotaurine was elevated drastically right after the surgery. Hepatic concentrations of polyamines were altered significantly by PHx. In the hepatectomized livers putrescine concentration was elevated rapidly, reaching a level 40- to 50-fold greater than normal in 6–12 h. Ornithine, the metabolic substrate for putrescine synthesis, was also elevated markedly. Spermidine was increased significantly, whereas spermine was depressed below normal, which appeared to be due to the increased consumption of decarboxylated SAM for spermidine biosynthesis. The results show that the metabolomics of sulfur-containing amino acids and related substances is altered profoundly in regenerating rat livers until the original weight is recovered. Hepatic concentrations of polyamines after PHx are closely associated with the alteration in the metabolomics of sulfur-containing substances. The implication of these changes in the progression of liver regeneration is discussed.

**Keywords** Sulfur-containing amino acids · Hepatectomy · Liver regeneration · Polyamines · *S*-adenosylmethionine · Glutathione

**Abbreviations**

| | |
|---|---|
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| C$\beta$S | Cystathionine $\beta$-synthase |
| CDC | Cysteine sulfinate decarboxylase |
| CDO | Cysteine dioxygenase |
| C$\gamma$L | Cystathionine $\gamma$-lyase |
| dcSAM | Decarboxylated *S*-adenosylmethionine |
| eIF5A | Eukaryotic translation initiation factor 5A |
| GCL | $\gamma$-Glutamylcysteine ligase |
| GSH | Glutathione |
| MAT | Methionine adenosyltransferase |
| PHx | Partial hepatectomy |
| ROS | Reactive oxygen species |
| SAH | *S*-adenosylhomocyteine |
| SAM | *S*-adenosylmethionine |

## Introduction

Liver regeneration is a fundamental response to loss of hepatic tissue. Hepatocytes can replicate rapidly, thus allowing the liver to recover from damage and to

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regenerate promptly after removal of its mass. The process of liver regeneration involves sequential changes in gene expression, growth factor production, and morphological structure (Michalopoulos 2010). It has been suggested that the mechanism of liver regeneration is associated with activation of multiple pathways which work closely one with another. At least three types of pathways have been identified in the regenerating liver: a cytokine pathway that is responsible for the entry of quiescent hepatocytes into the cell cycle (transition from the $G_0$ to the $G_1$ phase); a growth factor pathway that accounts for the progression of cell cycle (through the $G_1$ to the S phase); and a metabolic pathway that links metabolic signals with cell growth and proliferation (Fausto and Riehle 2005). Many growth factors and cytokines, most notably hepatocyte growth factor, epidermal growth factor, transforming growth factor-α, interleukin-6 and tumor necrosis factor-α, have been shown to play critical roles in this process (Fausto et al. 2006). Also the increased metabolic demands imposed on the liver remnant after hepatectomy are linked with activation of the machinery directly involved in DNA replication.

It has been noted that, in addition to the changes in gene expression and growth factor production, hepatocyte proliferation is closely associated with alterations in intracellular availability of some thiol- and/or sulfur-containing substances. Plating rat hepatocytes under low density, which stimulates hepatocytes to shift from the $G_0$ to the $G_1$ phase, was shown to result in an increase in hepatic glutathione (GSH), cysteine and γ-glutamylcysteine ligase (GCL) activity (Cai et al. 1995; Lu and Ge 1992). Numerous studies involving lymphocytes and fibroblasts indicate that an increase in GSH is required for the cells to enter the S phase (Shaw and Chou 1986; Messina and Lawrence 1989; Poot et al. 1995). In fact an increase in hepatic GSH was noted in an early period of liver regeneration in partially hepatectomized rats (Huang et al. 1998a). Meanwhile, it has been suggested that hepatic *S*-adenosylmethionine (SAM) has an important role in the regulation of hepatocyte growth (Mato and Lu 2007; Varela-Rey et al. 2009). SAM is the principal biological methyl donor and also a metabolic source for GSH via its conversion to cysteine in the transsulfuration pathway. Since oxidative stress is critically implicated in regenerating liver, the effects of SAM on the progression of liver regeneration have been frequently attributed to its function as metabolic supplier of cysteine that is essential for GSH synthesis. However, recent evidence suggests that intracellular SAM synthesis is critically regulated by growth factors, cytokines and hormones, and should be viewed as an intracellular control switch for essential hepatic function such as liver regeneration, differentiation, and apoptosis (Mato et al. 2002, 2007; Martínez-López et al. 2008). Also SAM is metabolically converted to decarboxylated SAM (dcSAM), which releases an aminopropyl group utilized in synthesis of polyamines that are necessary in cell proliferation and differentiation (Heby 1981; Jänne et al. 1991).

Both SAM and GSH are major metabolites in hepatic transsulfuration pathway, and accordingly, it is suspected that the metabolic reactions in this pathway would be tightly regulated during the process of liver regeneration. But extensive literature survey reveals that information regarding the changes in the transsulfuration reactions in regenerating liver is relatively scarce. In this study we examined the metabolomics of sulfur-containing amino acids and related substances in the remnant liver after partial hepatectomy.

## Methods

### Animals and treatments

Male Sprague–Dawley rats were purchased from Dae-Han Laboratory Animal (Seoul, Korea). The use of animals was in compliance with the guidelines established by the Animal Care Committee of this institute. Rats were acclimated in temperature (22 ± 2°C) and humidity (55 ± 5%) controlled rooms with a 12 h light/dark cycle for 1 week prior to use. Regular rat chow and tap water were allowed ad libitum until sacrifice. Rats were 9 weeks old and weighed 270–310 g when used in experiments.

Surgical procedures were performed according to the technique originally introduced by Higgins and Anderson (Higgins and Anderson 1931). Under light ether anesthesia rats were subjected to two-thirds partial hepatectomy (PHx). A midline incision was made in the sub-xiphoid area, the abdomen was opened, and the liver was mobilized. The median and lateral lobes were then removed. A separate group of rats which underwent laparotomy only served as a sham control.

### Assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined to estimate liver injury (Reitman and Frankel 1957).

Livers were homogenized in a fourfold volume of cold 1 M perchloric acid. Denatured protein was removed by centrifugation. Total GSH concentrations were determined using an enzymatic recycling method (Griffith 1980). Cysteine concentrations were estimated by the acid-ninhydrin method (Gaitonde 1967). SAM and *S*-adenosylhomocyteine (SAH) concentrations were measured using a HPLC method described elsewhere (Kim and Kim 2005).

For quantitative analysis of methionine, hypotaurine, and taurine, livers were homogenized in a fivefold volume

of cold methanol. Methionine, hypotaurine, and taurine were derivatized with *O*-phthalaldehyde/2-mercaptoethanol prior to quantification by HPLC with a fluorescence detector and 3.5 μm Kromasil C18 column (4.6 × 100 mm) (Eka Chemicals, Bohus, Sweden). Methionine was separated by using the method of Rajendra (1987). The method of Ide (1987) was used to separate hypotaurine and taurine.

Hepatic polyamine concentrations were determined using a HPLC method (Fu et al. 1998). Livers were homogenized in a fourfold volume of 1 M perchloric acid. Polyamine derivatives were separated on μBondapak C18 column (4.6 × 250 mm) (Waters, Milford, MA, U.S.A.), eluted with methanol and distilled water using a one-step linear gradient, and monitored by a fluorescence detector. Hexamethylenediamine was used as internal standard.

## Results

### Changes in parameters of liver injury

Liver/body weight ratio (LBWR) was reduced to less than one-third of control by PHx (Table 1). An increase in LBWR was significant from 24 h after the surgery. The liver appeared to recover its original weight in 2 weeks. Serum AST and ALT activities were measured to estimate the liver injury inflicted by the surgical process. Both AST and ALT activities were elevated rapidly, peaked in 12 h, and reduced slowly thereafter. But the serum enzyme activities were still higher than normal as late as 2 weeks after the surgery.

### Changes in concentrations of sulfur-containing metabolites

Hepatic methionine concentration was increased rapidly by PHx and remained elevated for longer than a week (Table 2). A change in SAM concentration appeared to be synchronized with that of methionine. Cystathionine was increased rapidly, reaching a level greater than 10 times of normal in 9 h after the surgery. Cysteine concentration was also elevated markedly, which was significant for as long as 10 days after PHx. Hepatic GSH concentration was increased gradually to a level almost 2 times of normal in 24 h and then returned slowly to normal. On the other hand, hepatic hypotaurine, the other major metabolic product of cysteine, was elevated rapidly, reaching a peak greater than 30 times of normal. However, there appeared to be no significant differences in taurine level, suggesting a fast removal of this substance from the liver.

### Changes in concentrations of polyamines

Putrescine concentration in liver was increased strikingly by the surgery (Table 3). Hepatic putrescine reached a peak level grater than 40 times of normal in 6–12 h after PHx, and decreased slowly to normal in a week. Hepatic concentration of ornithine, a metabolic substrate for putrescine synthesis, was also elevated markedly. Hepatic spermidine concentration was increased significantly from 6 h after the surgery and remained elevated for the next 10 days. In contrast, spermine, which increased transiently after PHx, was subsequently reduced to a level below normal.

## Discussion

The present results show that the metabolomics of sulfur-containing amino acids and related substances is altered significantly during liver regeneration in rats. Concentrations of methionine, SAM, cystathionine, and cysteine in liver were all elevated after PHx. Hepatic GSH was increased gradually, but generation of hypotaurine was drastically enhanced immediately following the surgery. The changes in the sulfur-containing metabolites in regenerating liver were mostly persistent until the liver weight was fully recovered. Almost all the parameters determined in this study including the liver toxicity indices, sulfur-containing metabolites in the transsulfuration pathway, and polyamines, returned to baseline nearly 2 weeks after the surgery, which coincided with recovery of the original liver weight.

In mammals the metabolism of sulfur-containing amino acids occurs primarily via the transsulfuration pathway in which methionine sulfur is ultimately transferred to GSH, taurine, or inorganic sulfate. The first step in the transsulfuration reactions is formation of SAM from methionine and ATP that is mediated by methionine adenosyltransferase (MAT). SAM serves as a major biological methyl donor and a co-product of transmethylation, SAH, is hydrolyzed to yield homocysteine, which is either remethylated to methionine or metabolically converted to cysteine in reactions mediated by cystathionine $\beta$-synthase (C$\beta$S) and cystathionine $\gamma$-lyase (C$\gamma$L), consecutively. Cysteine is irreversibly metabolized to either taurine, inorganic sulfate, or GSH. GSH is synthesized by successive actions of GCL and GSH synthetase. On the other hand, cysteine dioxygenase (CDO) mediates the oxidation of this amino acid to cysteine sulfinate that is mainly converted to taurine via hypotaurine. Metabolic fate of cysteine appears to be determined by its availability in liver. It has been suggested that low cysteine availability favors its utilization for GSH synthesis whereas high cysteine availability enhances its catabolism to inorganic

**Table 1** Changes in liver weight and serum enzyme activities after PHx

| | Time after PHx | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 3 h | 6 h | 9 h | 12 h | 24 h | 48 h | 96 h | 168 h | 240 h | 336 h |
| Body weight (g) | | | | | | | | | | | |
| Sham | 332 ± 3 | 323 ± 3 | 318 ± 4 | 323 ± 7 | 328 ± 2 | 344 ± 2 | 335 ± 5 | 351 ± 7 | 357 ± 7 | 347 ± 15 | 387 ± 6 |
| PHx | | 313 ± 4 | 313 ± 6 | 314 ± 3 | 319 ± 8 | 313 ± 5[a] | 321 ± 1 | 333 ± 11 | 367 ± 16 | 337 ± 6 | 380 ± 5 |
| Liver weight (g) | | | | | | | | | | | |
| Sham | 13.4 ± 0.5 | 12.8 ± 0.2 | 11.4 ± 0.4 | 11.6 ± 0.2 | 10.7 ± 0.4 | 13.0 ± 0.1 | 12.3 ± 0.3 | 13.1 ± 0.5 | 11.9 ± 0.2 | 13.2 ± 1.1 | 13.7 ± 0.6 |
| PHx | | 3.6 ± 0.2[b] | 3.6 ± 0.1[b] | 3.7 ± 0.3[c] | 3.8 ± 0.2[b] | 4.9 ± 0.3[b] | 6.3 ± 0.2[b] | 9.1 ± 0.1[a] | 9.7 ± 0.4[a] | 10.1 ± 0.7 | 11.7 ± 1.0 |
| LBWR (%) | | | | | | | | | | | |
| Sham | 4.04 ± 0.13 | 3.95 ± 0.04 | 3.58 ± 0.08 | 3.59 ± 0.09 | 3.25 ± 0.11 | 3.77 ± 0.04 | 3.69 ± 0.09 | 3.74 ± 0.17 | 3.32 ± 0.01 | 3.80 ± 0.15 | 3.54 ± 0.12 |
| PHx | | 1.16 ± 0.04[c] | 1.16 ± 0.04[c] | 1.18 ± 0.08[c] | 1.20 ± 0.03[c] | 1.56 ± 0.09[c] | 1.97 ± 0.06[c] | 2.74 ± 0.07[b] | 2.64 ± 0.02[b] | 3.00 ± 0.14[a] | 3.06 ± 0.22 |
| ALT (units/ml) | | | | | | | | | | | |
| Sham | 32 ± 4 | 21 ± 3 | 11 ± 2 | 11 ± 4 | 14 ± 1 | 19 ± 5 | 25 ± 3 | 24 ± 2 | 28 ± 4 | 21 ± 1 | 13 ± 3 |
| PHx | | 610 ± 162[a] | 924 ± 69[c] | 1097 ± 179[b] | 1578 ± 239[b] | 794 ± 205[a] | 245 ± 64[a] | 88 ± 8[b] | 32 ± 4 | 37 ± 5[a] | 21 ± 3 |
| AST (units/ml) | | | | | | | | | | | |
| Sham | 52 ± 2 | 67 ± 5 | 50 ± 1 | 54 ± 1 | 52 ± 4 | 56 ± 5 | 60 ± 3 | 51 ± 6 | 70 ± 5 | 49 ± 3 | 61 ± 2 |
| PHx | | 488 ± 113[a] | 548 ± 51[c] | 820 ± 29[c] | 1105 ± 278[a] | 615 ± 156[a] | 324 ± 63[a] | 253 ± 73 | 87 ± 13 | 80 ± 2[b] | 79 ± 1[c] |

Liver/body weight ratio (LBWR) was calculated as a percentage of the ratio of the remnant liver weight to body weight (100 × liver weight/body weight). Each value represents the mean ± SEM for 3 or 4 rats

[a, b, c] Significantly different from the sham control ($P < 0.05$, 0.01, and 0.001, respectively)

**Table 2** Changes in sulfur-containing substances in liver after PHx

| | Time after PHx | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 3 h | 6 h | 9 h | 12 h | 24 h | 48 h | 96 h | 168 h | 240 h | 336 h |
| Met (nmol/g liver) | | | | | | | | | | | |
| Sham | 60 ± 4 | 63 ± 6 | 63 ± 1 | 66 ± 2 | 63 ± 2 | 63 ± 2 | 68 ± 3 | 60 ± 2 | 57 ± 4 | 62 ± 3 | 69 ± 3 |
| PHx | | 106 ± 9[a] | 89 ± 4[b] | 89 ± 3[b] | 82 ± 7[a] | 81 ± 7 | 73 ± 5 | 75 ± 4[a] | 77 ± 2[a] | 82 ± 2[b] | 71 ± 5 |
| SAM (nmol/g liver) | | | | | | | | | | | |
| Sham | 85 ± 4 | 83 ± 2 | 89 ± 3 | 80 ± 2 | 78 ± 4 | 87 ± 4 | 85 ± 2 | 84 ± 4 | 87 ± 4 | 83 ± 4 | 91 ± 4 |
| PHx | | 97 ± 5 | 110 ± 8 | 122 ± 8[b] | 126 ± 3[c] | 152 ± 4[c] | 108 ± 3[b] | 96 ± 2 | 108 ± 7 | 106 ± 3[b] | 92 ± 5 |
| SAH (nmol/g liver) | | | | | | | | | | | |
| Sham | 26.2 ± 0.3 | 31.0 ± 0.8 | 29.8 ± 0.3 | 28.3 ± 1.1 | 29.4 ± 3.8 | 31.9 ± 0.7 | 27.4 ± 1.9 | 28.2 ± 1.0 | 27.8 ± 0.7 | 30.0 ± 1.0 | 32.9 ± 3.9 |
| PHx | | 45.3 ± 1.2[c] | 70.6 ± 7.5[b] | 70.9 ± 10.0[a] | 52.8 ± 4.4[a] | 34.7 ± 1.2 | 27.4 ± 1.4 | 26.2 ± 1.5 | 32.0 ± 4.9 | 39.7 ± 4.5 | 34.1 ± 1.0 |
| Cyst (nmol/g liver) | | | | | | | | | | | |
| Sham | 4.3 ± 0.5 | 3.7 ± 0.9 | 4.0 ± 0.5 | 4.0 ± 0.5 | 4.0 ± 0.5 | 3.9 ± 0.5 | 3.5 ± 0.5 | 3.7 ± 0.6 | 3.5 ± 0.5 | ND | ND |
| PHx | | 18.3 ± 6.8 | 26.2 ± 3.5[b] | 42.9 ± 11.8[a] | 38.5 ± 10.8[a] | 12.8 ± 1.5[c] | 13.1 ± 6.6 | 6.0 ± 0.4[a] | 5.0 ± 0.7 | ND | ND |
| Cys (nmol/g liver) | | | | | | | | | | | |
| Sham | 95 ± 5 | 100 ± 3 | 93 ± 5 | 80 ± 3 | 86 ± 6 | 97 ± 8 | 98 ± 3 | 102 ± 3 | 97 ± 4 | 91 ± 4 | 108 ± 15 |
| PHx | | 245 ± 19[b] | 250 ± 11[c] | 239 ± 18[c] | 234 ± 32[a] | 233 ± 20[b] | 215 ± 21[b] | 171 ± 28 | 149 ± 10[b] | 150 ± 15[a] | 119 ± 10 |
| GSH (μmol/g liver) | | | | | | | | | | | |
| Sham | 6.3 ± 0.1 | 6.3 ± 0.2 | 6.0 ± 0.1 | 5.0 ± 0.3 | 5.0 ± 0.5 | 6.2 ± 0.2 | 6.0 ± 0.3 | 6.4 ± 0.4 | 6.2 ± 0.4 | 6.2 ± 0.3 | 6.8 ± 0.2 |
| PHx | | 6.1 ± 0.1 | 7.1 ± 0.2[a] | 8.7 ± 0.3[c] | 9.3 ± 0.2[b] | 11.5 ± 0.5[c] | 9.8 ± 0.3[c] | 9.4 ± 0.1[b] | 7.2 ± 0.2 | 6.6 ± 0.3 | 6.3 ± 0.2 |
| HT (nmol/g liver) | | | | | | | | | | | |
| Sham | 193 ± 63 | 142 ± 6 | 151 ± 10 | 148 ± 28 | 171 ± 16 | 180 ± 41 | 227 ± 10 | 236 ± 11 | 176 ± 6 | 251 ± 24 | 250 ± 22 |
| PHx | | 2,061 ± 602[a] | 4,092 ± 919[a] | 4,798 ± 556[c] | 3,634 ± 438[b] | 2,213 ± 139[c] | 1,905 ± 640 | 1,484 ± 155[b] | 1,072 ± 69[c] | 633 ± 169 | 441 ± 81 |
| Tau (nmol/g liver) | | | | | | | | | | | |
| Sham | 2,843 ± 211 | 2,372 ± 315 | 2,023 ± 215 | 2,376 ± 199 | 3,285 ± 694 | 2,711 ± 352 | 3,063 ± 69 | 2,641 ± 267 | 3,086 ± 194 | 2,857 ± 325 | 2,529 ± 300 |
| PHx | | 1,743 ± 497 | 1,607 ± 128 | 2,482 ± 552 | 3,679 ± 412 | 3,759 ± 50[a] | 3,728 ± 256 | 3,441 ± 221 | 2,400 ± 202 | 2,869 ± 437 | 2,427 ± 161 |

Each value represents the mean ± SEM for 3 or 4 rats

*Met* methionine, *Cyst* cystathionine, *Cys* cysteine, *HT* hypotaurine, *Tau* taurine, *ND* not determined

[a, b, c] Significantly different from the sham control ($P < 0.05$, 0.01, and 0.001, respectively)

**Table 3** Changes in polyamines in liver after PHx

| | Time after PHx | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 3 h | 6 h | 9 h | 12 h | 24 h | 48 h | 96 h | 168 h | 240 h | 336 h |
| ORNT (nmol/g liver) | | | | | | | | | | | |
| Sham | 326 ± 85 | 468 ± 41 | 268 ± 60 | 332 ± 27 | 244 ± 22 | 429 ± 33 | 376 ± 51 | 271 ± 43 | 392 ± 12 | 408 ± 15 | 366 ± 56 |
| PHx | | 1,512 ± 204[b] | 1,169 ± 208[a] | 1,904 ± 350[a] | 1,773 ± 148[c] | 1,352 ± 117[b] | 976 ± 258 | 652 ± 46[b] | 510 ± 25[a] | 433 ± 109 | 423 ± 30 |
| PT (nmol/g liver) | | | | | | | | | | | |
| Sham | 4.5 ± 1.5 | 3.0 ± 0.3 | 2.4 ± 1.0 | 3.3 ± 1.3 | 2.7 ± 0.6 | 2.3 ± 0.8 | 3.2 ± 1.0 | 2.1 ± 0.6 | 2.1 ± 0.6 | 2.1 ± 0.6 | ND |
| PHx | | 26.3 ± 4.6[b] | 105.7 ± 19.1[b] | 143.1 ± 8.3[c] | 112.0 ± 15.8[b] | 43.8 ± 8.8[b] | 27.1 ± 6.2[a] | 16.6 ± 2.3[b] | 6.8 ± 3.3 | 5.1 ± 2.7 | ND |
| SPMD (nmol/g liver) | | | | | | | | | | | |
| Sham | 442 ± 6 | 452 ± 2 | 451 ± 9 | 435 ± 16 | 424 ± 11 | 419 ± 18 | 416 ± 9 | 431 ± 43 | 454 ± 10 | 409 ± 13 | ND |
| PHx | | 499 ± 17 | 530 ± 10[b] | 541 ± 10[b] | 553 ± 5[c] | 646 ± 24[b] | 679 ± 51[b] | 777 ± 40[b] | 676 ± 41[b] | 645 ± 50[a] | ND |
| SPM (nmol/g liver) | | | | | | | | | | | |
| Sham | 335 ± 22 | 343 ± 4 | 369 ± 15 | 350 ± 18 | 361 ± 12 | 336 ± 17 | 359 ± 16 | 363 ± 19 | 345 ± 10 | 360 ± 9 | ND |
| PHx | | 385 ± 7[b] | 325 ± 11 | 291 ± 13[a] | 282 ± 10[b] | 199 ± 13[b] | 209 ± 13[b] | 240 ± 5[b] | 287 ± 17[a] | 302 ± 23 | ND |

Each value represents the mean ± SEM for 3 or 4 rats

*ORNT* ornithine, *PT* putrescine, *SPMD* spermidine, *SPM* spermine, *ND* not determined

[a, b, c] Significantly different from the sham control ($P < 0.05$, 0.01, and 0.001, respectively)

sulfate and taurine (Kwon and Stipanuk 2001; Stipanuk et al. 2006).

It has been shown that hepatic SAM level changes markedly in regenerating liver (Huang et al. 1998b). In partially hepatectomized rat livers both MATIA mRNA, which encodes liver specific MATI/III, and MATIIA mRNA, which is the gene for non-liver specific MATII, were induced rapidly, but MATIA decreased in 12 h, whereas MATIIA remained elevated for as long as 72 h. Similarly, the activity of MATI/III was increased in 3–6 h, but returned to normal thereafter whereas MATII activity was greater than normal for 3 days. Therefore, there seemed to be an early increase in the overall MAT activity after PHx. However, hepatic SAM level reduced rapidly by the surgery, reaching a level lower than normal in 6–24 h. The authors attributed the inconsistency between hepatic SAM level and MAT activities to increased SAM utilization for polyamine synthesis and feedback inhibition of the enzyme by its product, SAM (Huang et al. 1998b). The activity of MATI/III is inhibited by NO through nitrosylation of cysteine 121, which results in a reduction of hepatic SAM level (Avila et al. 1997). Accordingly, the transient decrease in SAM after PHx was suggested to be an essential part of the priming event for hepatocyte proliferation to occur, probably via the NO-mediated inhibition of MATI/III (García-Trevijano et al. 2002; Lu and Mato 2005). Similar increases in MAT1A and MATIIA mRNA expression, and MAT activity immediately after PHx were demonstrated in a study conducted by Frago et al. (1998). In the latter study, however, hepatic SAM level was rapidly elevated as well when determined at 3 h following the surgery. In the present experiments hepatic SAM started to increase immediately after PHx, reaching a level significantly greater than baseline in 9 h, which is in agreement with the results of Frago et al. (1998). The reason for the discrepancy in the early change in hepatic SAM level after PHx remains unknown.

The present results suggest that the increased methionine availability and MAT activity in the hepatectomized rats may be directly responsible for the elevation of hepatic SAM and its demethylated product, SAH. Hepatic homocysteine concentration was not measured in this study, but the marked increases in cystathionine and cysteine imply that the metabolic reactions implicated in the transsulfuration pathway are mostly enhanced by the surgery. Of the two major metabolites generated from cysteine, the increase in GSH was delayed and less significant in comparison with its metabolic precursors, cystathionine and cysteine. Instead, hypotaurine was increased markedly, indicating that cysteine is preferentially converted to this substance rather than GSH in regenerating liver. However, taurine in liver was not elevated as much as its precursor, hypotaurine, which is probably due to a rapid transport of this $\beta$-amino acid into plasma. Enhancement of cysteine catabolism via the taurine pathway under high cysteine availability is in agreement with the study results of Stipanuk and colleagues (Kwon and Stipanuk 2001; Stipanuk et al. 2006). The physiological significance of increased hypotaurine generation during liver regeneration remains to be studied.

It has been known that the metabolism of polyamines is closely associated with cell proliferation and differentiation. Stimulation of polyamine synthesis was shown to precede the increase in synthesis of DNA, RNA, and protein (Heby 1981; Jänne et al. 1991). Likewise, cell proliferation was inhibited in the absence of sufficient polyamine pool, suggesting that polyamines may have an essential role in proper commencement of liver regeneration (Rasanen et al. 2002). The molecular mechanism of polyamine action in the cell growth is still largely unknown, except that spermidine, but not spermine, serves as the sole precursor for hypusine, an integral component of eukaryotic translation initiation factor 5A (eIF5A), which is the only protein known to contain this unusual amino acid residue (Park et al. 1981, 2006). Accumulating evidence not only reinforces a role for eIF5A in translation but also strongly supports its function in the elongation step of protein synthesis (Gregio et al. 2009; Saini et al. 2009). In this study PHx markedly elevated hepatic level of ornithine, the metabolic precursor of putrescine. Hepatic putrescine concentration was increased immensely, reaching a level greater than 40 times of normal. Spermidine, which is synthesized via addition of an aminopropyl group derived from dcSAM to putrescine, was also increased, but spermine, which also requires an aminopropyl group of dcSAM for its biosynthesis, was rather decreased by the surgery. This may be related to an increment in the need of dcSAM for production of spermidine, at the expense of spermine, during the process of liver regeneration.

In summary, the present results show that there are significant alterations in the metabolomics of sulfur-containing amino acids and related substances in regenerating liver. Most changes are persistent until the liver regains its original weight, suggesting their implication in the progression of liver regeneration. Hepatic methionine, SAM, SAH, cystathionine, and cysteine levels are all elevated markedly after the surgery. Cysteine catabolism to hypotaurine is enhanced rapidly whereas the increase in GSH level is delayed, indicating that cysteine is utilized preferentially for hypotaurine production during liver regeneration. Also the changes in the metabolomics of sulfur-containing substances are closely related to the metabolism of polyamines in the remnant liver. Particularly, the elevation of SAM availability appears to account for the increase in generation of spermidine, which is needed for the formation of eIF5A involved in protein biosynthesis.

Further studies on the cellular regulation of polyamine synthesis by sulfur-containing substances in liver regeneration are warranted.

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