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Gender differences in methionine accumulation and metabolism in freshly isolated mouse hepatocytes: Potential roles in toxicity

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ABSTRACT

L-Methionine (Met) is hepatotoxic at high concentrations. Because Met toxicity in freshly isolated mouse hepatocytes is gender-dependent, the goal of this study was to assess the roles of Met accumulation and metabolism in the increased sensitivity of male hepatocytes to Met toxicity compared with female hepatocytes. Male hepatocytes incubated with Met (30 mM) at 37 °C exhibited higher levels of intracellular Met at 0.5, 1.0, and 1.5 h, respectively, compared to female hepatocytes. Conversely, female hepatocytes had higher levels of *S*-adenosyl-L-methionine compared to male hepatocytes. Female hepatocytes also exhibited higher L-methionine-L-sulfoxide levels relative to control hepatocytes, whereas the increases in L-methionine-D-sulfoxide (Met-D-O) levels were similar in hepatocytes of both genders. Addition of aminooxyacetic acid (AOAA), an inhibitor of Met transamination, significantly increased Met levels at 1.5 h and increased Met-D-O levels at 1.0 and 1.5 h only in Met-exposed male hepatocytes. No gender differences in cytosolic Met transamination activity by glutamine transaminase K were detected. However, female mouse liver cytosol exhibited higher methionine-DL-sulfoxide (MetO) reductase activity than male mouse liver cytosol at low (0.25 and 0.5 mM) MetO concentrations. Collectively, these results suggest that increased cellular Met accumulation, decreased Met transmethylation, and increased Met and MetO transamination in male mouse hepatocytes may be contributing to the higher sensitivity of the male mouse hepatocytes to Met toxicity in comparison with female mouse hepatocytes.

Introduction

L-Methionine (Met), while an essential amino acid, is hepatotoxic when present at high concentrations. Met toxicity has been linked to total parenteral nutrition-associated cholestasis in infants (Moss et al., 1999) and may exacerbate hepatocellular necrosis and fibrogenesis in patients with chronic liver disease who often develop hypermethionemia (Finkelstein, 2003). Laboratory animals dosed with or fed high levels of Met may develop cholestatic liver disease (Moss et al., 1999; Shinozuka et al., 1971), hepatocellular ATP and GSH depletion (Shinozuka et al., 1971; Cox et al., 1973; Heyman et al., 1984), and exhibit increased markers of lipid peroxidation (Mori and Hirayama, 2000). In mice, Met adenosyltransferase (MAT) 1A knockout strains are hypermethionemic, have reduced GSH levels and altered gene expression, and are more prone to oxidative stress in the liver than wild type mice (Lu et al., 2001; Martinez-chantar et al., 2002). The mechanisms responsible for Met-induced hepatotoxicity, however, are not clear.

Our laboratory recently used freshly isolated mouse hepatocytes (FIMHs) to investigate Met toxicity. The results obtained regarding Met cytotoxicity in this model are summarized in Table 1. In male mouse hepatocytes, increased lactate dehydrogenase (LDH) leakage and decreased trypan blue (TB) exclusion, preceded by GSH depletion, were detected at Met concentrations ≥ 20 mM (Dever and Elfarra, 2008a). In contrast, female hepatocytes were completely insensitive to Met toxicity at Met concentrations as high as 30 mM and had increased cellular GSH levels compared to female hepatocytes incubated with vehicle alone. Addition of 3-deazaadenosine (3-DA), an inhibitor of *S*-adenosyl-L-homocysteine (SAH) hydrolase and the Met transmethylation (TM) pathway (Chiang et al., 1977) (Fig. 1) potentiated Met toxicity in male FIMHs while addition of aminooxyacetic acid (AOAA), an inhibitor of Met transamination (TA) (Mitchell and Benevenga, 1978) partially reduced Met toxicity (Fig. 2) (Dever and Elfarra, 2008a). The effect of AOAA and 3-DA were not investigated in female FIMHs due to the lack of toxicity of Met in

Abbreviations: Met, L-methionine; Met-D-O, L-methionine-D-sulfoxide; Met-L-O, L-methionine-L-sulfoxide; MetO, L-methionine-DL-sulfoxide; MsrA, methionine sulphoxide reductase A; MAT, methionine adenosyltransferase; SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; 3-MTP, 3-methylthiopropionic acid; GSH, glutathione; GSSG, glutathione disulfide; DTT, dithiothreitol; FIMHs, freshly isolated mouse hepatocytes; ATP, adenosine triphosphate; 3-DA, 3-deaazadenosine; AOAA, aminooxyacetic acid; TA, transamination; TM, transmethylation; SO, sulfoxidation; TB, trypan blue; LDH, lactate dehydrogenase; PhP, phenylpyruvate; DMEM, Dulbecco's modified Eagle's Medium; AUC, area under the curve; HPLC, high performance liquid chromatography.

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Table 1
Time course of the effect of Met (30 mM) with or without AOAA (0.2 mM) on the viability of male or female mouse FIMHs as measured by LDH leakage

Gender	% Viability			
	2 h	3 h	4 h	5 h
Female				
Vehicle alone	79 ± 7[a]	68 ± 13	67 ± 5	61 ± 1
Met	78 ± 2	69 ± 3	65 ± 8	65 ± 8
Male				
Vehicle alone	83 ± 4	75 ± 9	70 ± 11	60 ± 14
Met	66 ± 11[b]	45 ± 14[b]	26 ± 19[b]	20 ± 12[b]
Met + AOAA	74 ± 8	67 ± 8[c]	50 ± 22	40 ± 17

These data are adapted from a previous publication (Dever and Elfarra, 2008a).
[a] Data are expressed as mean ± SD ($n = 3$–4).
[b] Values are significantly lower than the corresponding values obtained in FIMHs of the same gender exposed to vehicle alone ($p < 0.05$).
[c] Values are significantly higher than the corresponding values obtained in FIMHs of the same gender incubated with Met only ($p < 0.05$).

that gender. These results and the finding that 3-methylthiopropionic acid (3-MTP) was nearly 100-fold more cytotoxic than Met in male FIMHs (Dever and Elfarra, 2008a) provided evidence for the involvement of Met TA metabolites in the cytotoxicity of Met. However, the biochemical basis for female FIMHs being less sensitive to Met toxicity was unclear. Since GSH depletion was detected in female FIMHs exposed to 0.3 mM 3-MTP, but not 30 mM Met, lack of 3-MTP formation rather than resistance to 3-MTP toxicity may have been a factor. Thus, events upstream of 3-MTP formation may have been contributing to the lower sensitivity of female FIMHs to Met toxicity in comparison with male mouse hepatocytes.

Met sulfoxidation (SO) was also a significant metabolic pathway in Met-dosed male and female mice with methionine-D-sulfoxide (Met-D-O) being the primary diastereomer detected in both genders (Dever and Elfarra, 2006). Methionine-DL-sulfoxide (MetO) toxicity in male FIMHs was characterized by increased LDH leakage, decreased TB exclusion, and GSH depletion at MetO concentrations ≥20 mM whereas female FIMHs were completely resistant to MetO

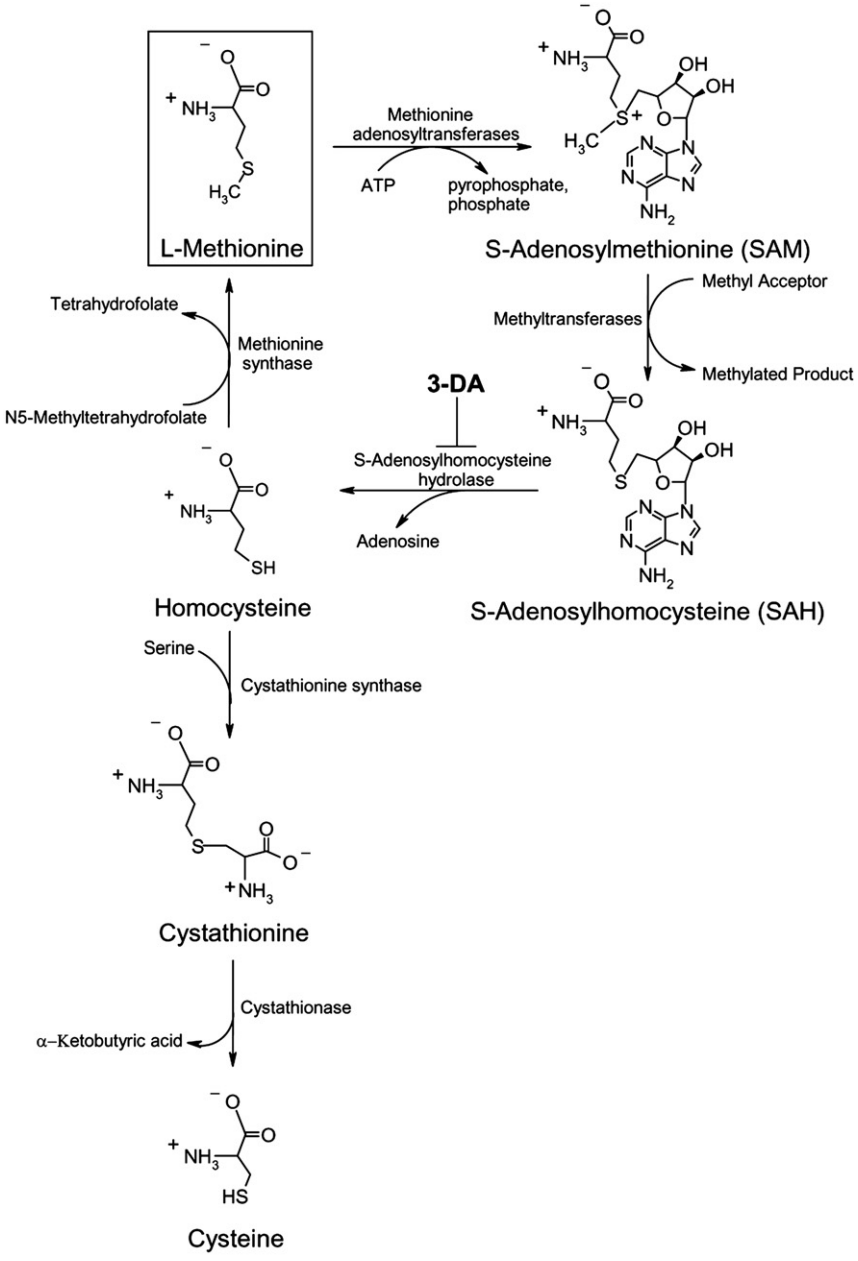

Fig. 1. Schematic of the Met transmethylation pathway, a proposed detoxification pathway for excess Met in FIMHs.