Gene expression profiles of murine fatty liver induced by the administration of methotrexate

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ARTICLE INFO

ABSTRACT

Methotrexate (MTX) is used to treat a variety of chronic inflammatory and neoplastic diseases. However, it can induce hepatotoxicity such as microvesicular steatosis and necrosis. To explore the mechanisms of MTX-induced hepatic steatosis, we used microarray analysis to profile the gene expression patterns of mouse liver after MTX treatment. MTX was administered orally as a single dose of 10 mg/kg (low dose) or 100 mg/kg (high dose) to ICR mice, and the livers were obtained 6 h, 24 h, and 72 h after treatment. Serum alanine aminotransferase, aspartate aminotransferase and triacylglycerol levels were not significantly altered in the experimental animals. Signs of steatosis were observed at 24 h after administration of high dose of MTX. From microarray data analysis, 908 genes were selected as MTX-responsive genes ($P < 0.05$, two-way ANOVA; cutoff ≥ 1.5-fold). Database for Annotation, Visualization and Integrated Discovery (DAVID) analysis revealed that the predominant biological processes associated with these genes are response to unfolded proteins, phosphate metabolism, and cellular lipid metabolism. Functional categorization of these genes identified 28 genes involved in lipid metabolism that was interconnected with the biological pathways of biosynthesis, catabolism, and transport of lipids and fatty acids. Taken together, these data provide a better understanding of the molecular mechanisms of MTX-induced steatogenic hepatotoxicity, and useful information for predicting hepatotoxicity through pattern recognition.

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1. Introduction

Methotrexate (MTX) is a synthetic analogue of dihydrofolate which is a potent inhibitor of dihydrofolate reductase catalyzing a key step in the production of tetrahydrofolate. Because tetrahydrofolate is required for the biosynthesis of purine nucleotides, MTX inhibits DNA synthesis and thereby blocks growth of rapidly dividing cells. Thus, MTX is used as a chemotherapeutic agent for a variety of human malignancies (Huennekens, 1994). MTX is the most frequently used anti-inflammatory drug for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis (Genestier et al., 2000). However, long-term therapy or a high cumulative dose of MTX is associated with an increased risk of liver injury (Richard et al., 2000). The histological features of MTX-induced hepatotoxicity include microvesicular steatosis, portal tract inflammation, focal liver cell necrosis, and fibrosis in the pericellular and portal tract regions (Kevat et al., 1988; Ahern et al., 1998; Richard et al., 2000), that resembles non-alcoholic steatohepatitis (NASH) (Langman et al., 2001). Therefore, the American Academy of Dermatology recommends monitoring hepatic safety with regular liver biopsies after an initial cumulative dose of 1500 mg of MTX, in patients with psoriasis (Roenigk et al., 1988). Although a clear relationship between hepatic injury and cumulative dose of MTX in patients, the underlying mechanisms that contribute to MTX-induced hepatic steatosis have not been studied

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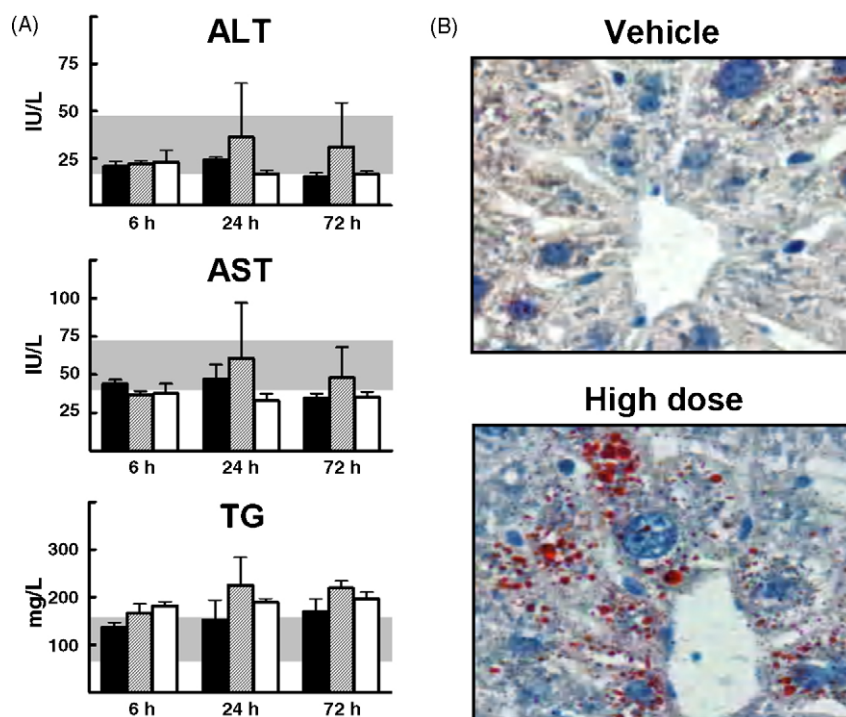E-mail address: molee@snu.ac.kr (M.-O. Lee).

Fig. 1. (A) Serum ALT, AST and TG levels after exposure to MTX. Shade area indicates the normal reference value (ALT 21–48 U/l, AST 40–71 U/l, and TG 60–143 mg/dl) (Song et al., 1989). (■) Vehicle, (▨) low dose, and (□) high dose. Result are mean ± S.D. (n = 3). (B) Histological assessment of hepatic steatosis after 24 h exposure to high dose MTX. Photomicrographs of Oil red O stained liver sections form a vehicle and high-dose MTX-treated mouse (400× magnification).

thoroughly. Variable dosage regimens and pre-existing liver conditions such as alcohol abuse, diabetes mellitus, and obesity make it difficult or impossible to ascribe the hepatotoxicity to MTX alone.

Drug-induced hepatotoxicity is an important healthcare issue because of the associated mortality and morbidity, which are difficult to predict (Kaplowitz, 2001). Evaluating the mechanism responsible for drug-induced hepatotoxicity is important and necessary for identifying risk factors and developing appropriate treatment regimens. Recently developed microarray assays provide highly sensitive and informative markers of toxicity and new information about the mechanism of action by analysing gene expression patterns provoked by toxicants (Nuwaysir et al., 1999). A large-scale analysis of gene expression profiling of hepatotoxicants using rat hepatocytes showed a correlation between the gene expression pattern and mechanism of toxicity (Waring et al., 2001a). Further hepatotoxicants could be clustered based on their mechanisms of toxicity using gene expression profiles in animal model (Waring et al., 2001b).

Here, we profiled the gene expression patterns of MTX-induced hepatic steatosis that is accompanied by clear histopathology of hepatic steatosis in a mouse model. Our gene expression profiling data show significant changes in the expression of genes important in the biosynthesis and catabolism of lipids and fatty acids. We compared our data with the gene expression profiles associated with other fatty liver-inducing drugs such as valproic acid and tetracycline. This information will be useful for developing tools to predict toxicity of unknown chemicals or new drug candidates, which will eventually contribute to improved processes for risk assessment and safety evaluation.

2. Materials and methods

2.1. Animals and study design

Male ICR mice, 6 weeks of age, were obtained from Japan SLC Inc. (Hamamatsu, Japan). The mice were housed three per cage in polycarbonate cages, and commercial

mouse chow (Certified Rodent Diet 5002; Purina Mills Inc., St. Louis, MO) and water were supplied *ad libitum*. The animal laboratory was located at the Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University (Seoul, Korea). It was maintained at a temperature of 20–23 °C, and humidity of 30–48%, with 12 h light and dark cycles. Upon arrival at the animal laboratory, the mice were allowed at least 4 days to acclimatize. Food was withdrawn for 4 h before and was resupplied 2 h after MTX administration. MTX was obtained from Sigma Chemical Co. (St. Louis, MO) and suspended in 0.1% carboxymethyl cellulose. Mice (n = 3) were dosed by oral gavage with 10 mg/kg and 100 mg/kg in 400 μl, and the livers were obtained at 6 h, 24 h, or 72 h after drug treatment. The doses of 10 mg/kg and 100 mg/kg were selected as low dose and high dose, respectively, by range-finding studies. High dose was determined as a minimal dose that induced microvesicular
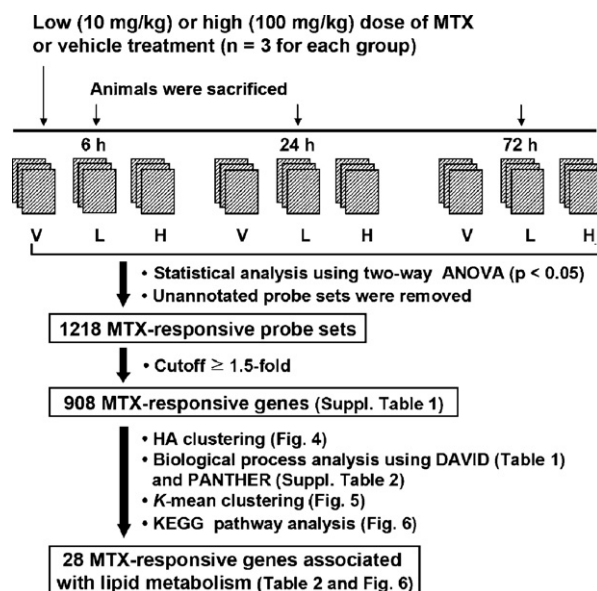

Fig. 2. Schematic representation of the experimental design and flow chart for this microarray data analysis.