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Title of research project	Establishment and refinement of novel evaluation system of hepatotoxicity, based on functional properties of hepatic macrophages
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【Abstract】

Hepatic macrophages (including Kupffer cells), consisting of 20% of liver elements, play an important role in homeostasis. The functional abnormalities of hepatic macrophages may primarily or secondarily influence chemically-induced hepatotoxicity. However, the evaluation system based on the characteristics of their functions is not yet established and mechanisms whereby hepatotoxicity is induced is not fully elucidated. Recently, a new concept of M1/M2-polarization was proposed for reactive macrophages; M1 type is induced by INF-γ showing high phagocytic activity, while M2 type is induced by IL-4 playing roles in reparative fibrosis by releasing IL-10 and TGF-β1. The purpose of this study is to establish a novel system for evaluation of chemically-induced hepatotoxicity, and to apply the evaluation system to elucidate mechanisms whereby chemicals exert hepatotoxicity through M1/M2-polarization macrophages. During hepatogenesis, CD68 M1 macrophages predominantly existed in embryos, whereas CD163 M2 Kupffer cells appeared along the sinusoids in neonates. These findings suggested different roles of macrophages for development of the liver. Treatment of the livers with liposome decreased the AST and ALT values by activating CD163 Kupffer cells, whereas treatment with clodronate resulted Kupffer cell-depletion and increase in the AST and ALT values. These data indicate that Kupffer cells are involved in clearance of liver enzymes, thus a condition of macrophages should be taken into consideration when hepatotoxicity is analyzed. In thioacetamide (TAA)-induced hepatic lesions, expression of INF-γ, THF-α and IL-6 that are related to M1 functions, and expression of IL-4 that is related to M2 functions were increased prior to histopathological changes. Then the expression of CD68 M1 and CD163 M2 types followed in injured centrilobular regions, and TGF-β1 and IL-10 were increased for reparative fibrosis. CD68 M1 type co-expressed MHC class II and Iba-1, whereas CD163 M2 type reacted to CD204 and Galectin-3. Under macrophage depletion by clodronate, TAA-treated livers showed delay of coagulation necrosis, and then developed dystrophic calcification. In α-naphthylisothiocyanate (ANIT)-induced bile epithelial injury, MHC class II-macrophages appeared exclusively, and the fibrosis was delayed in ANIT-induced lesions in Glisson's sheath. These findings indicated that different macrophages play distinct roles in TAA-induced centrilobular lesions (CD68 and D163) or ANIT-induced Glisson's sheath (MHC class II), and that depletion of hepatic macrophages influences hepatic lesion development. The *in vitro* addition of INF-γ for M1 and IL-4 for M2 to HS-P (rat macrophage cell line) well reflected conditions of *in vivo* hepatotoxicity, indicating usefulness of HS-P for mechanism analysis. In conclusion, this study provided novel information for the analysis methods of hepatotoxicity based on M1/M2 macrophage polarization. Relevant system would lead to refinement of ADI specification in the risk

assessment on food safety.