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論　文　内　容　要　旨

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学位論文題目	Studies on evaluation of bilirubin glucuronidation activity using hepatoma cell lines （肝癌細胞株を用いたビリルビングルクロン酸抱合活性評価に関する研究）		

内容要旨

In the first experiment, we conducted experiments to analyze and compare the bilirubin glucuronidation activity of HepG2 cells cultured in the usual 2D plates and 3D culture systems to exhibit the efficiency of the 3D-cultured hepatoma cell line for evaluation of bilirubin metabolism features. In this experiment, we found that bilirubin mono-glucuronide was detected at 1 h after initiation of the incubation in both 2D- and 3D-cultured HepG2 cells and the relative amounts of bilirubin mono-glucuronides elevated in a time-dependent manner up to 72 h especially in 3D-cultured HepG2 cells. The relative amounts of conjugated bilirubin in the HepG2 cells cultured by 3D culture systems significantly increased as compared with those generated by 2D plates after 10 h of incubation. Although bilirubin glucuronides were detected after 1 h-incubation, we judged that the amounts of glucuronides were too low to compare the bilirubin glucuronidation activity and to evaluate the utility of the 3D-culture systems. Therefore, in this experiment, we set longer incubation time up to 72 h to demonstrate the difference between 2D and 3D-culture conditions. Consequently, the HepG2 cells cultured by the 3D culture systems exhibited a higher metabolic activity of bilirubin glucuronidation than those by 2D culture plates, and we confirmed the efficacy of the 3D culture systems for the evaluation of bilirubin glucuronidation activity using hepatoma cell lines. It is crucial to establish the easy-to-use in vitro culture system for the comprehensive evaluation of bilirubin glucuronidation because it is well known that the impairment of bilirubin metabolism is related to many clinical outcome and detailed exploration for bilirubin metabolism can help to reveal these characteristics. With this evaluation system of 3D culture, we can conduct a detailed study for bilirubin glucuronidation easily using hepatoma cell lines.
In the second experiment, we tried to compare the bilirubin glucuronidation activity between humans and dogs by the microsome experiments and the experiment using the 3D culture systems established in the first experiment. In the microsomes experiment, bilirubin glucuronidation activity in DLMs was lower than that of HLMs in consistency with previous reports. In the 3D culture experiment, although bilir

ubin mono-glucuronide was generated in both HepG2 and canine hepatoma cells, r emaining bilirubin in canine hepatoma cell culture medium obviously decreased es pecially until 10 h after incubation. In contrast, the amount of remaining bilirubin in the HepG2 culture medium was almost stable throughout the culture period. The oretically, remaining bilirubin should change little in amounts because the amount of applied bilirubin is much larger than generated bilirubin glucuronides in this sy stem. Therefore, the behavior for the amount of residual bilirubin in canine hepato ma cell culture medium was not reasonable, and the conjugated bilirubin detected i n the culture medium of canine hepatoma cells might not reflect the correct activit y of bilirubin catabolism of the cells. The cell culture and bilirubin glucuronidatio n assay conditions need to be optimized further because there were adhesion of cel ls in the interstitial area between spheroids and also suspended cells that could aff ect the behavior of bilirubin and bilirubin glucuronides in canine hepatoma cell cu lture medium.

It is important to understand the similarities and differences in metabolism propert ies between humans and animals to extrapolate the results of non-clinical toxicity studies to humans in pharmaceutical compound development. In addition, if we co uld reveal the mechanism of species difference at the level of gene or protein, an e xtrapolation of animal data to humans would be more accurate. Regarding bilirubi n glucuronidation activity, the dog has been reported previously to have lower acti vity than human, and we have reconfirmed that in the microsome experiment of thi s study. Some genetic polymorphisms of UGT1A1 (enzyme for bilirubin glucuroni dation) have been reported in humans. For example, UGT1A1*6, a single nucleotid e substitution causing a missense mutation (Gly71Arg), is a common polymorphis m that causes a lower bilirubin glucuronidation activity. The 71th amino acid of U GT1A1 in the dog is also different from human, and the difference in the 71th ami no acid of UGT1A1 might be involved in lower bilirubin glucuronidation activity i n dogs. We believe that the 3D culture systems for bilirubin glucuronidation evalu ation using hepatoma cell lines will be helpful for the further experiments that add ress the detail for species difference at the level of gene or protein because hepato ma cell lines, including HepG2, are generally easier to obtain and handle than pri mary hepatocytes.