

## 論文内容要旨

報告番号	甲 先 第 , 465 号	氏 名	大森 久嘉
学位論文題目	Studies on evaluation of bilirubin glucuronidation activity in canine and human primary hepatocytes cultured in a 3D culture system (3次元培養系で培養したイヌおよびヒト初代肝細胞におけるビリルビングルクロン酸抱合活性の評価に関する研究)		
<p>内容要旨</p> <p>Species differences in bilirubin glucuronidation activity are observed between humans and dogs through liver microsomes and recombinant UDP-glucuronosyltransferase 1A1. Humans exhibit higher activity than that of dogs. In this study (Heading 2: Omori et al., 2022), bilirubin glucuronidation activity was examined in canine and human primary hepatocyte spheroids formed using a 3D culture system. When spheroid development in canine and human primary hepatocytes was evaluated on days 7 and 14 after the start of culture, canine primary hepatocyte spheroids had a more distinct spherical shape than human hepatocyte spheroids, irrespective of the culture period. Furthermore, mono- and di-glucuronide generation detected in spheroids were significantly higher (<math>P &lt; 0.05</math>) in human primary hepatocytes than in canine primary hepatocytes after 24 hours of incubation with bilirubin for each culture period. These results suggest that there are species differences in the bilirubin glucuronidation activity of primary hepatocytes with spheroid formation between humans and dogs, with the activity being higher in humans than in dogs.</p> <p>Bilirubin is excreted into the bile from hepatocytes, mainly as monoglucuronosyl and bisglucuronosyl conjugates, reflecting bilirubin glucuronidation activity. However, there is limited information on the in vitro evaluation of liver cell lines or primary hepatocytes. This study (Heading 3: Omori et al., 2023) aimed to investigate variations in the bilirubin metabolic function of canine and human hepatocyte spheroids formed in a three-dimensional (3D) culture system indicated by the formation of bilirubin glucuronides when protease inhibitors such as atazanavir, indinavir, ritonavir, and nelfinavir were treated with bilirubin. The culture supernatant was collected for bilirubin glucuronidation assessment, and the cells were used to evaluate viability. On day 8 of culture, both canine and human hepatocyte spheroids showed high albumin secretion and distinct spheroid formation, and their bilirubin glucuronidation</p>			

activities were evaluated considering cell viability. Treatment with atazanavir and ritonavir remarkably inhibited bilirubin glucuronide formation, wherein atazanavir showed the highest inhibition, particularly in human hepatocyte spheroids. These results may reflect the effects on cellular uptake of bilirubin and its intracellular metabolic function. Thus, primary hepatocytes cultured in a 3D culture system may be a useful in vitro system for the comprehensive evaluation of bilirubin metabolic function and risk assessment in bilirubin metabolic disorders for drug development.