Species differences of plasma esterase expression pattern and hydrolase activity were studied. By native polyacrylamide gel electrophoresis (PAGE), human, rhesus monkey, cynomolgus monkey and dog had shown two bands corresponding to paraoxonase (PON) and butyrylcholinesterase (BChE) while rabbit, rat and mouse had shown three bands including carboxylesterase (CES). PON is closely associated with HDL (high density lipoproteins) and is also calcium dependent. Hence, PON has slower mobility than BChE and by adding Ca\(^{2+}\) ions we can get more esterase activity which means more strong bands. For defining the substrate specificity, temocapril and propranolol derivatives were used. Temocapril hydrolase activity was considerably high in the mouse and rat plasma contrary to other plasma samples. From the inhibition experiment by using suitable inhibitors [ethopropazine and Bis[lp-nitrophenyl]phosphate (BNPP)], it was seen that both BChE and CES involved in the activity of mouse plasma. However, CES was the only effective enzyme in rat. Although rabbit plasma had CES, interestingly they did not show any activity for temocapril. Propranolol derivatives were highly hydrolyzed with R-preferential in all samples except human and dog plasma. In mouse and rat, both CES and BChE enzymes were involved in hydrolase activity but BChE had lower effect than CES. It was amazingly defined that monkey plasma had propranolol activity due to BChE enzyme. Nevertheless, human BChE enzyme didn’t show any activity for these two substrates. From these findings, it is clear that substrate specific hydrolase activities show differences at each animal and such differences must be considered when researching ester-type drugs intended for human use.

**[Purpose]** Simvastatin, a cholesterol-lowering drug, is a lactone ring-containing prodrug which undergoes hydrolysis to form an active hydroxyl acid. It has been reported that simvastatin hydrolysis is catalyzed by paraoxonase (PON) enzymes, which require Ca\(^{2+}\) to exert their activities. PONs are expressed in the liver and plasma. It has been reported that simvastatin hydrolysis in rat plasma is remarkably higher than in human plasma. In this study, we investigated whether PON is the only enzyme responsible for simvastatin hydrolysis in both liver and plasma, and explain the species differences between human and rat.

**[Methods]** We established baculovirus expression systems for human PON1, PON2, and PON3. Simvastatin hydrolase activities in liver microsomes, plasma, and serum albumin from human and rat as well as the recombinant human PONs were measured by HPLC. Inhibition studies were performed to characterize the enzymes.

**[Results and Discussion]** Among recombinant human PONs, only PON3 showed the simvastatin hydrolyase activity. Human and rat liver microsomes efficiently hydrolyzed simvastatin, and the activities were stimulated by the addition of CaCl\(_2\). In contrast, the activities in plasma were not affected by CaCl\(_2\) and EDTA. These results suggested that PON3 is responsible for the simvastatin hydrolysis in liver microsomes, but not in plasma. We found that serum albumin could hydrolyze simvastatin in human and rat. The simvastatin hydrolysis in rat plasma, but not in human plasma, was inhibited by DFP, a serine hydrolase inhibitor, indicating the involvement of B-esterase(s) in rat plasma. Collectively, it was predicted that the serum albumin would be the sole and major enzyme in human and rat plasma, respectively.

**[Conclusions]** We thoroughly characterized the enzymes involved in simvastatin hydrolysis in liver and plasma, which can explain the species differences between human and rat.