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Title of research project	Development of a risk assessment model based on the extrapolation of the metabolic profile of humanized-liver chimeric mice
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【Abstract】

Purpose

To improve the accuracy of risk assessment, it is important to understand how hazardous substances are metabolized in humans. However, the properties of drug-metabolizing enzymes are known to be very different between humans and rodents. To overcome this problem, we performed toxicity assessments of hazardous substances using humanized-liver chimeric mice, in which drug metabolism is similar to that in humans.

Methods

Male TK-NOG mice were injected intraperitoneally with ganciclovir (GCV) to ablate liver cells expressing the HSVtk transgene. One week after GCV treatment, the degree of liver damage was examined by determining serum alanine aminotransferase levels. Cryopreserved human hepatocytes (1.0×10^6cells) were injected intrasplenically using a Hamilton syringe with a 26-G needle. The successful engraftment of the hepatocytes was evaluated by measuring increases in the blood levels of human albumin (hAlb). The human-equivalent disposition and toxicity of two organophosphate pesticides (acephate and chlorpyrifos) and the synthetic growth promoter melengestrol acetate were evaluated using humanized-liver TK-NOG mice.

Results

When 300 mg/kg acephate was administered orally, the plasma concentration of acephate and methamidophos, which is the active metabolite of acephate, in humanized-liver TK-NOG mice was not significantly different from that of non-humanized TK-NOG mice. Chlorpyrifos and trichloropyridinol were significantly higher in humanized-liver TK-NOG chimeric mice than in control non-humanized TK-NOG mice at 0.5 and 2 h after the oral administration of chlorpyrifos (30mg/kg). Chlorpyrifos-oxone was detected in neither humanized-liver TK-NOG mice nor non-humanized TK-NOG mice. Melengestrol acetate elimination in humans was estimated to be slow compared with its elimination in rodents. Compared to non-humanized TK-NOG mice, humanized-liver TK-NOG mice had higher butyrylcholinesterase activity, at levels comparable to that of the normal range in humans. Butyrylcholinesterase activity in humanized-liver TK-NOG mice was inhibited by the

oral administration of acephate and chlorpyrifos in a dose-dependent manner.

Discussion

In our study of the disposition of chlorpyrifos and melengestrol acetate, we observed differences in the plasma concentration of each compound between humanized-liver TK-NOG mice and non-humanized TK-NOG mice. The higher plasma concentration of chlorpyrifos and melengestrol acetate in humanized-liver TK-NOG mice indicated the possibility that they may accumulate in humans. In this case, a species differences in their metabolism should be considered. Conversely, because there was no significant difference in acephate metabolism between humanized and non-humanized mice, consideration of a species difference would be sufficient with a safety factor of 10. In the present study, we found new uses for humanized-liver TK-NOG mice other than drug metabolism research by using “species differences.” Specifically, we found that cholinesterase, which is produced and secreted into the blood by human hepatocytes in humanized-liver TK-NOG mice, almost reaches the level observed in humans, and its enzyme activity is inhibited by the oral administration of organophosphorus pesticides. These results indicate that the inhibition of serum cholinesterase activity by organophosphorus pesticides found in humanized-liver TK-NOG mice can be extrapolated to humans, and it could lead to the development of new risk assessment methods.

Conclusion

The results of this study, in which a humanized-liver TK-NOG mouse model was developed, can be widely used as the basis of an experimental animal model for the safety evaluation of food and drug products in Japan.