The objectives of this research are to investigate the inhibitory effects (including mechanism of inhibition) of polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) on cytochrome P450 3A (CYP3A) in addition to determine the oral bioavailability (BA) enhancing effect of DHA on a CYP3A substrate, i.e., midazolam (MDZ), in rats. Moreover, the BA enhancing effect of DHA ethyl ester (DHA-EE) when it was incorporated as an oil ingredient in microemulsion formulation of cyclosporin (CsA) was also examined.

The inhibition of 6β-hydroxy testosterone formation from testosterone in rat liver microsomes was used as an index of CYP3A inhibition, using a NADPH-generating system in vitro. In the present study, among saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and PUFAs, the rank order of inhibitory effects of fatty acids on CYP3A activity was SFAs < MUFAs < PUFAs. Among the four PUFAs examined (linoleic acid, γ-linolenic acid, retinoic acid, and DHA), the highest inhibitory effect (94%) of CYP3A-activity was observed with DHA. Moreover, the percentage of inhibition (I) of fatty acids on testosterone 6β-hydroxylation was predicted from the number of double bonds and the carbon chain length of the fatty acids. It was found that the number of double bonds, as well as the carbon chain lengths of fatty acids, is important in the inhibition of
CYP3A with a good correlation between the predicted and observed I values.

The effect of DHA on CYP3A activity in rat intestinal microsomes was examined using MDZ as a CYP3A substrate. Lineweaver-Burk plots for the inhibition of 1'-OH midazolam and 4-OH midazolam formations by DHA demonstrated that DHA competitively inhibited CYP3A in a concentration-dependent manner with the inhibition constant ($K_i$) of 15.7 and 27.1 µM, respectively. In order to demonstrate DHA's effect on gut metabolism, the perfused everted intestinal segments from rats was employed by using MDZ as a CYP3A-substrate. The intestinal extraction ratio ($ER_E$), a marker indicating the extent of metabolism relative to the amount of drugs which interacted with the enzyme, of midazolam was determined to be 0.43 and decreased significantly to 0.12, 0.07, and 0.06 in the presence of 50, 100, and 200 µM DHA, respectively. To determine the effect of DHA on P-gp activity in the gut, the everted intestinal segment models were employed. The results show that DHA did not change the rhodamine-123 transport rate from the mucosal to serosal side across the everted intestinal segment, indicating that DHA did not affect the P-gp function in the gut epithelial cells.

The effect of DHA on the pharmacokinetics of MDZ was evaluated after oral or intravenous (i.v.) administration of drug to rats. Oral co-administration of DHA and MDZ markedly increased the $AUC_\infty$ and $C_{max}$ in a dose-dependent manner with DHA, without affecting the $T_{1/2}$, $V_{des}/F$, or $CL_{tot}/F$ as compared with the control. Oral bioavailability ($F$) of MDZ was significantly increased approximately 2 folds in comparison to control group. In contrast, no pharmacokinetic interaction was observed when MDZ was intravenously administered in rats dosed orally with DHA, suggesting that DHA dose not affect CYP3A metabolism in the liver in vivo. In these experiments, it was demonstrated that DHA inhibits CYP3A in vitro and the inhibitory effect DHA on CYP3A in the intestinal lumen, resulting in an enhancement of MDZ bioavailability in rats.

In order to determine whether or not DHA-EE incorporated in the microemulsion exhibits the oral BA-enhancing effect, a microemulsion formulation of CsA incorporating DHA-EE was developed. The novel CsA microemulsion formulation consisted of Tween-20, ethanol, water and DHA-EE (53.3/6.5/35.9/3.3 w/w%) (namely DHA-ME). DHA-ME was transparent and stable with the average particle size of 50 nm which was similar to that of the control formulation which contained
vitamin E instead of DHA-EE (namely VE-ME). Moreover, the solubility and rate of permeability of CsA obtained from both formulations were not significantly different. After oral administration of each formulation into rats, DHA-ME increased CsA blood concentration as compared with VE-ME to a comparable level with Neoral® (a positive control). C_{max} and AUC_{t→∞} of CsA in rats administered with DHA-ME were significantly increased by approximately 2-fold as compared with that of VE-ME. The relative oral bioavailability (F_r) of DHA-ME as compared with Neoral® was determined to be 114% while F_r of VE-ME was 60%. It was thus suggested that the use of DHA-EE as an oil excipient may be promising to develop a microemulsion formulation of CsA with improved oral bioavailability.

This study, for the first time, demonstrated that DHA inhibits CYP3A-mediated metabolism in the small intestine resulting in the increase of CYP3A substrates’ bioavailability. These results suggest that DHA and DHA-EE might be a general BA-enhancer agent for CYP3A substrates when they are employed either as a separate additive or as an excipient of microemulsions.