# Interactions among the components in amphetamine-type stimulant tablets in human intestinal Caco-2 cells and in oral administration to rats

KENJI KUWAYAMA[1], KENJI TSUJIKAWA[1], HAJIME MIYAGUCHI[1], TATSUYUKI KANAMORI[1], YUKO IWATA[1], HIROYUKI INOUE[1], TOHRU KISHI[1], SEIJI MIYAUCHI[2] and NAOKI KAMO[2]

[1] National Research Institute of Police Science

[2] Laboratory of Biophysical Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University

**AIMS:** There has been a considerable increase in the number of seizures of amphetamine-type stimulant (ATS) tablets in Japan. The ATS tablets contain one or more of active ingredients, which include 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), methamphetamine (MA), etc.. In addition, the tablets often contain other components with hallucinogenic and/or stimulant effects such as ketamine and caffeine. Various components in such tablets might interact in the body, leading to serious toxic symptoms. Therefore, the prediction of interactions among drugs is important for public health and forensic analysis. We examined the interaction among the components in ATS tablets using Caco-2 cells derived from human colons and using rats in order to investigate the interactions in absorption process on digestive tract that was the first step of pharmacokinetics after oral intake.

**METHODS:** Caco-2 cells were cultivated on dishes for about one week and on transwell membranes for about three weeks for uptake and permeation experiments, respectively. *Wistar* rats (male, 200-250 g body weight) were used for the experiment of *in vivo* drug administration. For the uptake experiment, the compounds related with ATS tablets such as MDMA, MDA, MDEA, MA, ketamine, caffeine, etc. (0.1-10 mM) were applies to the surface of cells. After the cells were incubated at 37°C for varying times (0.5-30 min), compounds uptaken into the cells were extracted with the solution of 25 mM ammonium acetate (pH 4.0) and acetonitrile in the proportion of 3 to 7. The samples were analyzed by HPLC with a photo diode array detector.

For the permeation experiment, the interaction among MDMA, MA, ketamine and caffeine were examined in detail. One or two compounds among the four were applied to apical sides of the cells. The compounds that permeated to basolateral sides through cells were taken at varying time periods. The permeates were diluted with the mobile phase for HPLC and the samples were analyzed by LC-MS/MS.

For the experiments of in *vivo drug* administration, the blood was taken from tail vein at varying time periods after one or two compounds among MDMA, MA, ketamine and caffeine (10-50 mg/kg) was administrated orally to rats. Plasma samples were hydrolyzed with hydrochloric acid and deproteinized with acetonitrile. After filtration, the samples were subjected to LC-MS/MS for determination of the unchanged compounds and the main metabolites under the selective reaction monitoring mode. Interactions among various compounds were evaluated using pharmacokinetic parameters.

DA-p-4

**RESULTS:** MDMA uptake into Caco-2 cells was inhibited by compounds with an amino group such as MDA, MDEA, etc.. Typical substrates of organic cation transporters (OCTs) such as tetraethylammonium and 1-methyl-4-phenylpiridinium, however, indicated no inhibition. MDMA, MA and ketamine inhibited their uptakes and permeations mutually. The results implied various compounds with similar chemical structure to ATS with the amino group are mediated by a common transport system that is different from OCT as reported previously. Caffeine enhanced permeation of MDMA although caffeine had no effect in the uptake experiment. It was considered that para-cellular transport was enhanced by caffeine.
In the experiments of *in vivo* drug administration, the maximal plasma concentration and area under the blood concentration-time curve (AUC) of MA significantly decreased by co-administrating with ketamine in comparison to MA alone, while those of MDMA significantly increased by co-administrating with caffeine in comparison to MDMA alone.

**CONCLUSION:** The results in rats were similar to those in Caco-2 cells. Therefore, in human, intakes of ATS tablets mixed with such components might bring serious interactions in adsorption process.

**KEYWORDS:** *MDMA, Amphetamine-type stimulant, Caco-2, rat, Interaction, Pharmacokinetics*

**Corresponding author:** kuwayama@nrips.go.jp