Viability-dependent xenobiotic metabolism in the liver tissue pieces (2) A novel, quick and quantitative diagnostic technique for a foreseeing of an unexpected side effects of a new drug or drug-drug interaction

Emiko Hayama*, Naomi Motoji*, Ryo Simizu*, and Akiyo Shigematsu**

* Biomedical Research Institute

** Institute of Whole Body Metabolism

Purposes

Not only Japanese but also many Asian situation are similar to use human biomaterials, and it is very strict within much more limited legal permission than U.S. or European situation. In Japan, Human Animal Bridge Discussion Group has been established in 1994 to search for our Japanese own way different from the other Western countries. We have a common consensus that the majority of human biomaterials must be used for diagnostic examination of the patient, and the resultant data must be useful for chemotherapeutic practice to help the patient donor. By this reason, the capital title must be urgent to be practical.

Materials and Methods

in vivo experiments

One of a few typical compounds, well known competitor to the predetermined ^{14}C-labeled compound, was daily dosed for 1 or 2 weeks before the analytical experiments. In this case, cimetidine and toriacetyloleandmycin were used for the test competitors.

in vitro experiments

The test competitor and ^{14}C-labeled compound were mixed each other in the buffered solution with a minute amount of the liver tissue

pieces of about 5 mg, under a given design of several combinations. After a given min. incubation at 37°C, reaction was stopped and the extract with methanol was spotted on a silica-gel TLC plate.

The liver specimens (an aliquot near 5 mg) were biopsied form the human patients, crub eating macaque monkeys, beagle dogs, and rats and they were incubated in 20 μl buffered solution containing reaction generator and ^{14}C labeled parent compound for 10~30 minutes at 37°C. The extract was placed on a predetermined point on a silica-gel TLC plate for developing. The resultant TLC plate was dried and placed in contact with a radiation sensor, Imaging Plate, IP, for 27 h in the dark. An image of the radioactive distribution recorded on IP was analyzed by a Bioimaging Analyzer, BAS2000, Fuji Photo Film Co. Ltd. As a sensitive metabolic reaction indicator, [1-^{14}C] acetic acid, [1-^{14}C] leucine, [7-^{14}C] benzoic acid, [2-^{14}C] diazepam, [6-^{14}C] 5 fluorouracil, or [^{14}C] 7 ethoxycoumarin, respectively, was used.

Results and Discussions

The metabolic activities of DZ, 7-EC, and 5-FU in the liver specimens biopsied from the rats, intraperitoneally injected cimetidine repeatedly with 50 mg/kg/day, decreased 60%, 50% and 75%, respectively, compared with those in the control specimens. However, the liver specimens incuvated with cold cimetidine *in vitro* did not change the metabolic activities towards DZ, 7-EC, and 5-FU.

The metabolic activities in the liver specimens obtained from the rats and monkeys administered triacetyloleandmycin orally with 1g/kg/day and intramusculally with 300 mg/kg/day, respectively, for 7 days decreased significantly compared with those in the control

specimens. Control rat liver specimen has a high metabolic activity for DZ and can produce high rate of temazepam, 3-hydroxy metabolite of DZ. The metabolic activity in the treated rat specimens towards 3-hydroxylation of DZ decreased significantly. The liver specimens incuvated with cold triacetyloleandmycin *in vitro* decreased the metabolic activities towards DZ, 7-EC, and 5-FU compared with the control. These results suggested that the techniques described in this report can be used to foresee an unexpected side effects of a new drug or drug-drug interaction.