Viability-dependent xenobiotic metabolism in the liver tissue pieces

(1) Effects of cold- and cryo-preservation on the liver tissue specimens of different mammarian species.

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Purposes

In near future, the human biomaterials will be procured by patient donors in good faith in case of not only autopsied samples but also sonopsied samples in Japan. Even if we have in optimistic prospect of the near future in our country, it is few possibility to use any human biomaterial procured from the brain death donors.

For prediction and prevention of strict side-effects of drug-drug interactions, it must be sure that a very minute amount of the human liver material is much more superiorly than massive amounts of the other mammarian materials. This consideration holds good in case of pharmacological and toxicological evaluations of new drugs.

It is, therefore, urgent necessity for our research group to study on the viability-dependent xenobiotic metabolism in the liver tissue pieces under conditions of cold- and cryo-preservations.

Materials and Methods

The liver specimens (an aliquot near 5 mg) were biopsied from the human patients, crab eating macaque monkeys, beagle dogs, and rats, and they were incubated in 20 μl of 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.

In order to compare superiority of a few preservation solutions, the liver tissue before/after perfusion was observed cytologically.

Results and Discussions

The viability was dependent on a species specific glucose concentration in a perfusion solution, even though an exchanging procedure was performed very smoothly and quickly from the blood flow into the cold (0°C) perfusion flow in the living liver anesthetized animals.

Under the cold preservation, there were two groups of different viabilities dependent on the radioactive substrates, very quick and exponential declination with time, and very mild ones. The former was indicated by incorporation of ^{14}C-leucine into protein or ^{14}C-acetate into succinate, and the latter was shown at a substrate dependent step by mutual metabolic activity involved in single or plural liver organella.

The metabolic activity under the cold preservation must be a good indicator of enzyme-specific or species-specific viability. Generally, some tested enzymes related to liver microsomal cytochrome P450 were used to be tolerable relatively to the long-term cold preservation, except diazepam metabolism.

For cryopreservation, 10 % DMSO and 2 % mannitol in the buffered solution were recommended, and the whole liver was perfused with its solution kept at 0°C prior to freezing at a constant descending rate of 1°C per min..

After a long-term cryopreservation, the frozen specimens must be defrosted before using for metabolic study. The defrosting procedure

was required to take specifically careful consideration by a multineedle weight method for momentary defrosting less than 1 min for the whole liver of 100 g. We succeeded that by this consideration, the fine cell structure could be kept intactly after defrosting.