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In vivo micronucleus assay - historical review and current improvement

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Genotoxicity plays an important role for the safety evaluation of chemicals. When the carcinogenicity is evident on a chemical, the threshold can be estimated only when genotoxic mechanism does not operate for carcinogenesis otherwise threshold cannot be set. Without genotoxic mechanism- non-genotoxic carcinogen-threshold can be estimated but with genotoxic mechanism-genotoxic- carcinogen-it cannot be estimated. It is well known that there are *in vitro* and *in vivo* assay systems for evaluation of chemical genotoxicity on different endpoints. Bacterial gene mutation test and chromosomal aberrations test using mammalian cultured cells are representative examples. It can be said that the genotoxicology field has been developed based on *in vitro* assay systems. It is, however, apparent that there are limitations of *in vitro* assay systems for chemical safety evaluation and risk assessment for human health and *in vivo* assay systems are becoming more important in the view point of weight of evidence. There are several *in vivo* assay systems have been developed and used for different endpoints. Among these, the rodent micronucleus test using haematopoietic cells has been most widely and frequently used to detect induction of chromosomal aberration. The historical aspects of development of in vivo micronucleus test and the characteristics of the test will be reviewed.

In the second part, the current development of the micronucleus test and future perspectives of the assay will be summarized and discussed. As previously mentioned, in vivo rodent micronucleus test is widely used to assess clastogenic and possibly aneugenic potential of chemicals for regulatory purpose. Haematopoietic cells, however, have been mainly targeted to evaluate cytogenetic effect and immature bone marrow erythrocytes or reticulocytes in peripheral blood cells have been observed. Bone marrow cells are effectively exposed by test chemical after treatment by intraperitoneal injection, gavage, intravenous injection because blood-bone marrow barrier is not effective if exists. Some chemicals, however, are metabolized in liver to active metabolites, some of those

metabolites have very short lifespan and cannot reach bone marrow cells. This is one of limitations of the micronucleus assay using hematopoietic cells. It has been desired to develop the genotoxic method targeted other than haematopoietic organs. Several test systems, e.g., transgenic animal model, comet assay, and also micronucleus test, have been developed but very few systems have been well validated to be used for assessment of chemical safety.

Micronucleus assay using other than bone marrow cells, for example, using liver, skin, testes, and colon cells instead of bone marrow cells, have been developed and validated successfully. The validation studies have been organized as collaborative studies of Mammalian Mutagenicity Study Group (MMS) belonging to Japanese Environmental Mutagen Society and some of them were published. The technical issues of micronucleus assay using colon and skin were established and the methods were evaluated by some model colon and skin carcinogens respectively. The methods could detect the model chemicals as micronucleus inducers. The assay system using testes and liver cells have almost established and validating at the moment. For the testes assay, we introduced double staining with DAPI and acridine orange. The staining made easy identification of spermatocyte from other cells at the different spermiogenesis stages. For the liver micronucleus assay, partial hepatectomy method has been used but we do not know the precise influence of hepatectomy. We developed the assay using young rat hepatocytes without partial hepatectomy and other pretreatment. We evaluated the system using some model liver carcinogens and the method could detect these chemicals as micronucleus inducers.

The other recent development of the micronucleus test is automated evaluation systems. Image analysis and flow-cytometry were introduced to the test system with great success. Now these automated methods are under validation for regulatory purpose. The use of these method, especially flow-cytometry, huge number of cells can be analyzed and resulting the overcome the limitation of spleen function of many mammalian species, including rat and human.

Finally, I will propose the in vivo multiple endpoint assay system. When we use transgenic mutagenicity models in the micronucleus assay, we can obtain information for both gene mutation and chromosomal aberrations concomitantly, and possibly DNA damage by the comet assay. Such multiple endpoint genotoxicity assay system should be highly appreciated.