16 In Vivo Rodent Micronucleus Assay

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Abstract

Genotoxicity plays an important role for the safety evaluation of chemicals. Chromosomal aberration is one of two major end points of genotoxicity. The rodent haematopoietic cell micronucleus assay is most widely used as an in vivo test to evaluate structural and numerical chromosomal aberrations. The historical aspects of the development of the in vivo micronucleus test, the mechanism of micronucleus formation, and the characteristics of the test are reviewed. One of the limitations of the micronucleus assay is the use haematopoietic cells and it is desired to develop the genotoxic method targeted at cells other than haematopoietic cells. The micronucleus assay using cells other than bone marrow cells, for example using liver, skin, testis, and colon cells, has been developed and validated successfully. The validation studies have been organized as collaborative studies of the Mammalian Mutagenicity Study Group belonging to the Japanese Environmental Mutagen Society and have been published. 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. These automated methods are being validated for regulatory purposes. The in vivo multiple end point assay system, which is more efficient, is also discussed here. When we use transgenic mutagenicity model animals in the micronucleus assay, we can obtain information on both gene mutation and chromosomal aberrations concomitantly, and possibly on DNA damage when the micronucleus assay is combined with the comet assay. Such a multiple end point genotoxicity assay system should be highly appreciated from the viewpoint of animal welfare.

16.1 Introduction

Genotoxicity plays an important role for the safety evaluation of chemicals. It is well known that there are in vitro and in vivo assay systems for the evaluation of chemical genotoxicity on different end points. The bacterial gene mutation test and the chromosomal aberrations test using mammalian cultured cells
are representative examples. It can be said that the genotoxicology field has been developed on the basis of 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 from the viewpoint of weight of evidence and risk assessment. Several in vivo assay systems have been developed and used for different end points. 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 the development of the in vivo micronucleus test and the characteristics of the test will be reviewed.

In addition, the current development of the micronucleus test and future perspectives of the assay will be summarized and discussed. As previously mentioned, the in vivo rodent micronucleus test is widely used to assess the clastogenic and possibly the aneugenic potential of chemicals for regulatory purposes. Haematopoietic cells, however, have been mainly targeted to evaluate cytogenetic effects and immature bone marrow erythrocytes or reticulocytes in peripheral blood cells have been observed. Bone marrow cells are effectively exposed by the test chemical after treatment by intraperitoneal injection, gavage, and intravenous injection because the blood–bone marrow barrier is not effective. Some chemicals, however, are metabolized in liver to active metabolites; some of those metabolites have a very short lifespan and cannot reach bone marrow cells. This is one of the limitations of the micronucleus assay using haematopoietic cells. It is desired to develop the genotoxic method to target organs other than haematopoietic organs. Several test systems, e.g. transgenic animal model, comet assay, and also the micronucleus test, have been developed, but only a few systems have been validated to be used for assessment of chemical safety.

Micronucleus assay using cells other than bone marrow cells, e.g. using liver, skin, testis, and colon cells, have been developed and validated successfully. The validation studies have been organized as collaborative studies of the Mammalian Mutagenicity Study Group (MMS) belonging to the Japanese Environmental Mutagen Society (JEMS) and some of them have been published. The technical issues of the 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 systems using testis and liver cells have almost been established and validated. For the testis assay, we introduced double staining with 4′,6-diamidino-2-phenylindole and acridine orange. The staining made identification of spermatocyte from other cells at the different spermiogenesis stages easy. For the liver micronucleus assay, the partial heptectomy method has been used, but we do not know the precise influence of heptectomy. We developed the assay using young rat hepatocytes without partial heptectomy and other pretreatment. We evaluated the system using some model liver carcinogens and the method could detect these chemicals as micronucleus inducers.