Practical Considerations in the Conduct of Micronucleus Test

K. Suryanarayana Rao

INTRODUCTION

Genotoxicity is commonly evaluated during the chemical safety assessment together with other toxicological endpoints. The micronucleus test is always included in many genotoxicity test guidelines for long time in many classes of chemicals, e.g., pharmaceutical chemicals, agricultural chemicals, food additives. Although the trend of the safety assessment of chemicals faces to animal welfare and in vitro systems are more welcome than the in vivo systems, the in vivo test systems are paid more attention in the field of genotoxicity because of its weight of evidence.

Chromosome defects are recognized as being a part or all of a chromosome. These chromosome aberrations in cells with which they have direct contact in a static system. Chemicals may react differently in vivo, however, where metabolic systems other than liver cytochrome P448/P450 operate, and where the dynamic processes of absorption, metabolism and excretion are involved. Chemicals should therefore also be tested for chromosome damaging activity in vivo.

The micronucleus test (MNT)—most widely used in vivo genotoxicity test is used as an index of clastogenicity, along with Ames test which is an index of point mutation. The rodent haematopoietic cell micronucleus assay is most widely used as an in vivo test to evaluate structural chromosomal aberrations. The micronucleus was recognized in the end of the 19th century when Howell and Jolly found small inclusions in the blood taken from cats and rats. The small inclusions, called Howell-Jolly body, are also observed in the erythrocytes of peripheral blood from severe anaemia patients. These are the first description of the micronucleus itself.

A micronucleus is the erratic (third) nucleus that is formed during the anaphase of mitosis or meiosis. Micronuclei (the name means ‘small nucleus’) are cytoplasmic bodies having a portion of acentric chromosome or whole chromosome which was not carried to the opposite poles during the anaphase. Their formation results in the daughter cell lacking a part or all of a chromosome. These chromosome fragments or whole chromosomes normally develop nuclear membranes and form as micronuclei as a third nucleus. After cytokinesis, one daughter cell ends up with one nucleus and the other ends up with one large and one small nucleus, i.e., micronuclei.

There is a chance of more than one micronucleus forming when more genetic damage has happened. The micronucleus test is used as a tool for genotoxicity assessment of various chemicals. It is easier to conduct than the chromosomal aberration test in terms of procedures and evaluation. Using fluorescent in situ hybridization (FISH) with probes targeted to the centromere region, it can be determined if a whole chromosome, or only a fragment is lost.

In 2014, the OECD TG 474, Mammalian Erythrocyte Micronucleus Test was updated, which I do not intend to elaborate any further here. This write up is not intended to describe the method or the assessment, rather to offer some perspective on the use of this test and the use of positive controls.

The in vivo test normally uses mouse/rat bone marrow or peripheral blood. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise a nucleated cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack a main nucleus. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage.

Advantages and Limitations of Micronucleus Assay

Micronucleus test does not take a direct observation of chromosomes in the cell metaphase. However, the micronucleus test is not suggested to be a simple alternative method for metaphase analysis or the results of the assay have less value compared to those of chromosomal aberration test. The characteristics of the micronucleus test compared to the metaphase analysis

1. In any dividing cell population can be used regardless of its karyotype
2. Accurate data can be obtained because its endpoint is simple and easy to identify
3. Response can be detected for longer duration
4. Spindle poisons can also be detected
5. The background frequencies of micronucleated cells are usually stable

Cite this article: Rao KS. Practical Considerations in the Conduct of Micronucleus Test. BEMS Reports, 2017;3(1): 15-6.
6. An additional treatment of chemicals other than test articles, e.g., colcemid or BrdU, is not required.
7. But the types of chromosomal aberration cannot be classified by micronuclei.
8. There is a possibility of appearance of pseudo-micronucleus under some circumstances.
9. Accurate data can be obtained because its endpoint is simple and easy to identify.
10. In the Micro nucleated erythrocyte, the micronucleus is only DNA component in the cell because the main nucleus of the cell is expelled during the erythropoiesis of mammals and the structure is simple.

11. The current revised OECD TG 474 proposed 4000 young erythrocytes should be analyzed, which is twice the number of cells stated in the former guideline. It is time consuming and tedious work to analyze 4000 young erythrocytes in the case of the microscopic analysis, but the flow cytometer can do that easily, granted it needs to be validated.

Positive Control
1. Many laboratories routinely use positive control in every study, which may be required in the early stages of establishing a lab and training of technical staff. When the test becomes routine, the historical positive control can assure the performance of the test, and concurrent positive control group is not necessary to include in all tests.
2. It is, however, recommended to use the positive control slides, which prepared separately, to certify the proper staining and observation. ICH S2 (R1) guideline and OECD TG 474 accepted the omission of the concurrent positive control. This approach reduces the number of test animals from each study.
3. A concurrent positive control is not needed and this is globally acceptable. Having a concurrent positive control will not address a study where the TA isn’t tested to an appropriate top dose.
4. I have used positive control slides from non-concurrent animals. Since these slides are just to confirm that the blinded evaluation can detect a significant increase, they do not have to be concurrent with the study being supported. I personally have not had study concerns when using a well-regarded and experienced CRO.
5. In Europe, avoiding unnecessary animal use is seen as a virtue, so based on experience of others I don’t think a concurrent positive control is needed for an experienced lab.

Incorporation of MNT in Repeat Dose Toxicity Studies
1. Up to recently, the in vivo genotoxicity tests have been run standalone as other animal toxicological studies. To approach the 3Rs concept, two or more assay systems are combined as the multiple endpoint assays. The difficulty was how to overcome the different optimal treatment regime for each test, especially sampling time after the last treatment. Originally, this point was overcome by using peripheral blood as target cells of the micronucleus test.
2. The guideline of ICH S2 (R1) recommends to integrate genotoxic endpoints, e.g., micronucleus formation, into general repeat dose toxicological study. Theoretically, we can take peripheral blood sample at any time during the study, and bone marrow cells at the termination of the study. Of course, there are several restrictions to perform the toxicological study completely, but we can use animals of the satellite group, if any.
3. If a study were conducted using both sexes, it would be acceptable to reduce the animal size to 5 animals/sex/group and combine the control group, as most often we do not see that there is no difference in control levels of Micronucleated Immature Erythrocytes (MIE) between males and females.

Integration of Dose for Micronucleus with the Toxicity Study Dose
1. The most important point of the integration study is the dose levels. Generally, the dose levels of the repeat dose study are lower than those of the standalone or combination micronucleus test. The ICH guideline suggests when mammalian cell assay gives positive or omits mammalian assay, several factors should be evaluated to determine whether the top dose is high enough for the appropriate genotoxicity evaluation. If one or more criteria listed below are considered to be sufficient to evaluate in vivo:
2. Maximum Feasible Dose (MFD) based on Physico-chemical properties of the drug in the vehicle is similar to that achievable with acute administration.
3. Limit dose of 1000 mg/kg for studies of 14 days or longer, if this is tolerated.
4. Maximal possible exposure demonstrated either by reaching a plateau/saturation in exposure or by compound accumulation.
5. Top dose is ≥50 % of the top dose that would be used for acute administration, i.e., close to the minimum lethal dose, if such acute data are available for other reasons. (The top dose for acute administration micronucleus tests is currently described in OECD guidance as the dose above which lethality would be expected.

SUMMARY
The micronucleus assay has been most widely used as an in vivo assay as the most reliable assay to assess the induction of chromosomal aberrations, which is one of two major endpoints of mutagenicity, for not only hazard identification but also risk assessment. Where possible it is advisable to incorporate MNT in the ongoing repeat dose toxicity studies. Also, it is a general consensus to avoid concurrent positive controls, particularly in well-established labs. However, reading coded non-concurrent positive controls will satisfy the integrity of the study. Most important is to make sure that MNT assessment is conducted at sufficiently high dose of the test item with good systemic exposure. Avoiding concurrent positive control and also integrating MNT in repeat dose toxicity study will reduce the use of animals and makes sense from a scientific point of view. There is no doubt that the assay has more weight in the course of risk assessment than other assays including in vitro mammalian chromosomal aberrations assay, although, it has shortcomings in the view point of animal welfare. At the present time, still animal studies are important for safety assessment of chemicals and the attempts mentioned above should be appreciated to reduce experimental animals.

DISCLAIMER
This newsletter is solely intended for educational purpose. Some of the contents of this newsletter may have been adopted without or with modification from other published resources.