B. 19. SISTER CHROMATID EXCHANGE ASSAY *IN VITRO*

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definition

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principle of the test method

The Sister Chromatid Exchange (SCE) assay is a short-term test for the detection of reciprocal exchanges of DNA between two sister chromatids of a duplicating chromosome. SCEs represent the interchange of DNA replication products at apparently homologous loci. The exchange process presumably involves DNA breakage and reunion, although little is known about its molecular basis. Detection of SCEs requires some means of differentially labelling sister chromatids and this can be achieved by incorporation of bromodeoxyuridine (BrdU) into chromosomal DNA for two cell cycles.

Mammalian cells *in vitro* are exposed to the test chemical with and without a mammalian exogenous metabolic activation system, if appropriate, and cultured for two rounds of replication in BrdU-containing medium. After treatment with a spindle inhibitor (e.g. colchicine) to accumulate cells in a metaphase-like stage of mitosis (c-metaphase), cells are harvested and chromosome preparations are made.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

-Primary cultures, (human lymphocytes) or established cell lines (e.g. Chinese hamster ovary cells) may be used in the assay. Cell lines should be checked for *Mycoplasma* contamination,

-Appropriate culture media and incubation conditions (e.g. temperature, culture vessels, CO_2 concentration and humidity) should be used,

-Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. The final concentration of a vehicle in the culture system should not significantly affect, cell viability or growth rate and effects on SCE frequency should be monitored by a solvent control,

-Cells should be exposed to the test substance both in the presence and absence of an exogenous mammalian metabolic activation system. Alternatively, where cell types with instrinsic metabolic activity are used, the rate and nature of the activity should be appropriate to the chemical class being tested.

Test conditions

Number of cultures

At least duplicate cultures should be used for each experimental point.

Use of negative and positive controls

Positive controls, using both a direct acting compound and a compound requiring metabolic activation should be included in each experiment; a vehicle control should also be used.

The following are examples of substances which might be used as positive controls:

-direct acting compound:

-ethylmethanesulphonate,

-indirect acting compound:

-cyclophosphamide.

When appropriate, an additional positive control of the same chemical class as the chemical under test may be included.

Exposure concentrations

At least three adequately spaced concentrations of the test substance should be used. The highest concentration should give rise to a significant toxic effect but must still allow adequate cell replication to occur. Relatively water-insoluble substances should be tested up to the limit of solubility using appropriate procedures. For freely water-soluble non-toxic substances the upper test substance concentration should be determined on a case-by-case basis.

Procedure

Preparation of cultures

Established cell lines are generated from stock cultures (e.g. by trypsinization or by shaking off), seeded in culture vessels at appropriate density and incubated at 37 ℃. For monolayer cultures, the number of cells per culture vessel should be adjusted so that the cultures are not much more than 50% confluent at the time of harvest. Alternatively, cells may be used in suspension culture. Human lymphocyte cultures are set up from heparinized blood, using appropriate techniques, and incubated at 37 °C.

Treatment

Cells in an exponential stage of growth are exposed to the test substance for a suitable period of time; in most cases one to two hours may be effective, but the treatment time may be extended up to two complete cell cycles in certain cases. Cells without sufficient instrinsic metabolic activity should be exposed to the test chemical in the presence and absence of an appropriate metabolic activation system. At the end of the exposure period, cells are washed free of test substance and cultured for two rounds of replication in the presence of BrdU. As an alternative procedure cells may be exposed simultaneously to the test chemical and BrdU for the complete culture time of two cell cycles.

Human lymphocyte cultures are treated while they are in a semisynchronous condition.

Cells are analysed in their second post-treatment division, ensuring that the most sensitive cell cycle stages have been exposed to the chemical. All cultures to which BrdU is added should be handled in darkness or in dim light from incandescent lamps up to the harvesting of cells in order to minimize photolysis of BrdU-containing DNA.

Harvesting of cells

Cell cultures are treated with a spindle inhibitor (e.g. colchicine) one to four hours prior to harvesting. Each culture is harvested and processed separately for the preparation of chromosomes.

Chromosome preparation and staining

Chromosome preparations are made by standard cytogenetic techniques. Staining of slides to show SCEs can be performed by several techniques, (e.g. the fluorescence plus Giemsa method).

Analysis

The number of cells analysed should be based on the spontaneous control frequency of SCE. Usually, at least 25 well-spread metaphases per culture are analysed for SCEs. Slides are coded before analysis. In human lymphocytes only metaphases containing 46 centromeres are analysed. In established cell lines only metaphases containing ± 2 centromeres of the modal number are analysed. It should be stated whether or not centromeric switch of label is, scored as an SCE. The results should be confirmed in an independent experiment.

2. DATA

Data should be presented in tabular form. The number of SCEs for each metaphase and the number of SCEs per chromosome for each metaphase should be listed separately for all treated and control cultures.

The data should be evaluated using appropriate statistical methods.

3. REPORTING

3.1. Test report

The test report shall, if possible, contain t he following information:

-cells used, methods of maintenance of cell culture,

-test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle used, incubation temperature, treatment time, spindle inhibitor used, its concentration and the duration of treatment with it, type of mammalian activation system used, positive and negative controls,

-number of cell cultures per experimental point,

-details of the technique used for slide preparation,

-number of metaphases analysed (data given separately for each culture),

-mean number of SCE per cell and per chromosome (data given separately for each culture),

-criteria for scoring SCE,

-rationale for dose selection,

-dose-response relationship, if applicable,

-statistical evaluation,

-discussion of results,

-interpretation of results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.