B. 25. MOUSE HERITABLE TRANSLOCATION

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 mouse heritable translocation test detects structural and numerical chromosome changes in mammalian germ cells as recovered in first generation progeny. The types of chromosome changes detected are reciprocal translocations and, if female progeny are included, X-chromosome loss. Carriers of translocations and XO-females show reduced fertility which is used to select F_1 progeny for cytogenetic analysis. Complete sterility is caused by certain types of translocations (X-autosome and c-t type). Translocations are cytogenetically observed in meiotic cells at diakinesis- metaphase I of male individuals, either F_1 males or male offspring of F_1 females. The XO-females are cytogenetically identified by the presence of only 39 chromosomes in bone marrow mitoses.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

The test chemicals are dissolved in isotonic saline. If insoluble they are dissolved or suspended in appropriate vehicles. Freshly prepared solutions of the test compound are employed. If a vehicle is used to facilitate dosing, it must not interfere with the test compound or produce toxic effects.

Route of administration

Routes of administration are usually oral intubation ot intraperitoneal injection. Other routes of administration may be appropriate.

Experimental animals

For the ease of breeding and cytological verification these experiments are performed with mice. No specific mouse strain is required. However, the average litter-size of the strain should be greater than eight and be relatively constant.

Healthy sexually mature animals are used.

Number of animals

The number of animals necessary depends upon the spontaneous translocation frequency and the minimal rate of induction required for a positive result.

The test is usually performed by analyses of male F_1 progeny. At least 500 male F_1 progeny should be tested per dose group. If female F_1 progeny are included, 300 males and 300 females are required.

Use of negative and positive controls

Adequate control data, derived from concurrent and historic control should be available. When acceptable positive control results are available from experiments conducted recently in the same laboratory these results can be used instead of a concurrent positive control.

Dose levels

One dose level is tested, usually the highest dose associated with the production of minimal toxic effects, but without affecting reproductive behaviour or survival. To establish a dose/response relationship two additional lower doses are required. For non-toxic chemicals exposure to the maximum practicable dose should be used.

Procedure

Treatment and mating

Two treatment schedules are available. Single administration of the test substanace is most widely used. Administration of the test substance on seven days per week for 35 days may also be used. The number of matings following treatment is governed by the treatment schedule and should ensure that all treated germ cell stages are sampled. At the end of the mating period females are caged individually. When females give birth, the date, litter size and sex of progeny are recorded. All male progeny are weaned and all female progeny are discarded unless they are included in the experiment.

Testing for translocation heterozygosity

One of two possible methods is used:

-Fertility testing of F_1 progeny and subsequent verification of possible translocation carriers by cytogenetic analysis,

-Cytogenetic analysis of all male F_1 progeny without prior selection by fertility testing.

(a) Fertility testing

Reduced fertility of an F_1 individual can be established by litter size observation and/or analysis of uterine contents of female mates.

Criteria for determining normal and reduced fertility must be established for the mouse strain used.

Litter size observation: F_1 males to be tested are caged individually with females either from the same experiment or from the colony. Cages are inspected daily beginning 18 days after mating. Litter size and sex of the F_2 progeny are recorded at birth and litters are discarded thereafter. If female F_1 progeny are tested the F_2 progeny of small litters are kept for further testing. Female translocation carriers are verified by cytogenetic analysis of a translocation in any of their male offspring. XO-females are recognized by the change in sex ratio among their progeny from 1:1 to 1:2 males *vs.* females. In a sequential procedure, normal F_1 animals are eliminated from further testing if the first F_2 litter reaches or exceeds a predetermined normal value, otherwise a second or third F_2 litter is observed.

F_1 animals that cannot be classified as normal after observation of up to three F_2 litters are either tested further by analysis of uterine contents of female mates or directly subjected to cytogenetic analysis.

Analysis of uterine contents: The reduction in litter size of translocation carriers is due to embryonic death so that a high number of dead implants is indicative of the presence of a translocation in the animal under test. F_1 males to be tested are mated to two to three females each. Conception is established by daily inspection for vaginal plugs in the morning. Females are sacrified 14 to 16 days later and living and dead implants in their uteri are recorded.

(b) Cytogenetic analysis

Testes preparations are made by the air-drying technique. Translocation carriers are identified by the presence of multivalent configurations at diakinesis-metaphase I in primary spermatocytes. Observation of at least two cells with multivalent association constitutes the required evidence that the tested animal is a translocation carrier.

If no breeding selection has been performed all F_1 males are inspected cytogenetically. A minimum of 25 diakinesis-metaphase I cells per male must be scored microscopically. Examination of mitotic metaphases, in spermatogonia or bone-marrow, is required in F_1 males with small testes and meiotic breakdown before diakinesis or from F_1 female XO suspects. The presence of an unusually long and/or short chromosome in each of 10 cells is evidence for a particular male sterile translocation (c-t type). Some X-autosome translocations that cause male sterility may only be identified by banding analysis of

mitotic chromosomes. The presence of 39 chromosomes in all of 10 mitoses is evidence for an XO condition in a female.

2. DATA

Data are presented in tabular form.

The mean litter size and sex ratio from parental matings at birth and weaning are reported for each mating interval.

For fertility assessment of F_1 animals, the mean litter size of all normal matings and the individual litter sizes of F_1 translocation carriers are presented. For analysis of uterine contents, the mean number of living and dead implants of normal matings and the individual numbers of living and dead implants for each mating of F_1 translocation carriers are reported.

For cytogenetic analysis of diakinesis-metaphase I, the numbers of types of multivalent configurations and the total number of cells are listed for each translocation carrier.

For sterile F_1 individuals, the total number of matings and the duration of the mating period are reported. Testes weights and cytogenetic analysis details are given.

For XO females, the mean litter size, sex ratio of F_1 progeny and cytogenetic analysis results are reported.

Where possible F_1 translocation carriers are preselected by fertility tests, the tables have to include information on how many of these were confirmed translocation heterozygotes.

Data from negative controls and the positive control experiments are reported.

3. REPORTING

.

3.1. Test report

The test report shall, if possible, contain the following information:

-strain of mice, age of animals, weights of treated animals,

-numbers of parental animals of each sex in experimental and control groups,

-test conditions, detailed description of treatment, dose levels, solvents, mating schedule,

-number and sex of offspring per female, number and sex of offspring raised for translocation analysis,

-time and criteria of translocation analysis,

-number and detailed description of translocation carriers, including breeding data and uterine content data, if applicable;

-cytogenetic procedures and details of microscopic analysis, preferably with pictures,

-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.