Critique of in vivo Cytogenetic Test Systems

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Experiments in laboratory animals in general are influenced by a number of factors that have to be taken into account in considering the results of cytogenetic studies. The points that are supposed to be pertinent are discussed in the following.

Selection of species

The animals used in cytogenetic tests should be easy to breed, and their chromosomes should be easily identifiable. Beagle dogs, a species widely used in toxicological work, have 72 chromosomes that are difficult to analyze. The 40 chromosomes of the mouse are achrocentric and very similar in size. However, this species has the advantage that a large amount of genetic information is available.

Rats, that are also widely used in toxicology seem to be more suitable animals for cytogenetic work than mice. Although the chromosome number of 42 is rather high the individual chromosomes are easily identifiable because of their different size and shape.

The Chinese hamster is considered to be the most suitable animal for cytogenetic studies. This species has 22 chromosomes, all of which are morphologically easily identifiable.

Species differences

According to cytogenetic investigations by RÖHRBORN et al. [27] mice proved to be more sensitive to cyclophosphamide than rats, and these were more responsive than Chinese hamsters.

In our own studies, 6-mercaptopurine induced a large number of aberrant metaphases in the bone marrow of mice and Chinese hamsters. Rats were much less sensitive to this antimetabolite (FROHBERG and BAUER [16b)].

Differences in sensitivity of various mammalian species to foreign compounds are mainly due to differences in pharmacokinetic behaviour and in metabolism. Therefore it may be important to obtain information on drug concentration in the target organs (e.g. testes, ovary or bone marrow) or in the cells that are evaluated (WHO, 1971).

Spontaneous incidence of chromosome aberrations

A number of reports are available on aberration frequencies in blood lymphocytes of persons who were not exposed to radiation or to known environmental mutagens. According to EVANS [15] the frequency of gaps ranges from 0.01 to 0.1% and the frequency of apparent deletions from 0.0033 to 0.094%. Other laboratories found much higher spontaneous aberrations. HARTWICH and SCHWANITZ [19] described a mean aberration rate in healthy persons of 5.1% and HAMPEL [18] of 6.2%.

In animals the type and frequency of spontaneous aberrations is species-specific and strain-specific. The spontaneous aberration rate of our own Chinese hamster strain was 0.3 and 0.4%, and the same figure is given by SCHMID et al. [28a]. MÜLLER and STRASSER [25b] found only gaps in their hamster experiments although they used animals from the same colony that was also studied by SCHMID. Similarly, among the mouse and rat strains used for comparison, differences in the spontaneous aberration rates between various mouse and rat strains are also described in the literature (Table 1).

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Influence of age

The frequency of numerical aberrations of lymphocytes in humans increases with advancing age (COURT BROWN [11], JACOBS et al. [20]).

Not enough data in animals are available on the effect of age. In mutagenicity experiments it is advisable to use only animals of defined age.

Exogenous factors

TOUGH et al. [31] were the first to report on the presence of aberrations in blood cells of individuals exposed to X-rays for therapeutic purpose. These observations were confirmed by a number of investigators. STEWART and SANDERSON [29] and CONEN et al. [10a, 10b] published that by diagnostic X-rays exposure aberrations can be induced in peripheral blood cells. Similar results were obtained in other laboratories. Therefore, if in vivo cytogenetic studies in humans are performed it should be taken into consideration that any form of X-ray exposure, even for diagnostic purpose, may induce chromosomal aberrations.

Apart from X-ray exposure, BARTSCH [4], GRIPENBERG [17] and other disease entities may be accompanied by chromosomal aberrations (BAUCHINGER et al. [5a]). Therefore, whenever in vivo cytogenetic investigations are carried out in humans, the patients treated with the test compound should be compared with a group of patients that have the same underlying disease (BAUCHINGER and SCHMID [5b]) and who are subjected to the same diagnostic and general therapeutic measures. The use of 'healthy' subjects as the sole controls cannot be recommended.

ARMENDARES and co-workers [2] reported that chromosome aberrations were significantly more frequent in malnourished children than in the general population. In starved mice the rate of chromosome aberration was not significantly increased in comparison to fed controls. The same was true for rats (Table 2). Our findings in laboratory animals are in agreement with THORBURN et al. [30] who found no evidence that malnutrition in children predisposes to chromosomal abnormalities.

Optimum time of examination

In experiments on rubidomycin, JENSEN and PHILIP [21] found most of the aberrations in rats 24 hours after the injection.

Of other alkylating compounds like cyclophosphamide and triaziquone, the optimum activity in Chinese hamsters was found at 8 or 6 hours after the second dose when two doses were given 24 hours apart (SCHMID et al. [28a]) (Table 3).

In mice, DATTA and SCHLEIERMACHER [12] found an optimum effect after 24 hours. According to our own in vivo cytogenetic studies (bone marrow) in mice (NMRI – EMD – SPF) with 6-mercaptopurine, a maximum aberration rate of 55.1% was found 48 hours after the administration. Furthermore, 2 and 3 days after injection of 6-mercaptopurine the most severe types of aberration such as translocations and multiple aberrations were observed (BAUER, FROHBERG [6]).

It is concluded that the optimum effect depends on the cellular cycle of the cytogenetic test system in the particular species of animal.

Table 1
Spontaneous chromosome aberration rate in animals: bone marrow method.

Species and strain	Number of		Aberrant	Metaphases	Authors
	animals	analyzed metaphases	metaphases* (%)	with gaps only (%)	
Chinese hamster	24	1800	0.3		SCHMID et al. [28a]
	10	1000	0.0	2.8	MÜLLER and STRASSER [25b]
	15	1500	0.4	0.9	BAUER and FROHBERG [6]
Mice					
F_1 (♂ C 3H × ♀ 101)	10	520	0.0	0.002	DATTA et al. [12]
NMRI-EMD	10	1000	0.7	0.5	BAUER and FROHBERG [6]
Rats					
Charles River	17	1090	1.2	1.8	ADLER et al. [1b]
Charles River	9	360	0.3	0.6	ADLER et al. [1b]
Wistar-AF/HAN-EMD	25	2500	0.3	0.2	BAUER and FROHBERG [6]

* Metaphases with gaps only are not included.

Dose response relationship

All pharmacological and toxic effects are dose-dependent, and one may ask whether the in vivo cytogenetic test systems that are currently used conform to this rule.

In vivo cytogenetic tests on the bone marrow performed by SCHMID et al. [28a] in Chinese hamsters with triaziquone demonstrated that the number of aberrant metaphases increased proportionally to the dose administered. The extent of other lesions was also dose-dependent.

A micronucleus test conducted by MATTER and SCHMID [24] with triaziquone in mice, rats, Syrian hamsters, guinea-pigs and Chinese hamsters, also demonstrated a marked dose dependence of effects.

In their studies on the bone marrow of the Chinese hamster MÜLLER et al. [25a] obtained about an equal number of anomalies by comparing the effect of cyclophosphamide between the micronucleus test and the cytogenetic assay of metaphases.

Table 2
Influence of transient starvation on the chromosome aberration rate in somatic cells: bone marrow method.

Species	Starvation period days	Animals Number	died	Metaphases % aberrant *	% with gaps only
NMRI-Mice	0	10	0	0.5	0.6
	3	27	2	1.2	0.8
	4	25	19	0.5	1.0
Wistar-Rats	0	25	0	0.25	0.2
	7	3	0	0.67	0.67
	8	4	0	0.0	1.0

* Metaphases with gaps only are not included

Relationship between mutagenic dose, toxic dose and pharmacologically active dose

For the evaluation of the potential risk of a drug, the relationship between mutagenic and therapeutic dose is decisive. In evaluating in vivo cytogenetic tests in animals the pharmacologically active and/or toxic dose should be compared with the mutagenic threshold dose in this species rather than the therapeutic dose in humans. Whereas some mutagens produce genetic lesions at subtoxic doses only, there are compounds that are effective at much lower dose levels (Table 4).

Table 3
Maximum aberration rate after compound administration. In vivo cytogenetics: bone marrow method.

Animal species	Compound	Dose (mg/kg)	Maximum activity hours after administration	References
Chinese hamster	Cyclophosphamide	2 × 64 p.o.	8**	SCHMID et al. [28a]
	Triaziquone	2 × 0.25 i.p.	6**	SCHMID et al. [28a]
	7, 12-DMBA	1 × 4 i.p.*	144	KATO et al. [23]
Rat	Rubidomycin	1 × 5 i.p.	24	JENSEN and PHILIP [21]
Mouse	Cyclophosphamide	1 × 200 i.p.	24	DATTA and SCHLEIERMACHER [12]
	6-Mercaptopurine	1 × 250 i.p.	48	FROHBERG and BAUER [16b]

* μm/animal
** Hours after last administration

Table 4
Relationship between toxic and mutagenic dose in the mouse.

Compound	LD_{50} i.p. (mg/kg)	Mutagenic dose referred to LD_{50}	References (LD_{50}-values)
TEM	3.0	1/60	OETTEL and WILHELM [26]
Triaziquone	0.8	1/26	KARRER and BOECKL [22]
6-Mercaptopurine	200.0	1/8	v. EBERSTEIN and FROHBERG [13]
Cyclophosphamide	275.0	1/4	WHEELER et al. [33]
EMS	450.0	1/3	EHLING et al. [14]

The mutagenic dose of triaziquone in mice and rats is about 3 times as large as the dose showing antineoplastic activity in transplanted tumours. For 6-mercaptopurine, the mutagenic dose is identical with the therapeutic dose in mice. By contrast, cyclophosphamide has a mutagenic effect in mice at 12 times the antineoplastic dose (Table 5).

Duration of treatment in mutagenicity tests

The majority of the compounds examined in long-term studies, such as caffeine (CATTANACH [9], ADLER and RÖHRBORN [1c], ADLER [1a]), Carbaryl (WEIL et al. [32]) and cyclohexylamine (BAILEY et al. [3]) gave negative results. After 2 to 4 weeks of daily oral administration of $^1/_{10}$ the LD_{50} of diazepam and medazepam the chromosome aberration rate was not elevated (SCHMID and STAIGER [28b]).

This raises the question whether long-term treatment, corresponding to the procedure used in long-term toxicological trials (FROHBERG [16a]) is appropriate.

In Chinese hamsters a 4 days' treatment with triaziquone (0.125 mg/kg i.p.) increased the number of aberrant metaphases in parallel with the number of injections (SCHMID et al. [28a]). It should be noted, however, that the doses administered were within the toxic range and the hamsters were moribund after the 4 treatments.

After 2 oral doses of 64 mg/kg cyclophosphamide 8 times as many aberrations were found in the bone marrow of Chinese hamsters than after a single administration. When the same single dose was administered 3 to 5 times, the aberration rate was again considerably lower (SCHMID et al. [28a]).

A 7-week experiment with Chinese hamsters receiving 8 mg/kg of cyclophosphamide daily, 5 times per week, demonstrated no increase in the aberration rate with increasing duration of treatment (SCHMID et al. [28a]).

The available experiments therefore do not permit the conclusion that a long-term treatment is feasible (see also the paper by W. Schmid presented at this meeting).

Advantages and disadvantages of the different in vivo cytogenetic tests

Somatic cells

The micronucleus test is simpler and takes less time to perform than any other in vivo cytogenetic method. As a result, a very large number of cells can be examined in a short period of time. In addition, experience with the test to date would seem to indicate that its sensitivity is not inferior to that of the bone marrow method.

Bone marrow is a good source for high numbers of mitotic cells in mammals. As compared with the micronucleus test, a large amount of experience is required to satisfactorily evaluate chromosomal changes.

Gonosomal cells

Mouse spermatogonia afford the means of detecting chromosome damage of mitotic cells. Often the testis of a mouse does not yield spermatogonial divisions in sufficiently high numbers. Furthermore, the sensitivity of the gonadal cells may be different during the different developmental stages so that mitotic as well as meiotic cells should be examined. With regard to sensitivity and dose response relationship the available experience of the spermatogonia method is as yet insufficient when compared with cytogenetic tests on somatic cells.

Table 5
Comparison of therapeutic and mutagenic doses of 3 antineoplastic drugs.

Compound	Mouse (mg/kg i.p.)		Rat (mg/kg i.p.)		Man (mg/kg)	References (td)
	td	md	td	md	td	
6-Mercaptopurine	25	25	50	50	2.5 oral	OETTEL and WILHELM [26]
Triaziquone	0.01	0.03	0.01	0.03	0.004 – 0.02 i.v.	BIERLING [7]
Cyclophosphamide	5	60	5	–	4–8 i.v.	BROCK [8]

td = therapeutic dose (in animals against transplantation tumours)
md = mutagenic dose

In concluding it is desired that a better knowledge of mutagenic threshold doses, and of the relationship between mutagenic and pharmacological threshold doses in the different test systems be obtained. Comparative investigations in different animal species with compounds of different chemical structure are necessary.

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