AGE-DEPENDENT RADIATION RISK IN MAN: CHROMOSOMAL INVESTIGATIONS

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The age-dependent reports about the chromosomal radiosensitivity are contradictory. Because chromosomal aberrations may be the source of both somatic and genetic disorders, age-dependent radiosensitivity is an important factor for radiation protection purposes.

The dicentric chromosome which is mostly used for those investigations will often result in the formation of anaphase bridges at the anaphase stage of mitosis. These bridges interfere with the mechanical separation of the two daughter cells. Such interference frequently leads to cell death. Therefore it is important that dicentrics are scored in first dividing cells after exposure. In earlier times the normal culture time for lymphocytes was 72h. Since it was learned that at this time many lymphocytes have passed the first cell division, culture time was reduced to about 56h and now the standard culture time is 48h. The aim is to avoid the inclusion of second division cells in the analysis as these would reduce the observed aberration yield. This reduction is a consequence of the multiplication of undamaged lymphocytes and the exclusion from the analysis of damaged cells.

It is now possible to identify reliably cells in their 1st, 2nd and subsequent in vitro division by using the technique of "Fluorescence plus Giemsa" (FPG) staining. The technique has also permitted to study the proliferating kinetic of lymphocytes in vitro. It is known that up to 60% of second mitosis (M2-cells) in cultures of human lymphocytes appear in 48h cultures. These findings have therefore cast doubts on the validity of much earlier cytogenetic research.

The following results present the cell cycle of lymphocytes in vitro, the dicentric rate in first and second dividing cells and the dicentric rate in different age groups.

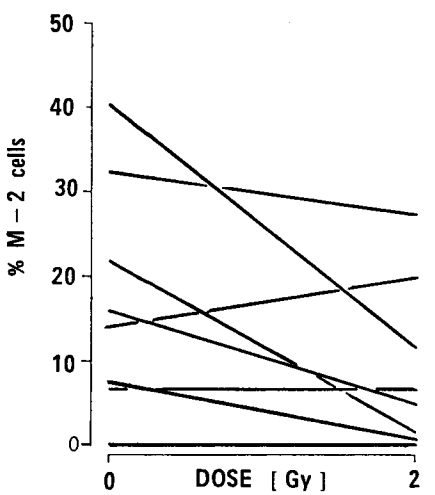
From 36 donors we have studied lymphocyte proliferation in non-irradiated and irradiated blood samples. Peripheral blood was irradiated in vitro with a single dose of 2 Gy and was cultured for 48h in the presence of BUdR (32 μM). As can be seen from Table 1 in non-irradiated samples, the proportions of M2-cells vary between 0 and 40%. In the donors aged 13 to 48 years, the proportions of M2-cells range, in most cases, between 0 and 10%. In contrast hereto it has been found that systematically 50% of cells with second cell cycle appeared in the 48h cultures of all cord blood samples (10 newborns). However, we have found only 30% in this particular age group.

Table 1. Proportions of M2-cells in non-irradiated (Co) and irradiated (2 Gy) blood samples

% M – 2	Co	2 Gy
	donors (13 - 85 yrs)	
0 - 10	16 (61.5 %)	22 (84.8%)
11 - 20	6 (23.2%)	2 (7.6%)
21 - 30	3 (11.5%)	1 (3.8%)
31 - 40	1 (3.8%)	1 (3.8%)
	26 (100%)	26 (100%)
	cord blood	
0 - 10	2 (20%)	8 (80%)
11 - 20	5 (50%)	1 (10%)
21 - 30	1 (10%)	1 (10%)
31 - 40	2 (20%)	0 (0%)
	10 (100%)	10 (100%)

After irradiation it can be seen that the proportions of M2-cells are reduced. This is to be expected, since it is known that cell cycle delay is induced by ionizing radiation. Moreover, regarding some individual cases, it is evident that there is no unique proliferation kinetic. In some cases the proportions of M2-cells after irradiation are lower up to 20% as compared with control values, in other cases the proportions of M2-cells are comparable to those in non-irradiated and irradiated samples. In one experiment the proportion of M2-cells is higher after irradiation, as compared with controls, for which fact we have no explanation.

Figure 1



These different proportions of M2-cells in cultures of 48h are of importance when assessing chromosome damage in lymphocytes after exposure to chromosome breaking agents. Accurate information on the aberration rate can only be gained from first metaphases, as shown in Table 2.

Table 2: Dicentrics in 1st and 2nd metaphases

division	scored cells	dic + ace	dic	dic + ace / cell ± S E	dic /cell ± S E	Σ dic /cell ± S E
1st	12.009	4.344	145	0.362 ± 0.005	0.012 ± 0.001	0.374 ± 0.006
2nd	459	57	30	0.124 ± 0.016	0.065 ± 0.012	0.189 ± 0.020
1st + 2nd	12.468	4.401	175	0.354 ± 0.005	0.014 ± 0.001	0.367 ± 0.005

M1-cells were identified by homogenous stained chromosomes. In 12.009 first mataphases, we have found 4.344 dicentrics with associated fragments and 145 dicentrics without fragments (3%). As second metaphases only those were scored where the sister-chromatids showed different staining patterns in all chromosomes of a metaphase. In 459 second metaphases we found 57 dicentrics with associated fragments and 30 dicentrics without fragments. It is remarkable that in our study we have found dicentrics without fragments in the first metaphases and dicentrics with associated fragments in second metaphases.

The dicentrics without fragments in M1-cells may perhaps indicate that some fragments are very small and not detectable. The dicentrics in M2-cells should not have an associated fragment, but it is known that dicentrics can be produced by isochromatid exchanges. The joining of the centric portions of two isochromatid deletions gives what appears to be a chromosome-type dicentric. It is only distinguishable from the true dicentric in case there are two fragments both of which show sister union, i.e. the chromosome-type dicentric can be distinguished from that which arises from chromatid deletions only be the shape of the fragments. Perhaps it may be assumed that the number of dicentrics with associated fragments partially reflects a toxic effect of BUdR. It is well known that treatment with BUdR causes a reduction in the number of metaphases and a slowing of the cell cycle. The dicentrics with associated fragments in second metaphases however seem to be of the chromosome type. This assumption is supported by the fact that we have found the same number of chromatid aberrations in the culture containing BudR as in those without BUdR.

Regarding the dicentric rates in M1- and M2-cells, it can be seen that in M2-cells only half of those dicentric rates are found in M1-cells. From this it can be concluded that in lymphocyte populations with high proportions of M2-cells a dilution of the dicentrics may take place.

For the investigation of a chromosomal age-dependent radiosensitivity, M1-cells only were scored for dicentrics (dic) and ringchromosomes (cr). The aberration yield ranges between 0.297 and 0.498 dic + cr per cell.

The age-dependent radiosensitivity is determined by comparing the mean yield of chromosome aberrations from 4 age-groups comprised of 47 donors (10 newborns, 11 donors 13-16 yrs, 11 donors 25-36 yrs and 15 donors 51-85 yrs). The incidence of dicentric chromosomes is following the Poisson distribution ($p = 0.36$) and the mean yield of chromosome aberrations is comparable in these 4 age groups (Table 3, $p=C.19$). The radiosensitivity seems not to be associated with the donors' sex (for males $p=0.30$ and females $p=0.20$). Therefore, these data cannot support an age-dependent radiosensitivity in man.

Table 3. Mean yields of chromosome aberrations, dic + cr (s.e) per 100 M1-cells, from 4 age-groups

Newborns	13-16 yrs	25-36 yrs	51-85 yrs	All groups
41.0 (6.5)	38.8 (3.3)	36.2 (4.1)	37.4 (5.0)	38.1 (5.0)

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