X-RAY-INDUCED CHROMOSOME ABERRATIONS IN THE LEUKOCYTES OF MOUSE AND MAN

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*Research supported by the Energy Research and Development Administration under contract with the Union Carbide Corporation.

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INTRODUCTION

In the past few years there has been considerable discussion of the sensitivity of the leukocytes of different species to the induction of chromosome aberrations by x-rays (see Sankaranarayanan, 1976 for a summary). This, in part, stemmed from the report by Brewen et al (1973) that there was a considerable variation in dicentric yield in the species that they studied, and also that for these particular species there appeared to be a linear relationship between dicentric yield and effective chromosome arm number at any particular dose. As they stated in their paper it was the relationship between aberration yields in man and mouse which was of particular interest. No explanation of the relationship between the dicentric yield and the effective chromosome arm number could be offered. Subsequently Savage and Papworth (1973) and Clifford (1976) have offered explanations. However, many other species have been studied, and it has become fairly clear that in some the yield is approximately that which would be predicted from the effective chromosome arm number, and in others it clearly is not. We feel that our original observation could in part be accounted for by a fortuitous choice of the species which were studied, as regards their DNA content, interphase chromosome volume, and perhaps repair characteristics. However, the fact that the dicentric yield was twice as high in human leukocytes as compared to the mouse, and that acentric fragment yields were similar in the two species appeared to be worth further study, particularly since the fixation times used for the mouse were rather late, and could result in a reduced yield due to the presence of second division cells. This became more important as a result of the study by de Boer et al (1977) in which they concluded that the dicentric yields were the same in mouse and human leukocytes, and that the

acentric yield was higher in the mouse at 200 rads. This paper presents a comparison made at early fixation times for both human and mouse.

MATERIALS AND METHODS

Buffy coat cultures were established from three human donors for the 42 hr fixations, from one donor for the 48 hr fixation, and from several mice. The human leukocytes were cultured according to our standard procedure as described elsewhere (Preston, Brewen and Gengozian, 1974). The technique used for culturing the mouse leukocytes was that of Triman, Davisson and Roderick (1975). Human cultures were fixed at 42 and 48 hrs and the mouse cultures at 36 and 48 hrs.

The cultures were irradiated prior to the addition of phytohemagglutinin. All irradiations were with a 250 kVp General Electric X-ray machine operated at 250 kv and 30 mA with 3 mm of additional Al fixation (h.v.l. 0.45 mm Cu). This dose rate was 100 R/min.

A minimum of 300 cells was analyzed at any one dose point except for the mouse 36 hr fixations. All aberrations were recorded, and a note was made of any exchange aberration which was not accompanied by an acentric fragment, so that some correction of the aberration yield could be made as a result of the presence of cells in their second mitosis after treated.

RESULTS

The yields of dicentrics and acentric fragments (terminal deletions, acentric rings, and small interstitial deletions) are given in Table 1 for the human at 42 hr and 48 hr fixations, and the data for dicentrics are shown graphically in Figure 1. The data were fitted to three models $Y = bD$, $Y = cD^2$ and $Y = bD + cD^2$ by least squares regression using Poisson variances and weights.

The best fit was to the model $Y = bD + cD^2$. Thespontaneous rate was in most cases zero and the curve was constrained to pass through the origin.

There is a significant difference in the dicentric yields at the two fixation times, with the yield at 42 hrs being higher than that at 48 hrs. for two donors (J.P. and T.H.). There was no difference between donor J.A. fixed at 42 hrs, and the data obtained for a different donor at 48 hrs, but no direct comparison could be made as no data were available for the same donor at the later fixation time.

The dicentric yields for the 48 hr fixation were corrected on the assumption that a cell containing a dicentric without an accompanying acentric fragment was at its second mitosis after treatment. The data are shown in Table 1, and it can be seen that the corrected yield was still significantly different from the yield in cells fixed at 42 hrs. This suggests that the increased yield at the earlier fixation time was not entirely due to the fact that only first division cells were being analyzed. Some other pattern of differential sensitivity of leukocytes to radiation is indicated.

The acentric fragment data are somewhat more confusing although it appears that there is essentially no difference in yield between cells fixed at 48 hrs and those fixed at 42 hrs.

The data for dicentrics and acentric fragments induced in mouse leukocytes by x-rays are shown in Table 2 and Figure 2, for both 48 and 36 hr fixations. These data were also fitted to the three models with the best fit at the 48 hr fixation being to the model $Y = bD + cD^2$.

If the dicentric yield at the 48 hr fixation was corrected for loss at the first division, by using the factor obtained from the frequency of cells containing dicentrics not accompanied by an acentric fragment, the yields obtained were still lower than those observed at 36 hrs. At this early fixation

(36 hrs) all cells with a dicentric also had an accompanying acentric fragment. The proportion of cells containing dicentrics without an accompanying acentric fragment in the mouse cells fixed at 48 hrs was higher than that obtained for human cells fixed at the same time (0.16 v. 0.06), suggesting a more rapid first division cycle in mouse leukocytes. This was borne out by the fact that sufficient metaphases were obtained in mouse cultures fixed at 36 hrs, whereas it was necessary to fix human cultures at 42 hrs to obtain sufficient metaphases.

A comparison of dicentric and acentric fragment yields in the human and mouse showed that for dicentrics the yield at 42 hrs in the human was between 1.5 and 2.1 as high as in the mouse fixed at 36 hrs, and the acentric fragment yields were comparable in the two species. If the corrected dicentric yields at 48 hrs fixation were compared, the human was about twice as high as the mouse. The acentric fragment yields were similar in the two, with perhaps a slightly higher value for the human.

DISCUSSION

It is clear that the original comparison that was made of aberration frequencies induced by x-rays in the leukocytes of mouse and man (Brewen et al, 1973) was based on cells that were in both their first and second mitosis after irradiation. It is also probable that more second division cells were present in the mouse cultures than in the human, since it is shown here that a higher proportion of cells containing dicentrics without fragments was present in mouse cultures than in human cultures when both were fixed at 48 hrs. This factor alone could influence the aberration frequencies differently in mouse compared to man, making the ratio of dicentrics and acentric fragments in the two different from those originally reported. However, when either the

influence of second division cells was accounted for as in the 48 hr fixations, or when only first division cells were compared at the earliest fixations a similar result to that already reported was obtained, i.e. human leukocytes in G_0 are twice as sensitive to dicentric production as mouse leukocytes, and the two have similar sensitivities to acentric fragment induction.

There are obviously some differences between these observations and those of de Boer et al (1977), who from a large study concluded that mouse and human leukocytes were equally sensitive to dicentric production and that the mouse was more sensitive to deletion production than man. How can we reconcile these results with the ones presented here? The data obtained for the mouse at 36 hrs in the present study show very good agreement at 100 and 200 R for dicentrics and deletions with those reported by de Boer et al (1977). The difference is in the results for human leukocytes. If comparisons are made at the same fixation time (48 hrs) it is clear that the yields reported here are considerably higher than those in de Boer et al (1977). For example, at 200 R the dicentric yields are 57.4% vs 34.0%, and the deletion frequencies at the same dose are 26.6% vs 18.5%. If comparisons are made between the yields at the earliest fixation used here (42 hrs) and the 48 hr fixation in de Boer et al (1977), the only one used, then the contrast is greater. The differences at the different fixation times and between the two sets of data need not only represent a difference in the proportions of first and second division cells, since when the data reported here at the 48 hr fixation are corrected for second division cells, the yields obtained are still lower than those actually observed at 42 hrs after treatment and stimulation. It would appear that it is possible that those cells reaching mitosis earliest are slightly more sensitive to aberration induction than those arriving later. This does not necessarily imply that there is a differential sensitivity of lymphocytes in G_0.

but perhaps that there is a differential stimulation of cells by phytohemagglutinin. It appears, therefore, that the differences between the present results and those of de Boer et al (1977) lie in the frequencies of aberrations in the human leukocytes, as a result of either different proportions of first and division cells or a different segment of the irradiated population being analyzed, or a combination of both.

It should be added that the yields reported here for the 42 hr fixation were similar for two different donors, and only slightly reduced for the third. This suggests that a donor to donor variation is not the most likely explanation of the differences discussed above.

From the data presented here, we suggest that indeed the yields of dicentrics induced by x-rays in human leukocytes are twice as high as in mouse leukocytes, and that acentric yields are similar in the two species.

These data are not presented as lending support to the so-called "arm number" hypothesis. Too many exceptions have been reported to make such a conclusion feasible. To use only chromosome aberration data obtained in leukocytes to make estimates of genetic hazard of radiation or chemicals to man could also be very misleading. The data of Sasaki (1975) using synchronized skin cells showed that mouse embryos and newborns were about one-half as sensitive to aberration induction by x-rays as human skin cells, but that the cells from 11 day old mice were equally sensitive to aberration induction as human cells. More will need to be known about the comparative induction of initial lesions and the extent and rate of their repair in different cells from different species before the full usefulness of data such as that presented here will be realized.

SUMMARY

In our earlier studies we showed that the frequency of dicentrics induced by x-rays in human leukocytes was about twice that induced in mouse leukocytes. The frequencies of deletions was similar in both species. However, the mouse cultures were fixed at 60 hrs and the human cultures at 54 hrs. In both cases it was likely that some of the cells analyzed were in their second post-treatment mitosis. Further studies were carried out using fixation times of 48 hrs for both human and mouse cultures. The same relationships held here, namely twice as many dicentrics in human. and similar deletion frequencies in both. The aberration frequencies observed were corrected to take account of second division cells, by assuming cells containing a dicentric without an accompanying fragment were in their second division. There were more such cells with the mouse. To further increase reliance on the conclusions, cultures were fixed at the earliest times that 300 cells per dose could be obtained - 36 hrs for the mouse, 42 hrs for human. The frequencies of dicentrics were increased in both, and a relationship of about 2:1 for human to mouse was obtained. Deletion frequencies were similar in both. Three different human donors were used. Since no dicentrics without fragments were obtained, it appeared that aberration frequencies in first division cells only were being compared.

Table 1 Chromosome aberration frequencies following x-ray exposure to human leukocytes; a) 48 hr fixation (Donor J.P.) b) 42 hr fixation (Donor J.P.) c) 42 hr fixation (Donor J.P.) d) 42 hr fixation (Donor T.H.)

Table 2 Chromosome aberration frequencies following x-ray exposures to mouse leukocytes; a) 48 hr fixation b) 36 hr fixation

Table 1.

Dose (R)	No. cells scored	% Dicentrics (± S.E.)	% Acentric fragments (± S.E.)	Corrected % Dicentrics*
a) 48 hr fixation (Donor J.P.)				
0	500	0	0.8 ± 0.4	0
25	500	3.8 ± 0.8	1.6 ± 0.6	4.0
50	500	7.2 ± 1.2	5.8 ± 1.1	7.6
75	500	13.6 ± 1.6	6.8 ± 1.2	14.4
100	500	16.8 ± 1.8	11.8 ± 1.5	17.8
150	500	33.4 ± 2.6	19.2 ± 2.0	35.3
200	500	57.4 ± 3.4	26.6 ± 2.3	62.2
250	500	77.4 ± 3.9	46.4 ± 3.0	81.8
b) 42 hr fixation (Donor J.P.)				
0	500	0	0.6 ± 0.3	
25	300	2.3 ± 0.9	1.7 ± 0.7	
50	300	11.0 ± 1.9	5.3 ± 1.3	
75	300	15.3 ± 2.3	7.0 ± 1.5	
100	300	27.3 ± 3.0	14.3 ± 2.2	
150	300	40.3 ± 3.7	11.7 ± 2.0	
200	300	68.0 ± 4.8	25.0 ± 2.9	
250	250	87.6 ± 5.9	26.4 ± 3.2	
c) 42 hr fixation (Donor J.A.)				
0	300	0	0	
50	300	8.0 ± 1.6	4.0 ± 1.1	
100	300	17.7 ± 2.4	11.0 ± 1.9	
200	300	54.3 ± 4.3	22.3 ± 2.7	
250	300	74.3 ± 5.0	40.3 ± 3.6	
d) 42 hr fixation (Donor T.H.)				
0	300	0	0	
50	350	9.4 ± 1.6	5.0 ± 1.2	
100	300	21.0 ± 2.6	11.3 ± 1.9	
200	300	66.3 ± 4.7	37.7 ± 3.5	

* The corrected frequency is calculated by multiplying the observed frequency by the total proportion of dicentrics without fragments (49/864 = 0.057), and adding this value to the observed frequency.

Table 2.

Dose (R)	No. cells scored	Dicentrics (± S. E.)	% Acentric fragments (± S.E.)	Corrected % Dicentrics *
a) 48 hr fixation				
0	500	0	0	0
25	500	2.2 ± 0.7	2.2 ± 0.7	2.6
50	400	3.0 ± 0.9	5.5 ± 1.2	3.5
75	500	5.8 ± 1.1	4.6 ± 1.0	6.8
100	500	7.8 ± 1.3	10.4 ± 1.4	9.1
150	500	14.4 ± 1.7	11.0 ± 1.5	16.8
200	400	22.5 ± 2.4	22.2 ± 2.4	26.3
250	500	38.8 ± 2.8	29.0 ± 2.4	45.3
b) 36 hr fixation				
0	300	0	0	
50	100	9.0 ± 3.0	5.0 ± 2.2	
100	300	12.7 ± 2.0	12.7 ± 2.0	
200	250	48.0 ± 4.4	27.2 ± 3.3	

* The corrected frequency is calculated by multiplying the observed frequency by the total proportion of dicentrics without fragments (75/447 = 0.168) and adding this value to the observed frequency.

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Figure 1 Dose-response curves for dicentric aberrations following x-ray exposures to human leukocytes. O 48 hr fixation (Donor J.P.); O 42 hr fixation (Donor J. P.); 42 hr fixation (Donor J.A.); 42 hr fixation (Donor T.H.). The curves are the fit of $Y = bD + cD^2$ to the 48 hr fixation and 42 hr fixation (Donor J.P.) data.

Figure 2 Dose response curves for dicentric aberrations following x-ray exposures to mouse leukocytes. 48 hr fixation; O 36 hr fixation. The dotted line is for the human data, 42 hr fixation (Donor J.P.).