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ORIGINAL ARTICLE

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Preferential reduction of dicentrics in reciprocal exchanges due to the combination of the size of broken chromosome segments by radiation

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Abstract Induction rates of the dicentrics and translocations involving chromosomes 2 and 4 in peripheral lymphocytes irradiated with X-rays at a dose of 3 Gy were examined using a conventional Giemsa staining method and a chromosome painting method. In total, 228 reciprocal exchanges detected in 982 metaphases were classified into three groups according to the break points of the original chromosomes. The incidence of both acentric fragments being larger than half of the original chromosome (combination 1) was only seven (3%) and did not contribute significantly to induction rates. When the broken acentric fragments of two affected chromosomes were smaller than half of the original chromosomes (combination 2), which was found in 175 (77%) rearrangements, the induction rates of dicentrics and translocations were about the same (86:89). But if the sizes of the broken segments were unequal in both chromosomes (combination 3: one with a larger acentric part and the other with a smaller acentric part), the yield of dicentrics was significantly lower than that of translocations (16:30). It was suggested that there was a special mechanism causing preferential reduction of dicentrics in reciprocal exchanges originated from the heteromorphic size of broken chromosomes in the last combination.

Keywords Dicentrics · Translocations · Induction rate · Chromosome rearrangements · Radiation · Human lymphocytes

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Introduction

Dicentrics and translocations in lymphocytes are the most useful indicators of radiation dose assessment. When breakages occur at one point in each of two chromosomes, an asymmetrical rearrangement leads to a dicentric and a fragment and a symmetrical rearrangement results in a translocation. If the rejoining occurs randomly, dicentrics and translocations will be produced in an equal ratio. However, the actual observed rates of radiation-induced translocations are higher in general than that of dicentrics (1.63–9 fold, Lucas et al. 1989; 1–12.33 fold, Cremer el al. 1990; 1.5–2 fold, Natarajan et al. 1992; 1–3 fold, Tucker et al. 1993; 1.67–4 fold, Boei et al. 1996; greater than 1, Virsik-Peuckert et al. 1997; 1.4 fold, Finnon et al. 1995; 1.3–3.75 fold, Luomahaara et al. 1999; 1.55–2.79 fold, Schmid et al. 1992; 1.11–3.67 fold, Bauchinger et al. 1993; an observed high number in translocations at most dose points, Nakano et al. 1993; 1.2 fold, Knehr et al. 1999). A study previously reported from our laboratory (Kanda et al. 1996), in which cells in the second division and misclassification of aberrations were carefully excluded, also showed a slightly higher number of translocations, although the difference was not statistically significant. Natarajan et al. 1994 suggested the difference in the mechanisms of the misrepair process as a possible explanation for the high incidence of translocations. The mechanism of what contributes to the formation of translocations and dicentrics resulting from pairwise interactions of two radiation-induced broken chromosomes is still unknown.

In order to understand why translocations were induced more frequently than dicentrics, we analyzed the lengths of the pieces of chromosomes involved in the rearrangements. We found that the yield of dicentrics was significantly lower than that of translocations when original chromosomes were heteromorphic in the size of broken segments; one chromosome consists of a small centric piece and a large acentric fragment, and the other

consists of a large centric piece and a small acentric fragment.

Materials and methods

Irradiation and chromosome preparation

Peripheral blood was drawn into heparinized vacutainers from a healthy 33-year-old man. It was immediately irradiated with 200 kV X-rays at a dose of 3.0 Gy and kept at 37°C for 3 h. Then the lymphocytes were separated from the blood and cultured in RPMI1640 containing 20% fetal calf serum for 48 h in the presence of Kanamycin (60 μg/ml), PHA (0.2%) and Colcemid (0.05 μg/ml). The cultured cells were treated with a KCl hypotonic solution (0.075 M) at 37°C for 20 min and fixed with acetic alcohol (1:3). Air-dried slides were made under warm and humid conditions (Hayata et al. 1992).

Image acquisition of chromosome rearrangements

The chromosome slides were stained with Giemsa's solution. Well-spread metaphases were photographed at high magnification (×60 objective) with a digital camera system (Olympus). The positions of metaphases on the slides were recorded using an automated stage system (Nikon). The Giemsa staining was washed out with acetic alcohol. The slides were processed following the method reported by Kanda et al. 1996. Chromosome painting by FISH with composite whole chromosome-specific DNA libraries for human chromosomes 2 and 4 (Cambio, Cambridge, UK) was performed according to the method described by Yamada et al. 1996 with a slight modification. Those chromosomes were chosen for painting because they were large and not metacentric. The recorded positions of metaphases in the Giemsa stained slides were relocated and the painted images of the same metaphases were photographed again by the digital camera system when there was a rearrangement in chromosomes 2 and 4.

Comparison of the sizes of rearranged segments

The size of the rearranged piece compared with the total length of the original chromosome was decided by comparing both images (Giemsa-stained and FISH-painted) using the Microsoft Windows 'Painter' program. If a piece was larger than half the original chromosome, it was called large; if it was smaller than half, it was called small. When both pieces were the same size, they were called medium. Rearrangement involving the medium pieces was not included in the present analysis. Therefore, all translocations and dicentrics in this study originated from three combinations of broken chromosome pairs (Fig. 1). Combination 1: Both chromosomes consisted of a small centric piece and a large acentric piece (SC + LA and SC + LA). Combination 2: One chromosome consisted of a small centric piece and a large acentric piece, and the other of a large centric piece and a small acentric piece (SC + LA and LC + SA). Combination 3: Both chromosomes consisted of a large centric piece and a small acentric piece (LC + SA and LC + SA). To assess the relationship between break-points and the incidence of aberrations, only reciprocal translocations [t(Ba), t(Ab)] and dicentrics with a fragment [dic(BA) ace(ba)] (Tucker et al. 1995) occurring as a simple rearrangement were used.

Statistical analysis

Assuming that dicentrics and translocations in each case are produced with equal frequency and that breaks and rejoins occur randomly, statistical tests based on the normal approximation to the binominal distribution were performed.

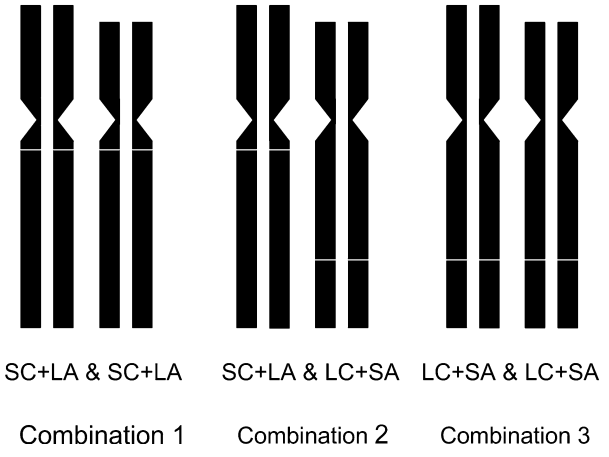

Fig. 1 Three combinations of original chromosomes according to the lesions induced by radiation. *LC* large centric, *LA* large acentric, *SC* small centric, *SA* small acentric

Results and discussion

One hundred six dicentrics and 125 translocations were found in 982 metaphases. However, 105 dicentrics and 123 translocations were subjected to the following analysis, as the rest involved the central chromosomal region in the rearrangements (see Materials and methods). The numbers of observed dicentrics and translocations involving chromosomes 2 and 4 are shown in Table 1, where expected numbers for respective rearrangements are also indicated.

The expected values were calculated as follows: According to Mayall et al. 1984, the relative size of chromosomes 2 and 4 are 4.19 and 3.31, and centromere indexes (10×long arm length/total length) of chromosomes 2 and 4 are 6.13 and 7.28, respectively. When we assumed that radiation induced breaks and joining randomly along these chromosomes, the ratio of chromosomal rearrangements involving each chromosome 2 and 4 was 559:441 [4.19/(4.19 + 3.31) versus 3.31/(4.19 + 3.31)]. If translocations and dicentrics are produced in the equal frequency, rearrangements involving chromosomes 2 and 4 are expected to be 63.7 and 50.3 cases in the total of 228 cases. Acentric fragments longer than half of the original chromosomes (SC + LA) are expected to be produced in 11.3 % (0.613–0.5) and 22.8% (0.728–0.5) for chromosome 2 and 4. As average centromere indexes of other chromosomes excluding either chromosome 2 or 4 are 6.72 and 6.63, respectively, acentric fragments longer than half of the original chromosomes (SC + LA) are expected to be produced in 17.2% and 16.3% of these cases. The probability of producing of LC + SA is 1 minus the probability of producing of SC + LA. Thus, the probabilities of respective combinations (1, 2, and 3 in Fig. 1) are obtained by the following formula: $(p_n \times p_{exn})$, $(p_n \times q_{exn} + p_{exn} \times q_n)$ and $(q_n \times q_{exn})$, where p_n and p_{exn} are the probabilities of producing of SC + LA with the painted chromosome (chromosome 2 or 4) and with other

Table 1 Incidences of dicentrics and translocations made from different original combinations in 228 rearrangements induced by radiation. Combination 1(SC + LA and SC + LA): Both chromosomes consist of a small centric piece and a large acentric piece. Combination 2(SC + LA and LC + SA): One chromosome consists of a small centric piece and a large acentric piece, and the other consists of a large centric piece and a small acentric piece. Combination 3(LC + SA and LC + SA): Both chromosomes consist of a large centric piece and a small acentric piece. Theoretical values by assuming the rearrangements occur randomly are shown in parenthesis

Original combination	Dicentrics involving chromosomes:			Translocations involving chromosomes:			Total
	2	4	2 and 4	2	4	2 and 4	
1(SC + LA and SC + LA)	1 (1.2)	2 (1.9)	3 (3.1)	2 (1.2)	2 (1.9)	4 (3.1)	7 (6.2)
2(SC + LA and LC + SA)	7 (15.7)	9 (15.9)	16[a] (31.6)	12[f] (15.7)	18 (15.9)	30 (31.6)	46 (63.2)
3(LC + SA and LC + SA)	50[d] (46.8)	36 (32.5)	86[b] (79.3)	53[g] (46.8)	36 (32.5)	89 (79.3)	175 (158.6)
Total	58[e] (63.7)	47 (50.3)	105[c] (114)	67[h] (63.7)	56 (50.3)	123 (114)	228 (228)

[a,b,c,] Dicentrics/(dicentrics + translocations) ± 95% confidence limits were 0.378 ± 0.144 for [a] (statistically significant, $p = 0.0390$); 0.491 ± 0.074 for [b] (not significant); and 0.461 ± 0.065 for [c] (not significant)

[d,e,f,g,h] Chromosomes 2/(2 + 4) ± 95% confidence limits were 0.581 ± 0.105 for [d]; 0.552 ± 0.095 for [e]; 0.400 ± 0.178 for [f]; 0.596 ± 0.103 for [g]: and 0.545 ± 0.088 for [h]. None were statistically significant

chromosomes, respectively; q_n and q_{exn} are the probabilities of producing of LC + SA with the painted and other chromosomes, respectively.

The observed number of total rearrangements involving chromosome 2 was 125 (58 + 67) and of chromosome 4 103 (47 + 56). Those values are in good agreement with the theoretical values of 127.4 (63.7 + 63.7) and 100.6 (50.3 + 50.3). The total observed number of translocations involving both chromosomes 2 and 4 was 123 and was slightly larger than 105 of dicentrics, although it is not statistically significant. When the original chromosome combination was SC + LA and LC + SA, the number of dicentrics and translocations were 16 and 30, respectively. The yield of dicentrics was significantly lower than that of translocations in this case. Observed numbers of dicentrics involving chromosomes 2 and 4 were 7 and 9. Both of those values are much smaller than the theoretical values of 15.7 and 15.9. Observed numbers of translocations involving chromosomes 2 and 4 were 12 and 18. Those values are not significantly different in comparison to the theoretical values of 15.7 and 15.9. On the other hand, if the original chromosome combination was LC + SA and LC + SA, the yields of dicentrics and translocations were 86 and 89. They are not significantly different from theoretical values. In combination 1 (SC + LA and SC + LA), the yields of dicentrics and translocations were 4 and 3 and do not contribute significantly to overall induction rates.

It seems that there is a special mechanism causing preferential reduction of dicentrics in reciprocal exchanges when original chromosomes were heteromorphic in the size of broken segments; one chromosome consists of a small centric piece and a large acentric fragment, and the other consists of a large centric piece and a small acentric fragment. Now that we could find such preferential reduction of dicentrics in reciprocal exchanges, we need to perform further studies, e.g., by painting other chromosomes to understand why such an interesting phenomenon is observed only in this combination.

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