5-Bromodeoxyuridine induced sister chromatid exchange frequencies in primate lymphocytes

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Summary. Comparisons of SCE frequencies at various BrdUrd concentrations showed significant interspecies differences with human lymphocytes being the most sensitive.

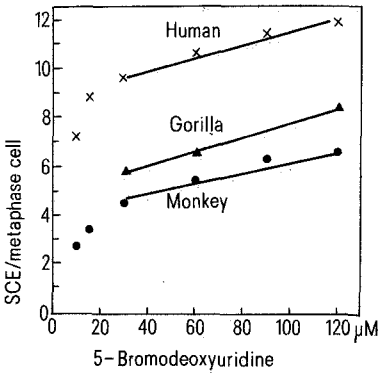
Established procedures for visualization of sister chromatid exchanges (SCE) with light microscopy require the incorporation of 5-bromodeoxyuridine (BrdUrd) during 2 consecutive rounds of DNA replication. BrdUrd is known to induce SCE[2-4]. Variation in sensitivity to BrdUrd among individuals does not appear to significantly influence baseline SCE frequencies[3-5], nor does diploid chromosome number[6]. Interspecies differences in SCE frequencies among cattle, pigs, sheep and humans have been demonstrated with increasing concentrations of BrdUrd[7]. We have conducted a series of experiments to determine interspecies differences in sensitivity to BrdUrd induction of SCE with lymphocytes from primates.

Peripheral blood samples were obtained in heparin from *Homo sapiens, Gorilla gorilla* (lowland), *Saimiri sciurens, Macaca malatta, Macaca fascicularis, Cynopithecus niger* and *Cercopithecus aethiops.* These were cultured in chromosome medium 1A with phytohemagglutinin (GIBCO, Grand Island, N.Y.) containing various concentrations of 5-bromodeoxyuridine (GIBCO, Grand Island, N.Y.). Cultures were incubated in the dark at 37 °C for 72 h. Colcemid (GIBCO, Grand Island, N.Y.) 0.1 µg/ml culture media was added for the final 2 h. Following hypotonic treatment with 0.075 M KCl at 37 °C for 10 min, the cells were fixed and washed 3 times with fresh, cold acetic acid:methanol (1:3). Differential staining was achieved by the fluorescence plus Giemsa method[8]. Sister chromatid exchanges were scored from 30 to 50 complete metaphases for each culture.

The overall mean frequencies of SCE/metaphase for the donors of each species at increasing BrdUrd concentrations are presented in the table.

An analysis of variance performed on these data indicated no significant differences in the BrdUrd induced SCE frequencies among the species of monkeys tested. With the 2 sample t-test at significance level 0.05, the critical t-value is 1.99. The t-value for monkey-gorilla was 1.38, indicating no significant difference. However the t-values for monkey-human ($t = 6.35$) and for gorilla-human ($t = 5.63$) indicated significant differences. More meaningful comparisons among species can be made on the basis of their response to increasing BrdUrd concentrations. The figure graphically summarizes these data. Data from all 17 monkeys were averaged at appropriate BrdUrd concentrations. At lower concentrations of BrdUrd, SCE frequencies rose sharply for all species tested. For this reason it was difficult to extrapolate to determine spontaneous SCE frequencies. However, for concentrations between 30 and 120 M BrdUrd a linear relationship was obtained. BrdUrd induction of SCE in all species tested is indicated by the fact that slopes of the regression lines were significantly different from zero ($p < 0.01$). Comparisons of these slopes among species showed the monkey to be significantly different from the human ($p < 0.01$) while the gorilla was not significantly different from the human. Thus, both the discrete point and the regression line slope differences indicated significant difference between the monkey and human.

Various experimental factors have been shown to effect SCE frequency. Among these are serum in culture media[9] and BrdUrd concentration/cell. However, neither of these was involved since the same serum batch and medium was used for culturing a standardized number of leucocytes/volume of medium. Giulotto et al.[10] have shown that SCE frequency appears to be independent of proliferation properties of cultured lymphocytes. In addition, both early and late proliferating cells appear to be equally sensitive to BrdUrd with respect to the induction of SCE[11]. Although a correlation between cellular DNA content and SCE frequency has been suggested[9], the differences in cellular DNA content among the primates tested is not the magnitude of the observed differences in SCE frequencies.



Sister chromatid exchanges in lymphocytes of human, gorilla and monkey cultured in media containing increasing concentration of BrdUrd. Data points show the means of 125 cells for humans, 50 cells for gorilla and a minimum of 500 cells for monkeys at each BrdUrd concentration.

Sister chromatid exchanges in lymphocytes cultured with varying concentrations of 5-bromodeoxyuridine[a]

Species	Donors	BrdUrd concentration (µM) 10	15	30	60	90	120
M. fascicularis	3	2.78(0.75)	3.17(0.83)	4.16(0.43)	5.55(0.42)	6.12(0.29)	6.69(0.30)
C. aethiops	5	3.04(0.66)	3.47(0.45)	4.78(0.52)	5.19(0.41)	6.06(0.44)	6.35(0.37)
M. malatta	2	2.49(0.36)	3.01(0.27)	4.25(0.41)	5.01(0.28)	6.04(0.27)	6.07(0.23)
S. sciurens	2	–	–	–	5.84(0.39)	–	–
C. niger	5	2.84(0.80)	3.83(0.74)	5.13(0.60)	5.90(0.48)	6.81(0.79)	6.92(0.45)
G. gorilla	1	–	–	5.89(0.41)	6.62(0.37)	–	8.40(0.49)
H. sapiens	4	7.26(0.38)	8.93(0.71)	9.64(0.61)	10.77(0.67)	11.52(0.58)	12.01(0.54)

[a] Each entry is the overall mean (± SE) for at least 30 metaphase cells/donor at each concentration.

A portion of the observed variation in SCE frequencies among primates might be the result of inherent differences in BrdUrd incorporation. Stetka and Carrano[12] found that the BrdUrd/base pair ratio correlated well with SCE frequency. The incorporation of BrdUrd is not linearly proportional to the molarity of BrdUrd in the culture medium, while the BrdUrd induced SCE frequencies are linearly proportional to the percentage substitution of BrdUrd for dThd[13]. It appears that BrdUrd competes with dThd for incorporation into DNA and that this competition determines the frequency of BrdUrd induced SCE. That mutagenicity of BrdUrd is not determined by the amount of the analog incorporated into DNA[14] might not be a valid factor for explaining interspecies differences in BrdUrd induced SCE since increased SCE frequency is not necessarily equivalent to increased mutagenicity[15,16].

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Red blood cell glutathione peroxidase in simple trisomy 21 and translocation 21/22[1]

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Summary. Red blood cell glutathione peroxidase activity was increased by about 50% in all Down's syndrome patients studied. It was slightly lower in translocation as compared with simple trisomy 21 but much higher with respect to controls.

Since the connection between trisomy 21 and Down's syndrome phenotype was postulated[4], numerous studies investigating enzyme activities in trisomy 21 have been carried out[5-9]. The most important recent discoveries in this field include the gene dosage effect[10], and localization of genes responsible for the phenotype of Down's syndrome and SOD-1 production[11,12]. However, activities of enzymes, being conditioned by many factors, seldom confirm theoretical predictions directly[13,14].

Inter alia, reports concerning the glutathione peroxidase (GSH-Px) activity are not consistent, some authors reporting its increased activity in Down's syndrome[15], and others finding no statistically significant increase[16]. In this report we give evidence for a statistically significant elevation of the GSH-Px level in erythrocytes of Down's syndrome patients.

Material and methods. 21 patients with Down's syndrome (19 with trisomy 21 and 2 with unbalanced translocation 21/22) were studied. Their karyotypes, determined according to Moorhead et al.[17], are shown in table 2. GSH-Px activity was estimated by the method of Beutler[18] in hemolysates prepared from heparinized blood. All absorbance measurements were performed using a Unicam SP 800 spectrophotometer (accuracy of 0.001).

Results and discussion. Data presented in tables 1 and 2 show an increase of about 50% in GSH-Px activity in patients with trisomy 21 as compared to controls. The difference is statistically significant ($p < 0.01$ using Student's t-test). Since in our previous work[19] we found a different behavior of SOD-1 activity in erythrocytes of patients with trisomy 21 and with unbalanced translocations 21/14 and 21/22, it seemed worthwhile to compare the GSH-Px activity in Down's syndrome due to translocation 21/22 with the activity in standard trisomy 21. Activity of the enzyme in erythrocytes of translocation patients was also increased but was slightly lower than in trisomy 21 (table 2). The low number of cases with translocation precludes detailed statistical analysis but the data obtained suggest some position effect.

Table 1. GSH-Px activity in erythrocytes of control subjects (units per g of hemoglobin)

No.	Sex	Activity
1	M	33.85
2	M	32.46
3	M	33.15
4	M	31.90
5	M	35.03
6	M	33.64
7	M	31.89
8	F	35.40
9	F	34.23
10	F	33.80
11	F	34.23
12	F	33.45
13	F	32.75
14	F	33.84
15	F	32.50
	Mean	33.45
	SD	0.39