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Assessment of Cell Cycle Duration on the Incidence of Sister Chromatid Exchanges in Somatic and Spermatogonial Cells of the Rat

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ABSTRACT

In order to examine the influence of the length of cell cycle on the incidence of sister chromatid exchanges (SCEs), average generation times and SCEs were studied in the presence of bromodeoxyuridine from spontaneously dividing rat lymph node, bone marrow, spleen, and spermatogonial cells. Average generation time differences among the three somatic cell types (lymph node, 7.6 h; bone marrow, 12.0 h; spleen, 14.9 h) were statistically significant as were the differences between the germinal cell (37.4 h) and each of the somatic cells. The SCE per cell frequencies were significantly higher in the somatic cells (lymph node, 6.8; bone marrow, 5.8; spleen, 6.1) as compared to the spermatogonial cells (1.6). However, no difference in SCE incidence was detected among the cells from the different somatic tissues. It was concluded that there was no simple relationship between cell cycle duration time and SCE formation.

INTRODUCTION

Although the precise molecular mechanism of SCE[2] formation and its biological significance remain to be ascertained, induction of SCEs in target tissues is becoming a popular tool for screening mutagenic/carcinogenic potential of chemicals. Among various *in vivo* target tissues, bone marrow, spleen, lymph node, and spermatogonium are generally analyzed for SCE induction. However, it is yet to be determined if individual cell cycle periods have any impact on SCE yield in these tissues.

Rat bone marrow cell cycle time estimated *in vivo* appears to be the same whether BrdUrd technology or tritium-autoradiographic methodology is employed (1). The cell cycle kinetics of bone marrow, spleen, lymph node, and spermatogonium of the rat were examined in the presence of BrdUrd. There were significant differences in AGTs between somatic and spermatogonial cells as well as among the three somatic cell types in a probe study presented earlier (2). This communication reports examination of SCEs and cell generation times in these multiple cell types *in vivo* to seek a relationship between the length of cell cycle and SCE incidence.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River, Wilmington, MA) approximately 10–12 weeks old were utilized.

BrdUrd powder (Sigma Chemical Company, St. Louis, MO) compressed into tablets (1–2 g BrdUrd/kg body weight) were implanted s.c. into the dorsal thoracic area of anesthetized rats, and the wounds were closed with stainless steel autoclips (3).

All the animals received an i.p. injection of colchichine (2.0 mg/kg) 3 h prior to sacrifice. The femoral bone marrow was aspirated in Hanks' balanced salt solution, treated with 0.075 M KCl hypotonic at 37°C, and fixed in 3:1 absolute methanol-glacial acetic acid. The spleen and lymph node were minced several times in the 0.075 M KCl solution prior to fixation. Spermatogonial cells were exposed to trypsin (0.1%) before KCl treatment and fixation. The cell pellets of both somatic and spermatogonial cells were air dried on slides and stained by FPG technique (4).

At the time of sacrifice (maximum, 72 h), any remaining BrdUrd tablets were removed, allowed to dry, and then weighed to estimate the amount of BrdUrd absorbed. When animals were implanted with 1 g BrdUrd/kg tablets, as low as 0.53 g/kg of absorbed nucleotide analogue resulted in adequate chromatid differentiation independent of the length of implantation. Doses lower than 1 g/kg showed lesser BrdUrd absorption and poor chromatid differentiation. Thus, 1 g BrdUrd/kg body weight was the dose chosen for the present investigation.

A majority of second division cells was observed in the spermatogonial preparations of the animals that had received BrdUrd tablets for 72 h. By this time, although the tablets were completely absorbed, the rats were very sick and additional tablets could not be used. Neither a larger tablet nor agar-coated tablets (5) proved practical.

The cell cycle kinetics of somatic and spermatogonial cells were determined by evaluating metaphases undergoing first, second, or third and subsequent divisions (6, 7). Harvest times were initially adapted from those of the mouse (3) and then adjusted to yield cells at different replication cycles. The AGTs for various tissue cell types were estimated following the formula of Ivett and Tice (8). SCE frequency was computed as the number of chromatid exchanges per chromosome and normalized to reflect SCEs/42 chromosomes (rat 2n = 42). Only second division metaphases were scored for SCE determination. As much as possible, the frequencies of these metaphase figures were optimized by selecting the appropriate harvest times.

The mean frequencies of AGTs and SCEs were computed per cell/animal and compared by one-way analysis of variance followed by Bonferroni's t test. As a supplemental analysis, the distribution of SCEs per cell within each tissue was compared by the statistical approach of Margolin and Shelby (9). The level of significance used in all comparisons was $\alpha = 0.05$.

RESULTS

BrdUrd-implanted somatic tissues harvested during 22–24-h intervals showed remarkable variations in the distribution of first-, second-, or third-division cells to the extent that 65% of lymph node cells had divided three times in comparison to only 8% of spleen and 13% in marrow (data not shown). Such a pattern of cycle kinetics of the cells in three somatic tissues suggested a much shorter cycle period for lymph node cells. Thus, as expected, the number of third-division cells was greatly decreased in the lymph node when the harvest times were reduced to 12–14 h. At these harvest times, none of the spleen or marrow cells had entered into a third-division cycle. Between 22 and 24 h of harvesting periods, however, a higher number of third-division cells was recorded in bone marrow, which suggests a slightly longer cycle period for spleen cells. These preliminary cell kinetic data indicated that the mean cell cycle periods of the three somatic tissues vary in the descending order of spleen, bone marrow, and lymph node.

Only first-division BrdUrd-exposed spermatogonial mitoses were recovered at the 48-h period, which presumably indicated a pattern of synchrony among certain spermatogonial cells entering into the replication cycles (10). As the time of harvest

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[2] The abbreviations used are: SCE, sister chromatid exchange; BrdUrd, bromodeoxyuridine; AGT, average generation time.

Table 1 *In vivo cell cycle kinetics and SCE frequencies in various tissues of male Sprague-Dawley rats*

Data are means ± standard deviations of five animals; data are in addition to those reported earlier (2).

Tissue	No. of animals	Harvest time (h)	Dose of BrdUrd (mg/kg)	% Metaphases in 1st division	% Metaphases in 2nd division	% Metaphases in 3rd division	AGT[a]	SCE data[b] SCE/cell	SCE data[b] H[c]
Lymph node	5	13.4[d]	0.72 ± 0.11	27.8 ± 6.8	67.6 ± 8.1	4.6 ± 4.8	7.6 ± 0.4	6.8 ± 0.6	1.0 ± 0.3
Bone marrow	5	24	0.85 ± 0.19	12.4 ± 9.4	74.2 ± 10.7	13.4 ± 7.0	12.0 ± 0.8	5.8 ± 1.0	1.4 ± 0.4
Spleen[e]	5	24	0.85 ± 0.19	56.0 ± 5.1	26.4 ± 5.0	17.6 ± 1.8	14.9 ± 0.5	6.1 ± 1.2	1.0 ± 0.2
Spermatogonia	5	72	0.92 ± 0.06	7.8 ± 2.5	92.0 ± 2.4	0.2 ± 0.4	37.4 ± 0.5	1.6 ± 0.2[f]	1.2 ± 0.3

[a] Average generation time = duration of BrdUrd (h)/1 × I + 2 × II + 3 × III, where I, II, and III represent the proportions of 1st, 2nd, and 3rd (or subsequent) division cells, respectively (8); the AGTs varied significantly ($\alpha \leq 0.05$) from one another among the tissues examined.
[b] 25 cells were scored from each animal for each tissue.
[c] Normalized variance of SCE/cell within each animal (H = mean/variance).
[d] The harvest time reported for lymph node is the mean. The harvest times for individual animals ranged 12.5–14.0 h.
[e] Spleens were collected from the same animals used for the bone marrow cells.
[f] Significantly different from all other tissues ($\alpha \leq 0.05$).

was increased, both second- and third-division cells started appearing. The 72-h period was selected for spermatogonial SCE determination to avoid the use of any additional BrdUrd tablets.

When data were pooled from five animals to determine the AGTs for the somatic and spermatogonial cells of the rat, statistically significant differences existed not only between the somatic and germinal tissues but even among the somatic tissues (Table 1). Among the somatic tissues, a spleen cell with an AGT of 14.90 h took the longest time to divide, whereas a 7.60-h AGT for the lymph node cell was the shortest. Spermatogonial AGT, on the other hand, was many times longer than that of any of the three somatic tissues examined. Although generation time of lymph node cells was estimated for the first time in the present investigation, comparable AGTs for other rat somatic cells and for rat spermatogonial cells have been reported earlier by employing the technique of tritium-autoradiography (11–15).

No significant difference in baseline SCE frequencies was noticed among the three somatic cell types (Table 1). However, when SCE frequencies were compared between the somatic and germinal cells, SCEs in each of the three somatic tissues were almost four times higher than in spermatogonia.

DISCUSSION

Since identical parameters were utilized to estimate the AGTs of various rat tissues, such AGT estimates can be applied to compare the cellular replication times of these tissues. Variations observed in replication times between nucleoside-labeled somatic and germinal tissue or among the somatic tissues have been reported previously by employing ^{3}H-autoradiographic methodologies (12, 15). However, our observation of a 7.60-h generation time for rat lymph node cells, which is about half of the observed spleen and bone marrow cell cycle periods, represents the fastest cell cycle time thus far reported for an *in vivo* replicating cell type. Most probably, this is why analysis of tritiated thymidine-labeled lymph node cells freshly cultured from rabbits were found to have a G_2 period approximately half that of similarly treated bone marrow cells (16).

No difference in SCE incidence was noted among the three somatic tissues, despite the fact that cell cycle lengths varied significantly among these tissues. Insignificant differences in baseline SCE frequencies have also been reported among various somatic tissues from the same animal for other mammalian species (3, 17, 18). The finding that cell cycle duration time has no significant effect on SCE formation in the whole animal is not consistent with other reports (19–22) showing that increases or decreases from optimal culture temperatures resulted in elevations in baseline SCEs in cultured cells with temperature-induced variable cycle lengths. These *in vitro* studies did not exclude the possibility that temperature itself may affect cycle lengths (23, 24) and SCE frequencies (25). In the present study, in contrast to the *in vitro* studies, the influence of cycle length on SCE formation was evaluated in an *in vivo* system, which is not susceptible to many of the artificial conditions commonly observed to affect *in vitro* results. Therefore, *in vivo* generated data are generally accepted as reflecting the normal physiological response.

Since the cells with longer generation time conceivably have greater opportunities for repair of spontaneously occurring DNA damage prior to SCE formation, it can be argued that such cells would contain fewer number of SCEs than cells with shorter generation times. However, no association of cell cycle length with SCE incidence was noted for the somatic tissues even though the spermatogonial cell was found to exhibit a longer cycle period and significantly fewer SCEs. Others have also reported low incidence of SCEs in the spermatogonium (17, 26). Since scoring of SCEs was based as much as possible upon optimal harvest times for 2nd-division cells in respective tissues, the much fewer SCEs in spermatogonial cells perhaps represent either the differentiation state of these cells or unequal BrdUrd substitution (BrdUrd-induced SCEs) between somatic and spermatogonial cells because of blood-testis barrier. Alternately, significantly lower numbers of SCEs in the spermatogonial cell reflect repair of a large amount of the background DNA damage prior to entering into the *S* phase by virtue of its cell cycle period many times longer than those of the somatic cells.

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