A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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DEGRADATION OF ^{3}H-TdR LABELLED DNA OF HUMAN LYMPHOCYTES DURING STORAGE

Influence of ^{3}H-thymidine incorporated into DNA of human peripheral blood lymphocytes on damage to lymphocyte DNA and possibilities of storage of ^{3}H-labelled culture of human lymphocytes in PBS (buffered physiological saline, pH 7.2) at 4^{o}C was studied by estimations of molecular weight of DNA in alkaline sucrose density gradient and determination of the amount of viable cells. The results obtained in estimation of molecular weight of DNA indicated that storage of ^{3}H-thymidine labelled human lymphocytes causes DNA degradation. The simultaneous lack of essential changes in lymphocytes survival suggest that during the storage-induced damage the DNA damage preceedes lymphocytes cell death.

Introduction

It has been shown in many studies of mammalian cells and bacteria that radioisotopes cause lethality and strand breaks when incorporated into DNA. The ^{14}C-decay induces potentially lethal lessions in bacterial DNA [17]. ^{125}Iodine incorporated into DNA as the thymine analogue 5-/^{125}I/-iododeoxyuridine causes lethality, mutations and double-strand breaks in E. coli [15], in phages T_1 and T_4 [19, 20, 29], and decreases survival of mammalian cells [2,7]. B o y d et al. [1] have shown that therapeutic doses of ^{125}I are capable of producing chromosome damage in human peripheral lymphocytes. The lethal and mutagenic effects of ^{32}P incorporated into biological systems have been also well dokumented [13, 14, 16, 22].

One of the most commonly used radioisotopes, ^{3}H-thymidine,

is a toxic agent when incorporated into DNA of mammalian cells, too. It has been estimated that the effect of tritium are similar to equivalent doses of X or gamma-radiation [6, 9, 28]. Tritium incorporated into Saccharomyces cerevisae induces reproductive death, gene conversions and mutations similar to those produced by an equivalent dose of gamma-radiation [4]. ^{3}H-Thymidine can produce chromosome aberrations when added to cultures of human lymphocytes in vitro [23]. ^{3}H-Tritium decay causes single-strand breaks in Chinese hamster cells V 79 [3]. Data on influence of ^{3}H-thymidine incorporated into DNA of human peripheral blood lymphocytes on the damage to DNA as well as possibilities of storage of labelled lymphocytes are lacking. We have been attempting to check whether the process of storage of ^{3}H-thymidine-labelled human lymphocytes in solution of PBS (phosphate-buffered saline) at 4°C influences the DNA degradation and cell survival. PBS was used as storage medium since PBS treatment does not produce toxicity nor produce degradation [33].

Material and methods

The lymphocyte cells used in these experiments were isolated from heparinized blood by the sedimentation method involving CM--cellulose. Granulocytes and macrophages were removed owing to their adhesion to glass at 37°C. The lymphocytes were cultured in a 199 medium containing 10% calf serum, antibiotic and phytohemagglutinin at 37°C for 48 h. The lymphocyte DNA was labelled with ^{3}H-thymidine with specific activity 5 mCi/mmol for 24 h, at a final activity of 2 μCi/ml. The cell survival was determined by Trypan Blue staining and labelling ^{51}Cr. Molecular weight of DNA was estimated by ultracentrifugation in alkaline sucrose density gradient according to M c G r a t h and W i l l i a m s [24]. 5-20% linear sucrose density gradients in 0.1 M NaOH, 0.9 M NaCl and 1 mM EDTA were used. The cells were lysed directly on the top of the gradient for 30 minutes at room temperature (the lysing solution contained 0.5 M NaOH and 0.1 M EDTA), and were centrifuged at 35 000 rpm in a Beckman SW 60 rotor for 2 h at

10°C. The gradients were calibrated with the phage T_4DNA (73S). The average molecular weight of DNA was estimated from the Studier's equation [31].

Results

Typical sedimentation profiles of ^{3}H-labelled DNA from human lymphocytes in 5-20% alkaline sucrose density gradient after different times of storage of lymphocytes are shown in Fig. 1.

The values of average molecular weight and sedimentation coefficient of human lymphocyte DNA after different times of cell storage are given in Tab. 1. The average molecular weight of DNA released from lymphocytes is equal to 26.10 ±2.87 x 10^7 daltons.

T a b l e 1

Effect of storage time of lymphocytes upon the average molecular weight and sedimentation coefficient of DNA

Wpływ czasu przechowywania limfocytów na masę cząsteczkową i stałą sedymentacji DNA

Влияние хранения лимфоцитов на молекулярный вес и константу седиментации ДНК

Storage time	n	M_w x 10^{-7}	Sedimentation coefficient, S
		m ±s	m ±s
0 h	11	26.10 ±2.87	121.0 ±4.9
24 h	10	5.08 ±0.45	63.1 ±2.5
48 h	9	2.71 ±0.47	48.6 ±2.6

This value corresponds to established values of subunit molecular weight of mammalian DNA in alkaline sucrose density gradients [5, 21, 26, 27]. The average molecular weight of DNA released from lymphocytes stored for 24 h is equal to 5.08 ±0.45 x 10^7 daltons, and of DNA from lymphocytes stored for 48 h to 2.71 ±0.47 x 10^7 daltons.

As it results from the data given in Tab. 1, the storage of lymphocytes results in a decrease of molecular weight of cell DNA. The decrease in the average molecular weight of lymphocyte

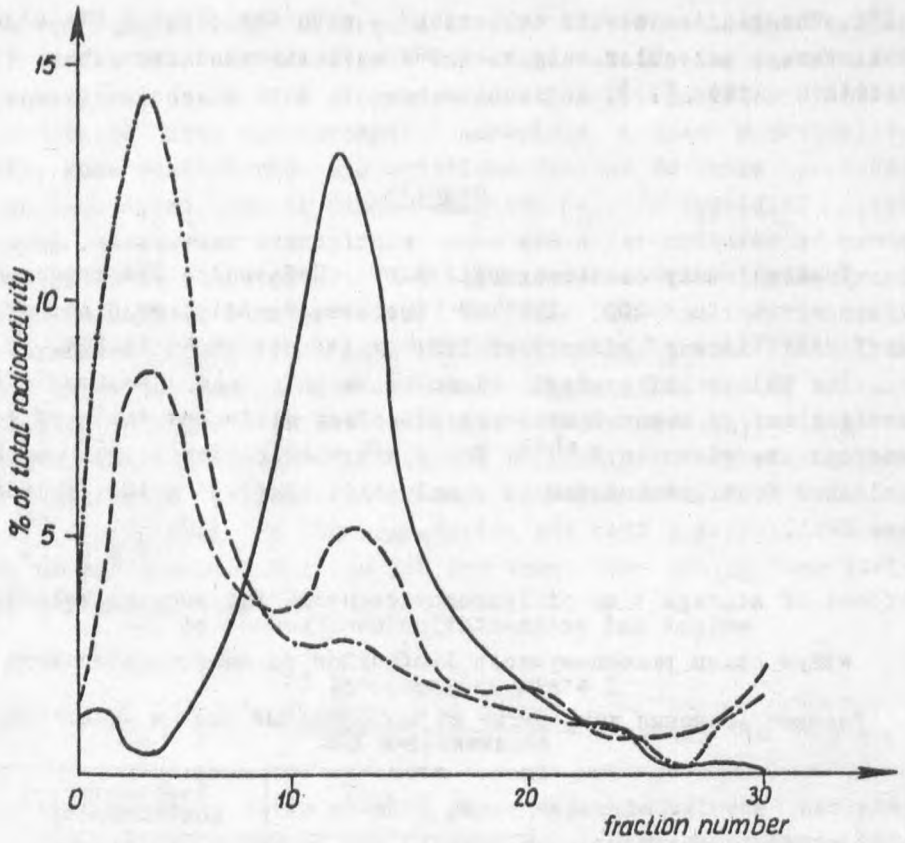

Fig. 1. Sedimentation profiles of ^{3}H-labelled human lymphocyte DNA in a 5-20% alkaline sucrose density gradient after various times of storage of lymphocytes. DNA was obtained by lysing of lymphocytes for 30 min before centrifugation. Lysing solution: 0.5 M NaOH, 0.1 M EDTA. Centrifugation: 35 000 rpm, 120 min, about 10°C, SW-60 rotor. Lymphocytes stored for —— 0 h, --- 24 h, -·-·-· 48 h

Profile sedymentacji znakowanego trytem DNA limfocytów ludzkich w 5-20% gradiencie gęstości alkalicznej sacharozy. DNA otrzymano w wyniku 30-minutowej lizy limfocytów na powierzchni gradientu bezpośrednio przed wirowaniem. Skład roztworu lizującego: 0,5 M NaOH, 0,1 M EDTA. Wirowanie: 35 000 rpm, 120 min, temp. 10°C, rotor SW-60. Limfocyty przechowywano przez —— 0 h, ---24 h, -·-·-48 h

Профили судиментации меченой тритом ДНК человеческих лимфоцитов в 5-20% градиенте плотности щелочной сахарозы. ДНК получали после 30-минутного лизиса лимфоцитов на поверхности градиента непосредственно перед центрифугированием. Состав лизирующего раствора: 0,5 М NaOH, 0,1 М EDTA. Центрифугирование: 35 000 оборотов/мин, 120 минут, температура 10°С, ротор SW-60. Лимфоциты хранили в течение —— 0 часов, --- 24 часов, -·-· 48 часов

DNA amounted to 81% for the time interval of 0-24 h, to 90% for the time interval of 0-48 h, and to 47% for the time interval of 24-48 h (Tab. 2). These changes are statistically significant.

T a b l e 2

Differences between the average molecular weights of DNA obtained from lymphocytes stored for different time intervals

Zmiany masy cząsteczkowej DNA otrzymanego z limfocytów przechowywanych przez różny okres czasu

Изменения молекулярного веса ДНК, полученной из лимфоцитов хранимых в различные промежутки времени

Time intervals	DNA molecular weight changes (in per cent)	p<
0 h ⟶ 24 h	81	0.001
0 h ⟶ 48 h	90	0.001
24 h ⟶ 48 h	47	0.002

The lymphocyte survival, as estimated on the basis of Trypan Blue staining amounted to 99% after a 24 h cultivation. After 24 and 48 h of storage of lymphocytes, their viability decreased to 90%. After the same time of storage, the amount of lymphocytes decreased by 5%. It seems that after storage of lymphocytes in PBS (phosphate buffered saline) at 4°C, viability and density of the cells were conserved because the observed changes are devoid of statistical significance. Similar results were obtained in experiments on cell survival estimated by measurements of ^{51}Cr release.

Discussion

^{3}H-Tritium incorporated into biological molecules can cause damage either due to beta-radiation of tritium decay, or from chemical changes associated with the transmutation from 3H to 3He. It has been shown that ^{3}H-decays in the nucleus are more effective than ^{3}H-decays in cytoplasm [5]. H o f e r et al.[12] showed that cytotoxic effects of ^{67}Ga-citrate, ^{3}H-thymidine and ^{125}I-iododeoxyuridine on L 1210 leukaemia cells labelled with various doses of these three radioisotopes result exclusively from nuclear damage. Therefore ^{3}H-thymidine decay demaged, first

of all, DNA. Tritiated thymidine (^{3}HTdR) is incorporated directly into DNA and thus conserved in the cell nucleus as a part of genetic material [3]. For this reason, it seems that it is necessary to avoid storage of ^{3}H-thymidine-labelled cell cultures in investigations of their DNA, because storage results in a decrease of molecular weight of this DNA due to its degradation. The results obtained in this paper indicate that average molecular weight of DNA markedly decreases after 48 h storage of ^{3}H-thymidine labelled human lymphocytes. Similarly, intracellular ^{14}C-decays in labelled V 79 Chinese hamster cells lead to strand breaks and to a progressive decrease in molecular weight of DNA with increased time of storage at -196°C [8] and ^{125}I-decay induces double-strand breaks in *E. coli* DNA when radiolabelled bacteria were stored at -196°C [18]. The sedimentation profiles of DNA obtained from HeLa cells treated with ^{3}H-thymidine was affected by the time of exposure of these cells to the radioisotope [11]. However it is worth to note that some reports suggested possibility of such degradation caused by nonradioactive processes [25, 30, 32]. In the light of these observation the influence of enzymic processes can not be excluded.

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DEGRADACJA ZNAKOWANEGO ^{3}H-TYMIDYNĄ DNA LIMFOCYTÓW LUDZKICH PODCZAS ICH PRZECHOWYWANIA

DNA limfocytów ludzkich z krwi obwodowej znakowano ^{3}H-tymidyną. Oznaczano wpływ ^{3}H-tymidyny na przeżywalność komórek i na właściwości sedymentacyjne DNA limfocytów przechowywanych w PBS w temp. 4°C przez różny okres czasu. Masę cząsteczkową DNA wyznaczano metodą ultrawirowania w alkalicznym gradiencie gęstości sacharozy. Stwierdzono, że przechowywanie znakowanych ^{3}H-tymidyną limfocytów powoduje degradację ich DNA. Nie zaobserwowano zmian w przeżywalności limfocytów, co sugeruje, że w stosowanych warunkach przechowywania uszkodzenie DNA poprzedza śmierć komórki.

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РАСПАД МЕЧЕНОЙ ^{3}H-ТИМИДИНОМ ДНК ЧЕЛОВЕЧЕСКИХ ЛИМФОЦИТОВ ВО ВРЕМЯ ИХ ХРАНЕНИЯ

ДНК человеческих лимфоцитов периферической крови метили ^{3}H-тимидином. Определяли влияние ^{3}H-тимидина на хранение клеток и на седиментационные свойства ДНК лимфоцитов, хранимых в PBS (буферный раствор физиологической солц) при температуре 4°C в различные промежутки времени. Молекулярный вес ДНК определяли методом дифференцированного ультрацентрифугирования в щелочном градиенте плотности сахарозы. Показано, что хранение лимфоцитов, меченых ^{3}H-тимидином вызывает распад ДНК лимфоцитов. Существенные отличия в переживании клеток не наблюдались, что свидетельствует о том, что в данных условиях хранения повреждение ДНК предшествует смерти клетки.