ACTA VET.BRNO,55,1986: 173-182

THYMIDINE SUICIDE ASSAY OF EXOGENOUS SPLEEN COLONY FORMING UNITS: INTERFERING INFLUENCE OF THE CHEMICAL CYTOTOXICITY OF 3H-THYMIDINE PREPARATIONS

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Received August 26, 1985

Abstract

V á c h a J., J. H o l á, V. Z n o j i l: *Thymidine Suicide Assay of Exogenous Spleen Colony Forming Units: Interfering Influence of the Chemical Cytotoxicity of 3H-Thymidine Preparations.* Acta Vet. Brno, 55,1986: 173-182.

Numerical values of thymidine suicide of CFU-S were compared using several 3H-TdR batches produced by the Institute of Production, Research and Application of Radioisotopes, Prague. The thymidine suicide values obtained are significantly higher using some batches than using other ones in the same cell suspension and they even rise over the biologically reasonable limit of 70 - 80%. The expected cytotoxicity of 3H-TdR preparations could not be induced by their gamma irradiation with doses corresponding to autoirradiation of preparations during long-term storing. Nor could toxic properties be cancelled by lyophilization and performing rechromatography of preparations by menas of H_2O as solvent on paper. Values of CFU-S 3H-TdR suicide in an array of inbred mouse strains under physiological conditions are also given.

Thymidine suicide of cells, CFU-S, cytotoxicity of 3H-TdR preparations, inbred mouse strains.

Several authors working with 3H-thymidine (further on 3H-TdR) of various origin suspect that some production batches of these preparations influence stem cells of haemopoietic tissues not only as a source of radiation causing the "thymidine suicide", but also cytotoxically as a result of the presence of some chemical impurities (personal communication of several European experimenters). As we ourselves have similar experience with the domestic 3H-TdR preparation, we shall refer to them and shall try to explain some aspects of this methodically important problem.

Materials and Methods

The Animals

Mice of four inbred strains (or interstrain hybrids) of our own conventional breed were used, whose health condition corresponds to category II according to K l í r et al. 1983, except for the C57BL/10cSnPh strain in which higher antibody titres against mouse hepatitis virus (MHV) were ascertained and which corresponds to category I. Donors of marrow grafts were all males about three months old, recipients males and females 2.5 to 4 months old. All experiments were carried out on intact animals only, under physiological conditions.

Irradiation

of marrow recipients was performed immediately before marrow administration on a Chisostat ^{60}Co source, mfr. Chirana, Prague, the midline tissue dose rate in open space was 0.58 - 0.70 Gy/min (measured on a Victoreen 555 exposimeter, Radocon II). The total dose varied from 8.01 to 9.57 Gy, according to the mouse strain used. Irradiation of 3H-TdR and non-labelled TdR solutions was performed on the same apparatus using doses of 1 000 and 3 000 Gy (dose rate 9.06 Gy/min).

T h y m i d i n e s u i c i d e (k i l l)

The procedure common in Paterson Laboratories, Manchester, U.K. (Dr. Lord, personal communication) was used with modifications. The marrow from both femours usually of 1 - 5 donors was flushed into 5 - 10 ml ice-cold medium (phosphate buffer solution with 1% serum albumin), the erythrocytes were haemolyzed with Zapoglobin and the nucleated cells were counted with Coulter Counter Model ZF (Coulter Electronic Ltd., U.K.). The nucleated cell suspension was diluted to give a concentration of $5x10^6$ - $2.4x10^7$ cells/ml, 0.8 ml of this suspension was pipetted into 10 ml test-tubes and 0.2 ml of the solution of the test substance (3H-TdR or unlabelled TdR) was added. The 3H-TdR preparations were supplied with a specific activity of 945 ± 22 GBq/mmol by the producer during our experiments; this did not change too much due to the long half-time of 3H (12.3 years). The solution supplied was diluted slightly with medium so that 0.2 ml of the resulting solution contained 7.4 MBq (=200 µCi) and about 7.83 nmol (= 1.92 µg) TdR. The solution of unlabelled TdR was prepared containing about the same quantity of the substance as the labelled solution. The specimens were incubated in a water bath for 30 min under slight shaking. Test tubes were then put into ice again and 5 ml of ice-cold unlabelled TdR at a concentration of 0.405 µM (= 100 µg) per ml of medium was added and the specimens shaken moderately for 15 sec and centrifugated at 280 g. The supernatant was discarded and the washing procedure with unlabelled TdR was repeated twice more. After discarding the supernatant from the last washing, 0.5 ml of unlabelled TdR solution in a concentration of 40.5 nM (= 10 µg) per ml of medium was added, the specimens were shaken and nucleated cells concentration in the suspension was measured again (which enabled us inter alia, to calculate the nucleated cell recovery).The cell suspension was then diluted according to the mean cellularity (calculated in all specimens together) with a medium to the desired concentration and administered to the irradiated recipients of the same strain, into tail veins in a volume of 0.1 ml (after whole-body heating of animals in a bulb-heated chamber).

Spleens were taken on day 8, wet-weighed and fixed in Bouin's solution. Spleen colonies were counted under a binocular preparation microscope with magnification 4x on the facies parietalis of the spleen, neglecting colonies of a diameter lesser that 0.5 mm (a criterion close to the "minimal criterion" of T i l l, 1972). Spleens of irradiated donors without marrow transplantation were examined parallelly ("controls").

P a p e r c h r o m a t o g r a p h y

of the 3H-TdR and unlabelled TdR specimens was performed with double-distilled water as a solvent on Whatman paper No. 1. The fractions corresponding to the maximum of radioactivity (thymidine peak) were eluted with water, concentrated by lyophilization and dissolved in the medium in such a way that the original radioactivity of 3H-TdR specimens was attained. 3H radioactivity was measured in a mixture of toluene, dioxan and ethanol with addition of scintillators, PPO and POPOP by means of Liquid Scintillation System Mark II Nuclear Chicago. Some specimens were lyophilized only and dissolved again without performing chromatography.

C h e m i c a l s

Bovine albumin lyophilized, SEVAC, Imuna, Šarišské Michaľany, Czechoslovakia; Zapoglobin, Coulter Electronics Ltd., U.K.; Thymidine Calbiochem, San Diego, Calif., U.S.A.; (6-3 H) Thymidine, Institute of Production, Research and Application of Radioisotopes, Prague - 2% ethanolic solution, sterilized, purified according to producer statement by means of paper chromatography by a mixture of butanol - acetic acid - water (4:1:5) and ethylacetate saturated with water, pH 5.0 - 5.5 (according to our measurements). Exceptionally (methyl-3H)thymidine of the same origin was tested. Production batches are designated arbitrarily in the following text as follows: batch No 1 - 4 is (6-3H)thymidine, years of production 1978, 1981, 1982, 1983, respectively; No 5 and 6 are identic batches of (methyl 3H)thymidine, produced in the year 1984; No 7 - 10 is (6-3H)thymidine, years of production 1982, 1983, 1984, 1985. (The experiments with all these preparation were carried out from June, 1984 to June, 1985).

E x p e r i m e n t N o 1 - t h y m i d i n e s u i c i d e o f C F U-S in s e v e r a l i n b r e d m o u s e s t r a i n s

The following inbred strains were used: BALB/c, B10.LP/Ph, A/Ph, C3H/DiSnPh, CBA/JPh, C57BL/10ScSnPh (in the following text we use only abbreviated names) and C57BL/10xCBA F_1 hybrids (in the following text F_1 only). 3H-TdR batches used were No 7, 8 and 10. Thymidine suicide was calculated according to the formula 100 (1 - colony counts per spleen after 3H-TdR/ colony counts per spleen after unlabelled TdR), having deducted colony counts per spleen in control mice in both groups. 10^5 nuclear cells/mouse were

administered to the mice in this experiment (with the exception of C57BL/10 mice, batch No 10, where 2x10^5 cells per mouse were administered). Figures indicated in Tab. 1 under n represent the number of donors; bone marrow of one donor (= two femurs) was administered to 8.0 ± 0.1 recipients in average.

Experiment No 2 - 3H-TdR suicide after various batches of 3H-TdR and a dose of 2x10^5 of nucleated cells per mouse

Bone marrow of 5 males F_1 10 weeks old was flushed into 7 ml of medium and incubated in the ratio of 0.8 ml of the cell suspension (concentration of 1.601x10^7 cells/ml) to 0.2 ml of 3H-TdR or unlabelled TdR solution. Marrow graft recipients were irradiated with a dose of 9.57 Gy. Batches used and numbers of recipients are indicated in Tab. 2.

Experiment No 3 - 3H-TdR suicide after various batches of 3H-TdR and a dose of 2x10^5 nucleated cells per mouse

Bone marrow of 5 males F_1 15 weeks old was flushed into 7 ml of medium and incubated in the ratio of 0.8 ml of the cell suspension (concentration of 1.517x10^7 cells/ml) to 0.2 ml of 3H-TdR or unlabelled TdR solution. Marrow graft recipients were irradiated with a dose of 9.57 Gy. Batches used and numbers of recipients are indicated in Tab. 3.

Experiment No 4 - 3H-TdR suicide after various batches of 3H-TdR and a dose of 1x10^6 (Exp. No 4 a) or 2.42x10^6 nucleated cells per mouse (Exp. No 4 b)

Bone marrow of 10 males F_1 10 weeks old was flushed into 8 ml of medium and incubated in the ratio of 0.8 ml of the cell suspension (concentration of 1x10^7 cells/ml - Exp. No 4a, 2.4x10^7 cells/ml - Exp. No 4b) to 0.2 ml of 3H-TdR or unlabelled TdR. Marrow graft recipients were irradiated with a dose of 9.57 Gy. Batches used and numbers of recipients are indicated in Tab. 4.

Experiment No 5 - suicide using gamma irradiated solutions of 3H-TdR and unlabelled TdR.

Bone marrow of 2 C3H males 11.5 weeks old was flushed into 5 ml of medium and incubated in the ratio of 0.8 ml of the cell suspension (concentration of 1x10^{7}cells/ml) to 0.2 ml of irradiated or intact solution of 3H-TdR (batch No 9) or of unlabelled TdR (gamma radiation doses of 1 000 and 3 000 Gy). Marrow graft recipients were irradiated with a dose of 8.61 Gy and received 1.46x10^5 cells/mouse. The numbers of marrow recipients are given in Tab. 5.

Experiment No 6 - suicide using lyophilized and chromatographically purified preparations of highly cytotoxic batch of 3H-TdR

Bone marrow of 5 F_1 males 15 weeks old was flushed into 7 ml of medium and incubated in the ratio of 0.8 ml of the cell suspension (concentration of 5x10^6 cells/ml) to 0.2 ml of solution of 3H-TdR (batch 6) - intact, lyophilized only and purified by means of paper chromatography using H_2O as solvent. All specimens were diluted before use to the same thymidine concentration, equal to the original one of the 3H-TdR preparation. Marrow graft recipients (their numbers see Tab. 6) were irradiated with a dose of 9.57 Gy, 2x10^5 cells per mouse was administered.

Experiment No 7 - suicide using chromatographically purified preparations of 3H-TdR and unlabelled TdR

Bone marrow of 5 F_1 males 11.5 weeks old was flushed into 8 ml of medium and incubated in the ratio of 1.6 ml of the cell suspension (concentration of 6.3x10^6 cells/ml) to 0.4 ml of solution of 3H-TdR (batch 10) and of unlabelled TdR intact or chromatographically purified with H_2O as a solvent. All specimens were diluted before use to the same thymidine concentration, equal to the original of the 3H-TdR preparation. Marrow graft recipients (their numbers see Tab. 7) were irradiated with a dose of 9.57 Gy, 2x10^5 cells per mouse were administered.

Results

Experiment No 1

Values of 3H-TdR kill of CFU-S in six inbred mouse strains under physiological conditions as dependent on a batch of 3H-TdR used are shown in Tab. 1. The differences between 3H-TdR batches are statistically significant in C57BL/10 strain (batch No 7 versus No 8: $P < 0.05$; batch No 7 versus No 10: $P < 0.01$). It is also evident that batch No 7 produces on the whole higher suicide values in the strains used than batches No 8 and 10. There is a significant difference in suicide values between A and C57BL/10 strains using batch No 7 ($P < 0.05$). Recovery of the original number of cells after incubation and repeated washing of cells in unlabelled TdR was 57 ± 1% and the colony numbers in specimens treated with unlabelled TdR corresponded well with our (unpublished) calibration curves (colony count per spleen versus cell dose) in individual strains.

Tab. 1

Thymidine kill of CFU-S in several inbred strains of mice as dependent on the 3H-TdR batch

Batch No	Strain					
	BALB/c	B10.LP	A	C3H	CBA	C57BL/10
7			16.6 ± 11.0 n=3	54.1 ± 17.7 n=4	51.5 ± 16.6 n=4	61.2 ± 9.0 n=7
8	20.0 ± 24.2 n=4	29.7 ± 29.1 n=5	-3.8 ± 27.1 n=3		8.22 ± 19.4 n=4	5.4 ± 21.4 n=8
10						1.95 ± 12.1 n=7

arithmetical means and standard errors are given, n - numbers of marrow graft donors

Experiment No 2

As can be seen from Tab. 2, all 3H-TdR batches used gave rise to highly significantly lower colony counts per spleen in comparison with the unlabelled TdR and thus also highly significantly higher values of kill. Recovery of cells after incubation and repeated washing was 55% in this experiment and did not differ between 3H-TdR batches used, nor between them and unlabelled TdR. 3H-TdR batches used gave rise to high suicide values in this experiment in spite of the fact that the recommended number of cells incubated with 7.4 MBq of 3H-TdR was exceeded three times and a higher dilution of the supposed cytotoxic components could therefore be except.

Experiment No 3

As could be seen from Tab. 3, the suicide values using 3H-TdR batches No 1 and 10 are considerably lower than are values obtained in the preceding experiment and batch No 1 gave significantly different results in comparison with both other batches used. Recovery of cells subjected to incubation was 55%.

Experiment No 4

The left-hand half of Tab. 4 (Experiment 4a) shows that batches No 5 and 6 gave rise to a highly significant drop of splenic colonies even in double quantity of cells was incubated with 7.4 MBq of 3H-TdR compared to the original method and if a number of cells (1×10^6) highly exceeding usual quantities is administered to the recipients after incubation. Batches No 3 and 10 give reasonable colony counts per spleen and suicide values. Analogous results also follow from the right-hand half of the table (Experiment No 4b), where even the depressive influence of batch No 7 is lost, in connection with the enhancement of the number of the cells administered

Tab. 2

Number of exogenous colonies per spleen and of thymidine kill values of CFU-S in C57BL/10xCBA F_1 mice as dependent on the 3H-TdR batch (dose $2x10^5$ nucleated cells per mouse)

Batch No	CFU-S				kill %	
	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.
2	1.72	0.47	16	P < 0.01	85.2	4.8
3	1.41	0.73	16	P < 0.01	88.3	7.2
4	2.375	0.87	16	P < 0.01	78.6	8.7
5	1.685	0.825	15	P < 0.01	85.5	8.2
6	1.595	0.58	16	P < 0.01	86.4	5.8
7	0.64	0.23	14	P < 0.01	96.0	2.3
9	2.42	0.81	19	P < 0.01	78.2	8.1
unlabelled TdR	10.24	0.85	21			
controls	0.235	0.18	15			

$\bar{x}$ - arithmetical mean, S.E. - standard error, n - number of marrow graft recipients, t-test: difference between a batch and unlabelled TdR

Tab. 3

Number of exogenous colonies per spleen and of thymidine kill values of CFU-S in C57BL/10xCBA F_1 mice as dependent on the 3H-TdR batch (dose $2x10^5$ nucleated cells per mouse)

Batch No	CFU-S				kill %	
	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.
1	9.605	0.99	19	P < 0.01[a]	54.8	6.9
5	0.16	0.12	19	P < 0.01[a]	99.2	0.6
				P < 0.01[b]		
10	14.395	2.03	19	P < 0.05[a]	32.2	12.2
				P < 0.05[c]		
unlabelled TdR	21.235	2.38	19			
controls	0.00	0.00	20			

t-test: a - difference between a batch and unlabelled TdR, b - batch No 1 versus 5, c - batch 1 versus 10

Tab. 4

Number of exogenous colonies per spleen and of thymidine kill values of CFU-S in C57BL/10xCBA F_1 mice as dependent on the 3H-TdR batch (dose $1x10^6$ nucleated cells per mouse - left, $2.42x10^6$ - right)

Batch No.	CFU-S				kill %		CFU-S				kill %	
	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.
3	13.535	0.90	15	N.S.[a] P < 0.0.1[b]	18.3	10.7	13.10	1.57	5	N.S.[a]	23.6	13.7
4							4.00	1.41	6	P < 0.01[c]	77.6	8.8
5	0.165	0.06	15	P < 0.01[c]	100.4	0.4	0.40	0.32	5	P < 0.01[c]	99.0	1.9
6	0.38	0.18	16	P < 0.01[c]	99.0	1.1	0.10	0.10	5	P < 0.01[c]	100.7	0.6
7	5.905	0.73	16	P < 0.01[a]	65.2	6.0	16.30	1.65	5	N.S.[a]	4.7	15.8
10	16.00	1.23	15	N.S.[a] P < 0.01[d]	3.22	13.2	14.25	3.15	6	N.S.[a]	16.8	21.5
unlabelled TdR controls	16.525	1.86	18				17.085	2.25	6			
							0.225	0.12	20			

t-test: a - difference between a batch and unlabelled TdR, b - batch No. 3 versus 7, c - difference between a batch and batches No. 3, 7, 10, unlabelled TdR, d - batch No. 7 versus 10

Tab. 5

Number of exogenous colonies per spleen and of thymidine kill values of CFU-S in C3H/DiSnPh mice assayed by gamma irradiated solutions of 3H-TdR (batch No. 9) and of unlabelled TdR

	CFU-S			kill %	
	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.
3H-TdR	12.585	1.535	18	42.1	8.9
3H-TdR 1000 Gy	16.215	1.215	19	25.4	9.0
3H-TdR 3000 Gy	16.765	1.63	17	22.9	10.4
medium only	21.735	2.04	15		
unlabelled TdR	12.97	1.88	18	26.9	14.6
unlabelled TdR 1000 Gy	14.53	2.215	17	18.1	16.8
unlabelled TdR 3000 Gy	11.785	2.135	16	33.6	15.1
medium only	17.75	2.425	14		

Tab. 6

Number of exogenous colonies per spleen and of thymidine kill values CFU-S in C57BL/10xCBA F_1 mice assayed by lyophilized and chromatographically purified preparations of highly cytotoxic batch of 3H-TdR (No. 6)

	CFU-S				kill %	
	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.
3H-TdR intact	0.35	0.15	20	$P < 0.01$	98.7	1.0
lyophilized	0.575	0.20	20	$P < 0.01$	97.3	1.4
chromatograph. purified	0.30	0.15	10	$P < 0.01$	99.1	1.0
medium only	15.275	1.47	18			
controls	0.16	0.09	28			

t-test: difference between a batch and single medium

Tab. 7

Number of exogenous colonies per spleen and of thymidine kill values in C57BL/10xCBA F_1 mice assayed by chromatographically purified preparations of 3H-TdR (batch No. 10) and unlabelled TdR

	CFU-S				kill %	
	$\bar{x}$	S.E.	n	t-test	$\bar{x}$	S.E.
3H-TdR intact	2.10	0.42	20	N.S.[a]	64.2	8.6
3H-TdR chromatograph. purified	3.60	0.54	15		38.7	12.4
unlabelled TdR intact	5.26	0.80	19	N.S.[a]	10.4	18.2
unlabelled TdR chromatograph. purified	1.95	0.39	19		66.8	8.0
medium only	5.87	0.79	15			
controls	0.00	0.00	24			

t-test: a - difference between this and immediately following preparation

($2.42x10^6$ cells per mouse). Recovery of cells was 57% and 63% in Exp. No 4a and 4b, respectively.

E x p e r i m e n t   N o 5

It follows from Tab. 5 that the irradiation both of the 3H-TdR solution and of unlabelled TdR solution with the two doses chosen did not give rise to a significant lowering of colony counts per spleen in comparison with the effect of non-irradiated preparations. Recovery here was 54%.

E x p e r i m e n t   N o 6   a n d   7

As can be seen from Tab. 6, neither lyophilization alone nor an attempt at chromatographical purifying of batch No 6 brought a lowering of its profound depressive effect on the number of colonies per spleen. Tab. 7 shows that chromatographical purification gave rise to significant changes in colony counts neither in case of 3H-TdR (batch No 10) nor with unlabelled TdR. Recovery was 48% and 33% in these experiments, respectively.

Discussion

If the use of various production batches of 3H-TdR (whether (6-3H)TdR or (methyl-3H)TdR) gives rise to statistically significant differences in colony counts per spleen and in kill values, it is necessary to suppose the presence of cytotoxically acting chemical impurities in some batches (Exp. No 1, 3, 4). So, e.g., batches No 4, 5 and 6 appear as significantly toxic, batch 7 as toxic. Batches No 8 and 10, on the other hand, give rise to suicide values compatible with the presupposition of zero toxicity (i.e., kill could be produced exclusively by a radioactive decay of 3H-TdR in this case). As CFU-S kill measured by spleen-colony essay is in the case of some 3H-TdR batches identical to that ascertained by incubation of cells with hydroxyurea (Dr. Nečas, Prague, personal communication), it follows that at least some of these batches from our source are not chemically toxic.

From a consideration of the minimal duration of the cell cycle in the population of haemopoietic stem cells (6 hrs) and the S-phase (in less than 5 h) there follows as the theoretically maximal suicide value a figure of 80% (if we neglect the case of a totally synchronous stem cell proliferation which is not the case under physiological conditions at least because of the biological variability). As the suicide values obtained using batches No 4, 5, 6 exceed this figure very frequently, it is apparent that some cytotoxically active component(s) is (are) contained in these preparations. The influence of this component(s) is so pronounced that it could not be decreased even by enhancing the number of transplanted marrow cells by one order of magnitude over the usual quantity (Exp. No 4a, 4b). It appears, on the other hand, that the influence of less toxic batches is depressed with rising doses of transplanted cells (compare results given in Tab. 2 and 4) which is probably connected with the presence of a plateau on calibration curves expressing dependence of colony counts per spleen on cell dose. This way of depressing the toxicity of preparations could not be, of course, employed practically with the thymidine suicide method.

The influence of radiation and chemical toxicity could not be distinguished from each other with the method used as the cell recovery following use of chemically toxic and non-toxic preparations is the same (e.g., Exp. No 2 and others). Toxically damaged cells survive incubation and subsequent washing with the same relative decrease as non-damaged cells, and their shortage appears only in a loss in the ability to form splenic colonies.

Gamma irradiation of the solutions of 3H-TdR and unlabelled thymidine with dose corresponding approximately to the autoirradiation of 3H-TdR preparations under a year's or longer storage did not lead to enhancement of toxicity of preparations (Exp. No 5). It therefore appears that toxicity is not brought on by products of thymidin radiolysis (organic peroxides etc.; this is also confirmed by the fact that toxicity is not dependent on the age of a preparation in our experiments.

An attempt to remove cytotoxic components by means of paper chromatography with H_2O as a solvent did not succeed; still less did mere lyophilization of preparations suffice for the separation of toxic components. As 3H-TdR used by us is purified by the producer chromatographically by means of several organic solvents, it is not

impossible that cytotoxic effects are due to trace quantities of these substances.

Before further progress is made regarding the question of 3H-TdR preparation toxicity, it is necessary to test the possible toxicity of individual batches in advance and use only attested batches.

Acknowledgement

The authors thank MUDr. V. Drášil, DrSc. (Institute of Biophysics, Brno) for the chromatographical separation of thymidine preparations.

Metoda tzv. thymidinové smrti u exogenních jednotek tvořících kolonie ve slezině: rušivý vliv chemické toxicity preparátů 3H-thymidinu

Byly porovnávány číselné hodnoty thymidinového killu CFU-S při použití několika 3H-TdR šarží produkovaných Ústavem pro výzkum, výrobu a využití radioisotopů, Praha. U některých šarží jsou získané hodnoty killu signifikantně vyšší než u jiných při téže buněčné suspensi a stoupají i přes biologicky přípustnou mez 70 - 80%. Tuto předpokládanou cytotoxicitu 3H-TdR preparátů nelze vyvolat jejich gamma ozářením dávkami odpovídajícími autoirradiaci preparátů při dlouhodobém skladování. Toxické vlastnosti se nepodařilo odstranit lyofilisací a rechromatografováním preparátů pomocí H_2O jako rozpustidla na papíře. V práci jsou uvedeny také hodnoty CFU-S 3H-TdR killu u řady inbredních kmenů myší za fysiologického stavu.

Метод т.н. "тимидинового самоубийства" у экзогенных единиц образующих колонии в селезенке: вредное влияние химической токсичности препаратов 3Н-тимидина

Сравнивались нумерические величины тимидинового самоубийства КОЕ-с при применении нескольких шаржев 3Н-TdR выпускаемых Институтом для исследований, производства и применения радиоизитопов в Праге. В случае применения некоторых шаржев были получены величины самоубийства достоверно более высокие по сравнению с другими при одной и той же суспензии клеток и повышающиеся над биологически допустимый предель 70 - 80%. Эта предполагаемая токсичность 3Н- TdR препаратов не может быть вызвана их облучением гамма лучами в дозах соответцтвующих самооблучению препаратов при длительном сохранении. Токсические свойства не удалось устранить ни при помощи лиофилизации, ни рехроматографией препаратов при помощи H_2O на бумаге. В работе также приводятся величины КОЕ-с 3Н- TdR самоубийства у мышей ряда инбредных линий при физиологическом состоянии.

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