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Calibration dose-response relationships for cytogenetic biodosimetry of recent and past exposure to low dose gamma-radiation

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Калібрувальні залежності «доза — ефект» для цитогенетичної біодозиметрії недавнього та віддаленого гамма-опромінення в низьких дозах

Цель работы: Построение базовых калибровочных кривых «доза — эффект» для цитогенетической биодозиметрии в ранние и отдаленные сроки после γ-облучения в низких дозах с использованием классического хромосомного анализа и техники флуоресцентной *in situ* гибридизации (FISH).

Материалы и методы: Образцы крови человека были подвергнуты острому γ-облучению *in vitro* в диапазоне доз от 0 до 1 Гр. Цитогенетический анализ клеток 48 ч культуры лимфоцитов осуществлялся с применением рутинного окрашивания и техники FISH (комбинации хромосом 1, 2 и 4 или 6, 9, 15 и 21; пан-центромерные зонды). Регистрация стабильных аберраций производилась с использованием модифицированной PAINT-номенклатуры, учитывающей каждый полный межхромосомный обмен как одно событие. Для построения кривых «доза — эффект» применялся метод итеративно взвешенных наименьших квадратов.

Результаты: Зависимость «доза — эффект» для выхода радиогенных аберраций, выявляемых рутинным методом или техникой FISH, хорошо соответствовала классической линейно-квадратичной модели. Наблюдалось совпадение калибровочных кривых для выхода дицентриков и кольцевых хромосом, построенных по данным рутинного и FISH-исследования; в обоих случаях линейный коэффициент составлял около $3 \cdot 10^{-2} \cdot Гр^{-1}$, квадратичный — около $8 \cdot 10^{-2} \cdot Гр^{-2}$. Путем исключения из анализа неполных транслокаций в клетках с реальным или предполагаемым отсутствием части хромосомного материала была построена дозовая зависимость для выхода действительно радиационно-индуцированных транслокаций, оказавшаяся очень близкой к кривой для дицентриков. Калибровочная кривая для суммы транслокаций и инсерций в «стабильных» клетках характеризовалась линейным коэффициентом $1{,}4 \cdot 10^{-2} \cdot Гр^{-1}$, что в 3 раза превышало значение соответствующего параметра кривой, построенной только для полных транслокаций. С использованием полученных калибровочных зависимостей было показано, что минимальная доза, достоверно детектируемая рутинным дицентрическим методом при анализе 1000 клеток, составляет 0,14 Гр острого и 0,20 Гр хронического γ-облучения. Относительная чувствительность FISH-биодозиметрии (при анализе 1000 геном-эквивалентов) варьирует от 0,22 до 0,31 Гр острого и от 0,50 до 0,85 Гр хронического облучения с учетом повышения спонтанной частоты стабильных аберраций с возрастом в интервале 20–60 лет.

Выводы: Оба хромосомных коктейля, использованные для FISH-анализа в настоящей работе, позволяют адекватно оценивать уровень аберраций хромосом в расчете на весь геном при построении калибровочных кривых. Совпадение зависимости «доза — эффект» для выхода дицентриков и действительно радиационно-индуцированных транслокаций хорошо подтверждает гипотезу об идентичности механизмов формирования этих двух типов межхромосомных обменов в облученных лимфоцитах человека. Калибровочная кривая для суммы радиационно-индуцированных обменов в «стабильных» клетках обладает большей потенциальной информативностью для практической цитогенетической биодозиметрии, по сравнению с кривой для выхода только полных транслокаций, особенно в условиях хронического облучения.

Ключевые слова: дицентрики и центрические кольца, транслокации, флуоресцентная *in situ* гибридизация, лимфоциты, калибровочные кривые, биологическая дозиметрия.

Objective: To generate the basic calibration curves for chromosomal biodosimetry of recent and past exposure to low dose γ-irradiation using conventional cytogenetic analysis and fluorescence *in situ* hybridization (FISH) technique.

Material and Methods: Human blood samples were acutely irradiated *in vitro* to γ-rays within dose range 0-1.0 Gy. Cytogenetic analysis was carried in 48 h lymphocyte cultures using conventional method and FISH highlighting chromosome combinations 1, 2 and 4 and 6, 9, 15 and 21 and centromeres of all chromosomes. Stable aberrations were recorded with modified conventional-PAINT descriptive nomenclature, accounting each complete chromosome exchange as an entity. The dose response curve fitting was performed by iteratively reweighted least squares method.

Results: The dose response for both conventionally and FISH-measured chromosome aberration yields showed a good fit to the classic linear-quadratic model. Calibration curve for the full genome dicentrics plus centric rings yield estimated on FISH coincided with that of estimated by conventional analysis, both displaying a linear term about $3 \times 10^{-2} \times Gy^{-1}$ and quadratic term about $8 \times 10^{-2} \times Gy^{-2}$. Excluding incomplete translocations in cells with actual or assumed partial lack of chromosomal material from data treatment provided a dose response for truly radiation-induced translocations, which was very close to that of for dicentrics. The calibration curve for translocations plus insertions in 'stable' cells had a linear coefficient about $1.40 \times 10^{-2} \times Gy^{-1}$ that was 3 times higher than a respective parameter for complete translocations alone. The calibration data showed that the minimum dose, which can be statistically detected by conventional dicentric method with 1000 cells scored, was about 0.14 Gy of acute and 0.20 Gy of chronic γ-exposure. The relative sensitivity of FISH biodosimetry with 1000 genome equivalents analyzed varied from 0.22 to 0.31 Gy of acute and from 0.50 to 0.85 Gy of chronic irradiation according to the increase of spontaneous yield of stable chromosome exchanges with persons' age within 20-60-year interval.

Conclusion: For the calibration purposes, both chromosome cocktails used in our survey appeared to be suitable for detecting the true level of aberrations in full genome. The coincidence of the dose response curves for dicentrics and truly radiation-induced translocations confirmed the hypothesis about identical mechanisms of balanced and non-balanced interchromosomal exchange formation in irradiated human lymphocytes. The calibration curve constructed for truly radiation-induced chromosome exchanges in «stable» cells seems to be more appropriate in practical retrospective biodosimetry, in compare with complete translocations alone, especially for chronic exposure conditions.

Key words: dicentrics and centric rings, translocations, fluorescence *in situ* hybridization, lymphocytes, calibration curves, biological dosimetry.

During last four decades the cytogenetic analysis of human peripheral blood lymphocytes has been widely used as the biological technique for detecting and quantifying radiation exposure in man. The main approach of cytogenetic biodosimetry for assessing the absorbed dose is based on referring the yield of aberrations observed *in vivo* to a calibration curve generated *in vitro* with an appropriate reference radiation [1]. Since the basic technique of metaphase preparation from human PHA-stimulated lymphocyte cultures was elaborated, a large body of data concerning the dose-response relationship for chromosomal rearrange ments induced by ionizing radiation of different quality has been accumulated using this test-syste m [2].

Two types of chromosome rearrangements have been established as the end-points of choice for measuring the radiation load to an individual. The first type includes dicentrics and centric rings, which can be identified easily using the routine cytogenetic method with uniform staining of all chromosomes. The only disadvantage is declining of their yield with time following exposure. The second type consists of translocations and insertions, which may persist in human lymphocytes many years after irradiation and can be visualized with a fluorescence *in situ* hybridization (FISH) technique. Thus, acute exposure accompanied by deterministic clinical effects or resulted from the accident in the work place and detected shortly after the incidence should be evaluated with dicentric measure ment. Evidently, it is the past or long term chronic exposure that will be preferentially investigated by FISH assay [1, 3].

However, in contrast to the progress in calibrating the dicentric test system and its successful application in numerous incidents, a lot of questions still exist concerning the utility of translocations as a retrospective biodosimeter. One of the main problems with FISH-measured stable aberrations is related to the insufficient standardization of the scoring criteria and differences in the methods of calibration curve constructing. The most frequent and popular types of dose response relationshi ps were those built for all translocations, but the curves generated for the combination of complete and some of incomplete translocations, complete translocations alone or translocations in "stable" cells only could also be found in the literature [4-16]. Alternatively to the fitting a calibration curve from the data of aberration scoring at each dose point, a computational way for deriving the translocation dose response has been proposed. It converts the relatively easy-to-establish curve for dicentrics into curve for translocations applying the translocations to dicentrics ratio as a correction factor, which could be determined following *in vitro* or *in vivo* irradiation [3, 4, 8, 17]. Due to all these variations the dose effect curves obtained in different laboratories with FISH are not easy to compare.

The world experience has shown that producing *in vitro* calibration curves is necessary for each cytogenetic laboratory undertaking a biodosimetry, because any tiny inter-laboratory variability of aberration scoring criteria could cause a meaningful difference in fitted dose-response coefficients and resultant dose estimations, especially within the low dose range [2, 5, 18]. Taking into account that the results of cytogenetic experiments *in vitro* could be significantly influenced by variations in physical dosimetry and cell cultivation technique, the dose effect curves should be set up where possible in frame of collaborative projects, with aberration scoring quality assurance and standard irradiation conditions guaranteed. The most extensive co-operative work on intercomparison and unification of the dose response curves in different laboratories of the former Soviet Union was carried out by the international CEC-CIS Experimental Collaboration Project No 6 "Biological dosimetry for persons irradiated by the Chernobyl accident" (ECP-6) [5].

The task of the present work is to display our calibration curves for conventionally scored chromosome aberrations, to describe the original approach for setting up the calibration curve for FISH-detected rearrange ments and to determine the theoretical magnitude of sensitivity for both methods of biodosimetry.

Material and Methods

The material for constructing the basic calibration curves in our laboratory was obtained during participating in the international CEC-CIS Experimental Collaboration Project "Biological dosimetry for persons irradiated by the Chernobyl accident". The details of irradiation procedure and cell cultivation were published earlier [5]. Briefly, blood

from two healthy male donors was irradiated with Co^{60} γ-rays at a dose rate of 0.5 Gy · min^{-1} to the following doses: 0.00, 0.10, 0.25, 0.50, 0.75 and 1.00 Gy. Setting up PHA-stimulated lymphocyte cultures and metaphase harvesting at 48 h were performed according to the standard method [1]. Replicated coded slides were prepared from the fixed cells and used for further analysis.

For conventional cytogenetic analysis the slides from each dose and donor were stained by conventional Giemsa method. All types of chromosomal rearrangements detected by routine staining with light microscopy were recorded in metaphases containing 46 centromeres; those were dicentrics and centric rings accompanied by acentric fragments, excess acentric fragments, chromatid breaks and exchanges.

The preparations from one donor's blood irradiated at 0.00, 0.25, 0.75 and 1.00 Gy were painted by FISH technique following the procedure adopted by Cytogenetic Laboratory of National Radiological Protection Board of the United Kingdom [4, 8]. Combinations of commercial FITC-labelled whole chromosome probes for chromosomes number 1, 2 and 4 or 6, 9, 15 and 21 (Cambio) plus a biotin-labelled pancentromeric probe highlighted with Texas Red (Oncor) were used and other chromosomes were counterstained blue with DAPI. Although FITC was used for all painted chromosomes, they could be distinguished by a combination of size, centromere position and intensity of fluorescence. The FISH-painted slides were examined using the fluorescence microscopes (Nikon, Zeiss) equipped with separate filters for DAPI, FITC and and triple-band-pass filter for simultaneous vizualization of all three fluorochromes.

Cells were only accepted for scoring if they contained exactly 46 centromeres and normal diploid amount of painted material from FITC-highlighted chromosomes. Unstable aberrations scoring was concentrated on centric rings and dicentrics involving painted chromosomes, both accompanied by an acentric fragment. Stable aberrations were recorded using the modification of hybrid conventional plus PAINT descriptive nomenclature and accounting each exchange (either complete or incomplete) as an entity. Translocation was recorded as complete if its reciprocal bicolored counterpart was observed. Incomplete translocations were described according to the PAINT system [19]. Bicolored chromosomes with the centromere located in the non-painted part and accompanied by a painted truncated chromosome were termed t_{inc}(Ab). Incomplete translocations with DAPI signal on the end of painted chromosome, i.e. t_{inc}(Ba), were subdivided into three subgroups depending on the completeness and disposition of the painted material in the cell. The terminal exchange accompanied by an acentric fragment from the painted chromosome was classified as t_{inc}(Ba+ac). The situation when the painted chromosome involved in translocation was not noticeably shorter comparing with its homologue and a fragment was absent was recorded as t_{inc}(Ba*). At last, if the translocation occurred in a painted chromosome, which appeared to be distinguishably shorter than its non-aberrant homologue, and no missing painted fragment were detected somewhere in the cell, the exchange was recorded as t_{inc}(BaMP), i.e. "missing part". Insertions of both (Aba) and (Bab)-types were pooled into one category. Markedly shortened painted chromosomes with a segment absent were recorded for completeness.

For data comparison the aberration yields at each dose were expressed per 100 cells. Numbers of the actual cells scored by FISH were converted into genome equivalents applying the monocolor version of the formula devised by Lucas et al [20]. Calculations of the painted fraction of male genome were performed using the sum of DNA content in highlighted chromosomes [21]. The randomness of aberration-per-cell distribution was checked with dispersion to mean ratio (σ^2/Y) and Papworth's *u*-test [22]. For data comparison at different dose points Student's *t*-test was applied. The dose response curve fitting was performed by the method of iteratively reweighted least squares using the computer program kindly provided by A.A. Edwards (NRPB, UK).

Results and discussion

The results of aberration scoring in conventionally stained lymphocytes of two donors' blood irradiated at 0-1.00 Gy are presented in Table 1. The increase of the aberrant cell yields with radiation dose was clearly seen in both donors' cultures. For structural rearrange ments that was related to chromosome type aberrations only, meanwhile the chromatid rearrangements level remained nearly constant around the control values. For dicentrics, excess acentrics and total chromosome type aberrations the statistical increase above the spontaneous levels was detected at the 0.25 Gy dose point for the given numbers of aberrations and cells scored ($p<0.01$-0.001). The chromosome exchanges and fragments appeared to be randomly distributed amongst the scored cells in consistence with Poisson statistics, that was confirmed by the values of dispersion-to-mean ratio and Papworth's *u*-test ($\sigma^2/Y\approx1$, $u<1.96$). Cells containing >1 chromosome type aberration were found at dose points 0.25, 0.75 and 1.00 Gy, but in each case not more than 2 separate aberrations were seen in one metaphase.

Due to the absence of any significant difference between individual aberration yields at each dose point, the results derived from cultures of two donors were combined and the data were fitted to the linear-quadratic model

$$Y = c + \alpha \cdot D + \beta \cdot D^2, \qquad (1),$$

where Y is the aberration yield, c is the background incidence and D is dose in Gy. Table 2 shows the resultant dose-response coefficients with their standard errors for the different kinds of chromosome type aberrations or their combinations.

The derived coefficients for background levels of unstable exchanges and excess acentrics were within the range of the known values for control populations [23-25]. The linear coefficients of the dose response equations for dicentrics and dicen-

trics plus centric rings appeared to be nearly identical, but the quadratic term was 1.3-times higher for the total unstable chromosome exchanges than for dicentrics alone. The curve for excess acentrics yield was more linear and less quadratic comparing with the regression for exchanges. The equation coefficients for total unstable chromosome aberrations were close to the sum of respective values from regressions for exchanges and fragments.

It should be noted that our dose response data for dicentrics showed a remarkably good fit to the classic linear-quadratic model (χ^2=1.62; p=0.66 for 3 degrees of freedom), and no sign of the plateau for this end-point within the dose range 100-250 mGy was detected. The presence or absence of such plateau has been actively discussed during last two decades, because the phenomenon of possible distortion of the monotonous dose response regression might limitate the sensitivity of cytogenetic biodosimetry at low doses [26, 27].

The dose response coefficients obtained in our work for conventionally scored unstable chromosome exchanges are in very good agreement with the large body of the dose response data for γ-radiation, that have been published by different laboratories. In those experiments where sufficient scoring at low doses was performed the linear coefficients for γ-induced dicentric curves varied from 0.90 to $5.9 \cdot 10^{-2} \cdot Gy^{-1}$, and the quadratic term ranged from 2.9 to $7.11 \cdot 10^{-2} \cdot Gy^{-2}$ [2, 6-8, 12, 28-33]. Both coefficients for dicentrics estimated in our laboratory fall well in the middle of these intervals.

The means of the linear and quadratic term for dicentric dose response obtained under standard irradiation conditions by different participants of ECP-6 project varied from 1.17 to $5.77 \cdot 10^{-2} \cdot Gy^{-1}$ and from 2.07 to $9.58 \cdot 10^{-2} \cdot Gy^{-2}$, respectively. Coefficients estimated by our laboratory were the closest to the corresponding parameters of the overall dicentric curve provided by pooling the data from all laboratories participated in the ECP-6: $\alpha = 2.71 \cdot 10^{-2}$, $\beta = 6.95 \cdot 10^{-2}$ [5].

Table 3 shows the data derived from the FISH-processed preparations scored for aberrations involving the painted chromosomes, with aberration yields expressed per 100 genome equivalents. Metaphases with ≥2 aberrations or complex rearrangements, except rare insertions, were not observed. According to the dispersion-to-mean ratio and Papworth's *u*-test, the aberration-per-cell distribution didn't differ from that of expected from Poisson statistics at each dose point ($\sigma^2/Y \approx 1$, $u < 1.96$).

The FISH-measured dicentric plus centric ring yields were close to the respective means obtained with routine staining at respective dose points. The sum of incomplete translocations exceeded the number of complete ones at doses 0.00 and 0.25 Gy, but the difference disappeared at higher doses. The ratio of incomplete translocations t(Ab) to that of t(Ba) type was 1:1 in the control sample and remained nearly constant at value 1.1 : 1 through the studied dose range.

Among different types of FISH-detected chromosome aberrations the positive dose dependence was shown for complete translocations, incomplete translocations of t(Ab), t(Ba*) and t(Ba+ac) types, but not for incomplete t(BaMP) translocations and deleted chromosomes with fragment lost. Assuming the identity of the mechanisms of formation for t(Ab) and t(Ba) incomplete exchanges and taking into account their constant ratio within the studied dose range, the virtual proportions of different kinds of t_{inc}(Ab) were calculated. It was made with multiplying the number of total t_{inc}(Ab) translocations by the respective fractions of t_{inc}(Ba*), t_{inc}(Ba+ac) and t_{inc}(BaMP) within total t_{inc}(Ba) at each dose point. The obtained values were rounded off to the integer numbers and used for calculating the full-genome yields (Table 4, columns 3-5). The standard errors were estimated assuming the Poisson per-cell distribution for each aberration type.

For further data treatment the incomplete t(Ab) and t(Ba) translocations were combined into FP ("full presence") or MP ("missing part") categories depending on whether all the material from the chromosome involved into the translocation was assumed to be present in the cell (columns 6 and 7 in Table 4). No statistical elevation of the t_{inc}(MP) yield above the control was detected even at 1.00 Gy (t=1.03; p>0.05), meanwhile the sum of incomplete FP-type translocations showed a sufficient exceeding the

Table 1

Chromosomal aberrations scored by conventional analysis in lymphocytes following low dose ^{60}Co γ-irradiation in vitro

Dose, Gy	Donor	Cells scored	Numbers of abnormalities and their yiel					
			Ab Cells	Dic fr	CR fr	Ac fr	A Cs	A Ct
0.00	I	1000	24	1	0	6	7	18
	II	1000	21	1	0	10	11	10
	I+II	Yield	2.25±0.33	0.10±0.07	0.00	0.80±0.20	0.90±0.21	1.40±0.26
0.10	I	1000	28	2	1	9	12	16
	II	1000	43	5	0	17	22	21
	I+II	Yield	3.55±0.41	0.35±0.13	0.05±0.05	1.30±0.25	1.70±0.29	1.85±0.30
0.25	I	1000	52	9	3	25	37	16
	II	1000	55	17	0	29	46	15
	I+II	Yield	5.35±0.50	1.30±0.25	0.15±0.09	2.70±0.37	4.15±0.47	1.55±0.28
0.50	I	425	47	13	2	3	38	9
	II	500	44	17	0	17	34	10
	I+II	Yield	9.84±0.98	3.24±0.58	0.22±0.15	4.32±0.67	7.78±0.88	2.05±0.47
0.75	I	500	86	35	3	47	85	13
	II	400	54	24	2	29	55	6
	I+II	Yield	15.56±1.21	6.56±0.90	0.56±0.25	8.44±0.99	15.56±1.35	2.11±0.48
1.00	I	560	120	53	10	58	121	13
	II	425	90	34	11	42	87	10
	I+II	Yield	21.32±1.31	8.83±0.93	2.13±0.46	10.15±1.01	21.12±1.42	2.34±0.48

SE – standard error derived from aberrations-per-cell distribution; Ab Cells – cells with chromosomal rearrangements (either chromosome or chromatid type); Dic fr – dicentrics accompanied by an acentric fragments; CR fr – centric rings accompanied by an acentric fragments; Ac fr – excess acentrics; A Cs – total unstable chromosome type aberrations; A Ct – chromatid type aberrations.

Table 2

Dose response equations for unstable chromosome type aberration yields in human lymphocytes following ^{60}Co γ-irradiation at low dose range in vitro

Aberrations	Coefficients of the regression $Y = c + \alpha \cdot D + \beta \cdot D^2$ (per 100 cells, D – dose, Gy)		
	C (±SE_c)	α (±SE_α)	β (±SE_β)
Dicentrics	0.087 (±0.046)	3.052 (±0.787)	6.236 (±1.133)
Dicentrics + Centric Rings	0.093 (±0.029)	2.984 (±0.492)	8.049 (±0.717)
Excess Acentrics	0.760 (±0.152)	6.333 (±1.529)	3.509 (±1.875)
Total Unstable Chromosome Aberrations	0.852 (±0.169)	9.279 (±1.922)	11.589 (±2.477)

spontaneous level at 0.25 Gy dose point ($t=2.39$; $p<0.05$). The same magnitude of statistical difference between this dose point and zero dose was observed for combination of the complete and incomplete FP-type translocations ($t=2.54$; $p<0.05$), as it is shown in column 8 of Table 4. The index displayed in the final column is the frequency of stable chromosome exchanges with actual or assumed full presence of chromosomal material in cells without unstable aberrations, that represents the sum of insertions, complete translocations and those incomplete FP-type translocations, which involved unshortened chromosome, i.e. actual t_{inc}(Ba*) and calculated t_{inc}(Ab*).

The data for FISH-detected aberrations and their combinations, which showed the positive dose dependence, were fitted to the linear-quadratic equation (Table 5). The coefficients for the full genome dicentrics plus centric rings yield measured by FISH-technique are in a very good agreement with those estimated from conventional scoring. The linear term for complete translocations alone appeared to be sufficiently lower than that of for conventional dicentrics or FISH-detected dicentrics plus rings. The dose dependence for incomplete FP-type translocations had a quadratic coefficient nearly equal to the linear one. The dose-response coefficients for the sum of complete and incomplete FP-type translocations nearly coincide with the respective means for the dicentrics plus centric rings curve

Table 3

Full genome aberration yield measured by fluorescence in situ *hybridization in lymphocytes after γ-irradiation* in vitro

Dose, Gy	Genome equivalents scored	Aberration yield per 100 cells ± SE (actual number of aberrations involving painted chromosomes is shown in parenthesis)							
		Dic+CR fr	t_{comp}	t_{inc}(Ab)	t_{inc}(Ba*)	t_{inc}(Ba+ac)	t_{inc}(BaMP)	Ins	Del Chs
0.00	1070	0.09±0.09 (1)	0.37±0.19 (4)	0.28±0.16 (3)	0.09±0.9 (1)	0.00 (0)	0.19±0.13 (2)	0.00 (0)	1.31±0.35 (14)
0.25	459	1.31±0.53 (6)	0.87±0.43 (4)	0.65±0.38 (3)	0.22±0.22 (1)	0.44±0.31 (2)	0.22±0.22 (1)	0.22±0.22 (1)	1.53±0.57 (7)
0.75	505	6.93±1.13 (35)	4.36±0.91 (22)	2.18±0.65 (11)	0.59±0.34 (3)	0.59±0.34 (3)	0.20±0.20 (1)	0.00 (0)	1.39±0.52 (7)
1.00	225	11.11±2.10 (25)	7.11±1.72 (16)	3.11±1.16 (7)	1.33±0.77 (3)	1.78±0.88 (4)	0.44±0.44 (1)	0.89±0.63 (2)	1.78±0.88 (4)

SE – standard error derived from aberrations-per-cell distribution; Dic+CR fr – dicentrics and centric rings accompanied by an acentric fragments; t_{comp} – complete translocations; t_{inc}(Ab), t_{inc}(Ba*), t_{inc}(Ba+ac), t_{inc}(BaMP) – incomplete translocations; Ins – insertions; Del Chs – deleted chromosomes with fragment absent somewhere in the cell.

Table 4

Full genome frequencies of chromosome exchanges with full presence (FP) or missing part (MP) of chromosomal material in lymphocytes after γ-irradiation in vitro

Dose, Gy	Genome equivalents scored	Aberration yield per 100 cells ± SE						
		t_{inc}(Ab*)	t_{inc}(Ab+ac)	t_{inc}(AbMP)	Σt_{inc} (MP)	Σt_{inc} (FP)	t_{comp} +t_{inc} (FP)	St Exch
0.00	1070	0.09±0.09	0.00	0.19±0.13	0.37±0.19	0.19±0.13	0.56±0.23	0.56±0.23
0.25	459	0.22±0.22	0.22±0.22	0.22±0.22	0.44±0.31	1.09±0.49	1.96±0.65	1.53±0.57
0.75	505	0.79±0.39	0.99±0.44	0.40±0.28	0.59±0.34	2.97±0.76	7.33±1.16	5.74±1.04
1.00	225	1.33±0.77	1.33±0.77	0.44±0.44	0.89±0.63	5.78±1.56	12.88±2.24	10.67±2.06

SE – standard error derived from Poisson statistics; t_{inc}(Ab*) – translocations with assumed full involvement of unpainted chromosome; t_{inc}(Ab+ac) – translocations with assumed accompanying by unpainted acentrics; t_{inc}(AbMP) – translocations with assumed involving shortened unpainted chromosome and absence of its fragment somewhere in the cell; Σt_{inc}(MP) – sum of t_{inc}(AbMP) and t_{inc}(BaMP); Σt_{inc}(FP) – sum of t_{inc}(Ab) and t_{inc}(Ba) translocations with actual or assumed full presence of chromosomal material in the cell; t_{comp} – complete translocations; StExch – chromosome exchanges with actual or assumed full presence of chromosomal material in "stable" cells (t_{comp} + t_{inc}(Ab*) + t_{inc}(Ba*) + ins).

constructed from either conventional or FISH data. In case of chromosome exchanges with full presence of chromosomal material in "stable" cells the quadratic coefficient was similar to the respective value for previous end-point, but the linear term was intermediate between the respective parameters for complete plus incomplete FP-type translocations and complete translocations alone.

The coincidence of the full genome dicentrics plus rings yield in FISH-painted and conventionally stained preparations showed that the total involvement of the painted chromosome combinations into unstable exchanges did not deviate from that of expected from their combined length. This result confirmed the assumption about the randomness of the aberration formation in human chromosomes according to their DNA content. The similar conclusion was achieved in several investigations with different combinations of labeled chromosomes [4-9, 11, 12, 34-37]. So, for the calibration purposes, both cocktails used in our survey appeared to be suitable for detecting the true level of dicentrics and rings in full genome, and that agreement gives confidence that measured translocation yields can be corrected to the full genome equivalent by the same formula.

The approach suggested in our work for calibrating the system of FISH-measured translocation was based on the main principle of cytogenetic biodosimetry, that was setting up the calibration curve for those types of chromosome rearrangements, which show the radiation specificity and dose dependence. In case of translocations this question is strictly related to the mechanisms of their formation in irradiated human lymphocytes and to correspondence of these mechanisms to that of for dicentrics. Apart from the pure academic interest, the problem of translocations to dicentrics ratio has a practical side. As mentioned earlier, this parameter can be applied as a correction factor for deriving the

Table 5

Dose response equations for chromosome aberration yields measured by fluorescence in situ *hybridization in lymphocytes after γ-irradiation* in vitro

Aberrations	Coefficients of the regression Y per 100 cells = c + α · D + β · D² (D – dose, Gy)		
	c (±SE_c)	α (±SE_α)	β (±SE_β)
Dic+CR fr	0.093 (±0.007)	2.835 (±0.205)	8.278 (±0.275)
t_{comp}	0.373 (±0.016)	0.449 (±0.222)	6.388 (±0.289)
t_{inc}(FP)	0.193 (±0.104)	2.433 (±1.911)	2.455 (±2.431)
t_{comp} + t_{inc}(FP)	0.568 (±0.105)	2.972 (±1.621)	8.720 (±2.071)
St Exch	0.570 (±0.122)	1.401 (±1.682)	8.021 (±2.149)

calibration coefficients for translocations from the corresponding parameters of the relatively easy-to-establish dose response for dicentrics [3, 4, 8]. However, during such computations one should keep in mind that total dicentrics measured by conventional analysis represent the sum of "complete" and "incomplete" ones (i.e. with bicolored and monocolored acentrics) if visualized by FISH [4, 8]. Obviously, complete translocations are analogues of those dicentrics, which are accompanied by bicolored fragment. The situation with incomplete translocations is more complex and requires some comments.

The truly incomplete translocations are represented by bicolored chromosomes and acentric fragments, which are unjoined to their reciprocal chromosomal counterpart. That could arise the same way as dicentrics accompanied by monocolored fragments; the mechanism of both kinds incomplete exchange formation may involve a palindrome repair of broken DNA ends [38]. The most probable explanation for occurring incomplete translocations, which are not accompanied by fragment but involve unshortened chromosome with joined counterstained material – t_{inc}(Ba*), is related to the assumption that they actually represent reci procal exchanges involving a small telomeric region, which is beyond the limits of visual resolution by FISH. The comprehensive support for this idea was obtained in the studies where the telomeric probes were used and the data showed that a dominant majority of all one-way exchanges appeared to be actually complete ones resulted from breaks occurring at or near to the telomeres of chromosomes [39]. Surely, if the segment translocated from painted to unpainted chromosome is smaller than required for detecting by eye, the translocation will be scored as t_{inc}(Ba*).

The problem with t_{inc}(BaMP) translocations was recognized recently as a part of general question of choosing metaphases for chromosomal analysis by FISH [40]. Our recording of these translocations corresponds to designations "Tri-", "t(Ba)-ac(b)" or "pp-" (painted piece missing) proposed by other authors [10, 19, 40]. The occurrence of such kind translocations could be caused by the lost of chromosome fragment during the mitosis of those lymphocyte precursors where an incomplete exchange of t(Ba+ac)-type initially arose. So, the obvious reason for the absence of any dose dependence for the yield of t_{inc}(BaMP) translocations in our *in vitro* experiment was the prevalence of the first post-irradiation mitosis in the standard 48-h lymphocyte cultures, that limited the chance of loosing the fragments. But alternatively, t_{inc}(BaMP) may appear as the result of chromosome segregation derived from the balanced chromatid exchange in lymphocyte precursor during it's subsequent mitotic division. The preference of the second mechanism was supported by the fact of minority of t_{inc}(BaMP) among all translocations found in persons who were exposed to high dose radiation, and including this type of rearrange ments in data treatment may distort the result of retrospective dose assessment [40]. When such chromosome abnormalities are observed *in vivo* their exact predecessors are usually unknown, so the designation t_{inc}(BaMP) see ms to be more appropriate than t(Ba - ac) which restricts the origins of these aberrations to initial incomplete t(Ba+ac) exchanges only.

The main assumption applied in our study for constructing the calibration curve for incomplete translocations was the identical mechanisms of formation for t_{inc}(Ab) and t_{inc}(Ba), implying that the same three categories of incomplete exchanges distinguished within total t_{inc}(Ba) must be also present amongst t_{inc}(Ab). The correctness of this idea was deduced from the stability of the t_{inc}(Ab) : t_{inc}(Ba) ratio through the studied dose range. Evidently, a slightly more frequent oc-

currence of total t_{inc}(Ab) rearrangements comparing with total t_{inc}(Ba) ones was caused by the known difference between minimum size of detectable segment for painted and unpainted material, that was 11.1 Mb for painted and 14.6 Mb for unpainted chromosomes [41]. The ratios of t(Ab) to t(Ba) rearrangements reported by different authors range from 1.09:1 to 1.29:1 [4, 9, 42, 43], that are rather similar to our data.

Thus, the proportions of different types of t_{inc}(Ab) exchanges were calculated from the respective percentages of t_{inc}(Ba*), t_{inc}(Ba+ac) and t_{inc}(BaMP) within all t_{inc}(Ba) translocations; and the sum of complete translocations, actual t_{inc}(Ba*) and t_{inc}(Ba+ac) and virtual t_{inc}(Ab*) and t_{inc}(Ab+ac) represented the total yield of translocations truly induced by radiation. This end-point seems to be the most adequate for comparison with radiation-induced dicentrics, and the resultant similarity of the dose-response coefficients for dicentrics and the sum of complete and FP-type incomplete translocations is one of the most intriguing facts in our study.

Despite all the attention paid to the comparison of dicentrics and translocations radiation output, this question has been still unsolved. Sometimes the dose response curves for translocations and for dicentrics were rather close, but in other experiments the radiation induction of translocations appeared to be higher than that of for dicentrics [4-9, 11, 12, 20, 34, 35, 44-46]. This variability was caused by several reasons, such as different nomenclatures used for dicentrics and translocations scoring and difference in the control level between these two end-points, especially when their actual yields with nonsubtracted spontaneous incidence were compared at low doses. Also, some discrepancies were related to the technical question whether the centromere probes were applied for providing the unambiguous distinguishing between translocations and dicentrics. But strong evidence arises from our data that the previous results of dicentrics *versus* translocations comparison were contradictory mainly because of somewhat inappropriate choice of the FISH-measured end-points. The approach suggested in our work for estimating the total radiation output of complete and truly radiation-induced incomplete translocations provides a good confirmation for a frequently quoted opinion about the identity of the mechanisms of balanced and non-balanced interchromosomal exchange formation. Thus, the parameters of a calibration curve for total translocation yield in human lymphocytes irradiated at G_0-phase can be easily predicted now from the dicentric dose response applying 1:1 converting factor.

The dose response relationships, which could be found in the literature for total translocations, were built either for all translocations including those with part of chromosomal material missing or for the sum of complete and all t(Ab)-type incomplete translocations [4-15]. Our present work seems to be the first time the splitting of t_{inc}(Ab) translocations into FP and MP categories has been proposed for calibration curve constructing. Therefore our calibration curve constructed for total translocations could not be compared directly with those obtained in other laboratories, but such comparison is available in case of the dose response for complete translocations alone. The reported estimations of the linear term for complete translocations are: $1.19 \cdot 10^{-2} \cdot Gy^{-1}$ (our computation from their dicentric curve parameter) [8], $1.51 \cdot 10^{-2} \cdot Gy^{-1}$ [7] and $1.41 \cdot 10^{-2} \cdot Gy^{-1}$ (for 2B rearrangements according to S&S nomenclature) [16]. It should be noted that two latter values markedly exceed our estimation ($0.45 \cdot 10^{-2} \cdot Gy^{-1}$), but they both were accompanied by quite low means of the quadratic coefficients — $3.47 \cdot 10^{-2} \cdot Gy^{-2}$ and $1.83 \cdot 10^{-2} \cdot Gy^{-2}$, respectively. Taking into account a known inverse correlation between linear and quadratic terms of the dose regression [2], a refitting of these two equations with a quadratic term set at higher values (about $5\text{-}8 \cdot 10^{-2} \cdot Gy^{-2}$, as for dicentric curves) will cause the respective declining of the linear term.

A calibration system, which was constructed in present work for practical purposes of retrospective FISH-biodosimetry, is not based on total radiation-induced translocations, because the potential stability of aberrations needs to be also taken into account. Obviously, the acentrics induced as a part of t_{inc}(Ba+ac) and t_{inc}(Ab+ac) translocations in lymphocyte precursors are subjects for elimination in subsequent mitotic divisions, that may cause either mitotic death or the resultant occurrence of incomplete translocations

with "missing part" in mature lymphocytes. Therefore, these two types of exchanges were withdrawn from the data during fitting a calibration curve for potentially stable aberrations, but insertions were added instead, that compensated the decrease of the aberration number in statistical analysis. The resultant practically applicable calibration curve for total stable exchanges has a significantly higher linear term comparing with that of for complete translocations alone, that is especially valuable for biodosimetry at low doses.

In trying to derive such a curve in a computational way from the dicentric dose response, one has to keep in mind that truly incomplete dicentrics accompanied by a monocolored fragment representing the segment of only one chromosome involved (equivalents of t_{inc}(Ba+ac) and t_{inc}(Ab+ac) translocations) are not easy to distinguish from 'false' incomplete ones with visibly monocolored fragment, which actually carry a non-detectable by eye segment of the counterstained chromosome (equivalents of t_{inc}(Ba*) and t_{inc}(Ab*) translocations). Applying the telomeric probes can solve this problem probably, but the conductivity of such assay need to be evaluated in practice. From other side, it seems appropriate to use the empirical ratio of total stable exchanges to dicentrics as a converting factor, as it was originally proposed for generating curves for total translocations [3, 4, 8, 17]. It should be noted that an establishing of this factor *in vitro* has to be done at low doses for avoiding a possible data distortion by saturation effects and significant occurrence of complex rearrange ments. The converting factors coming from the ratios of linear coefficients in our work is 0.47 for total stable exchanges and 0.15 for complete translocations only.

Using the equations presented in Tables 2 and 5, the sensitivity of conventional and FISH techniques for practical biodosimetry was estimated. The acute γ-radiation dose that may cause doubling of the spontaneous level of conventionally scored aberrations is about 0.03 Gy for dicentrics plus centric rings and 0.11 Gy for excess acentrics. From that a higher sensitivity of unstable chromosomal exchanges than acentrics as biodosimeters becomes obvious. A minimum γ-dose, which could be detected on the level of statistical significance ($t=2.00$; $p<0.05$; assuming the Poisson dispersion) with dicentrics and rings scored in 1000 cells in both irradiated and control samples, is 0.14 Gy of acute irradiation and 0.20 Gy of chronic exposure. Estimation of minimum detectable dose of prolonged exposure was performed with the quadratic term of the basic acute curve set to zero. It is a usual practice recommended by IAEA, if the duration of radiation exposure exceeds 24 hrs and the quadratic term becomes reduced by the Lea and Catchisid's function to negligibly small value [1].

In case of FISH-measured stable aberrations a comparison was carried out for end-points practically applicable in retrospective biodosimetry, those were complete translocations and total chromosome exchanges with assumed full presence of chromosomal material in "stable" cells. For strengthening the conclusions the known effect of accumulation of stable aberration spontaneous level with persons' age was taken into account [25, 47-49]. The data describing the yields of FISH-measured aberrations in three age groups of control donors investigated in our laboratory had been recently published [50]. These results were utilized in the present work for calculating the virtual FP-fraction within the spontaneous yield of t(Ab) incomplete translocations, as it was done for *in vitro* dose response. The numbers of complete translocations, t(Ba*) and t(Ab*) incomplete translocations and insertions were pooled within each age group of donors, and the age dependence for the control level of the chromosome exchanges with full presence of chromosomal material in "stable" cells was fitted to the quadratic model. This resulted in the equation

$$Y_{S\rho} = 1.10 + 2.68 \cdot 10^{-3} \cdot A^2,$$

where $Y_{S\rho}$ is the full genome yield per 1000 cells and A is age in years. The coefficients of the age dependence for the spontaneous yield of complete translocations only were 1.68 and $1.24 \cdot 10^{-3}$ per 1000 cells, respectively [50]. The quadratic shape of the regression in our work is in total agree ment with data obtained by other authors [48, 49].

Table 6 presents the doses, which may double the control level of these two end-points, and doses, which may cause a statistical difference in aberration yield between irradiated and control sa-

mples each of 1000 genome equivalents, scored in individuals of different age. The chosen age values represent the range of most frequent cases of radiation overexposures known from the literature data and our own experience. For predicting the yield of stable aberrations under protracted exposure the quadratic term of the calibration curve was reduced in the same manner as for dicentrics plus rings. The suitability of this approach was deduced from the coincidence of the acute dose response for total translocations and dicentrics, that implied the identity of mechanism of both types interchromosome exchange formation and an equality of DNA primary breaks interaction time as a factor for curve modification.

The results show that the age-related accumulation of the spontaneous level are compensated more effectively by a radiation incidence of total stable chromosome exchanges, comparing with complete translocations alone. The difference is especially pronounced at chronic exposure conditions, where the linear term of the dose response plays a critical role. That clearly indicates that despite somewhat sophisticated manner of measuring and calibrating, the combination of complete plus FP-type incomplete translocations and insertions in "stable" cells could become an end-point of choice for retrospective FISH biodosimetry.

Conclusions

From the results of conventional and FISH measure ments of chromosome aberration yield in human blood lymphocytes after low-dose γ-irradiation *in vitro* the following conclusions may be drawn.

1. The dose response relationship for unstable chromosome exchange yield under low dose radiation exposure showed a good fit to the classic linear-quadratic model. The calibration curve coefficients for the full genome dicentrics plus centric rings estimated from the FISH data coincided with that of estimated by conventional analysis. No sign of the plateau for aberration yield within the low dose range was detected.

2. A new approach to data treatment was suggested and applied for constructing a calibration curve for translocations. It consisted in combining the complete translocation yield with mathe matically derived fraction of truly radiation-induced incomplete translocations. The dose response curve generated in this manner appeared to be very close to that of for dicentrics, confirming a hypothesis about an identity of the mechanisms of balanced and non-balanced interchromosomal exchange formation in irradiated human lymphocytes.

3. The end-point of choice for the practically applicable FISH biodosimetry syste m represented the sum of insertions, complete translocations and calculated fraction of those incomplete translocations, which were probably complete ones with indistinguishably small translocated segment. The calibration curve constructed for this end-point had a higher linear term comparing with complete translocations only, that may increase significantly the sensitivity of retrospective FISH biodosimetry, especially in chronic exposure conditions.

Table 6

A comparison of the FISH-measured end-points' resolution power for biological dosimetry depending on individuals' age

Age, Years	Control level, per 1000 genome equivalents		γ-dose doubling the control level, Gy				γ-dose detected with 1000 genome equivalents scored, Gy			
			Acute		Chronic		Acute		Chronic	
	t_{comp}	St Exch	t_{comp}	St Exch	t_{comp}	St Exch	t_{comp}	St Exch	t_{comp}	St Exch
20	2.18	2.17	0.15	0.10	0.49	0.16	0.30	0.22	1.50	0.50
30	2.80	3.51	0.18	0.14	0.62	0.25	0.32	0.24	1.70	0.60
40	3.66	5.29	0.21	0.18	0.82	0.38	0.32	0.26	1.80	0.65
50	4.78	7.80	0.24	0.24	1.07	0.56	0.34	0.29	2.00	0.80
60	6.14	10.75	0.28	0.29	1.37	0.77	0.36	0.31	2.20	0.85

t_{comp} – complete translocations; St Exch – chromosome exchanges in "stable" cells ($t_{comp} + t_{inc}(Ab^*) + t_{inc}(Ba^*) + ins$).

4. With practically acceptable number of cells scored, the routine cytogenetic analysis allows an unbiased detecting the γ-doses about 0.14 Gy of acute irradiation and 0.20 Gy of chronic exposure. The resolution power of FISH biodosimetry relies on the age-dependent spontaneous level of stable aberrations and varies from 0.22 to 0.31 Gy of acute irradiation and from 0.50 to 0.85 Gy of chronic exposure within the studied age range (20-60 years).

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