

Dose and dose-rate effects of long-term exposure to low-dose rate gamma rays on unstable-type chromosome aberration frequencies in mouse splenocytes ¶

**Kimio Tanaka¹, Atsushi Kohda¹, Kenichi Satoh²,
Yoichi Oghiso¹, Akihiro Shima¹**

¹Dept. Radiobiol., Inst. Environ. Sci., Japan

²Dept. Environment. Biometrics, Inst. Radio. Biol. Med., Hiroshima Univ., Japan

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Abstract

- **Objectives:** The present study was performed to investigate whether dose and dose-rate effects are observed on unstable-type chromosome aberration rates in mice exposed to wide ranges of doses (100~8,000mGy) and dose-rates, i.e. low dose-rates (LDRs:1, 20 mGy/22h/day) to medium dose-rates (MDR:200, 400 mGy/22h/day).
- **Methods:** SPF C3H female mice were continuously exposed to gamma-rays with LDRs and MDRs for different periods from the age of 8 weeks to a maximum of 657 days. Lymphocyte chromosome spreads were prepared from splenocytes from these mice.
- **Results:** Incidences of dicentrics (Dics) plus centric rings detected by conventional Giemsa staining and those of Dics detected by FISH increased almost linearly up to a total accumulated dose of 8000 mGy at 20 mGy/day. The values of regression coefficients for linear regression lines for these aberrations, obtained by multiple linear regression analysis adjusted for age-related differences, decreased as the dose rate was lowered from MDRs to LDRs, suggesting the presence of dose-rate effects in the range of 400-folds different dose rates (1-400 mGy/day) as well as discrepancy with the current formula to estimate dose and dose-rate effectiveness factor (DDREF). DDREF of 4.5 was obtained for Dics by comparing chromosome aberration rates at the same total dose of 100 mGy, reached by 890 mGy/day and LDR(20 mGy/day). Further, DDREF varied (4.5~17.8) with accumulated dose ranges (100~1000 mGy).
- **Conclusions:** DDREF for Dic increased with reducing dose-rates in low dose range.

Background

Dose and dose-rate-effects are not well studied at lower dose rates ranging from 1 mGy/22h/day (0.045 mGy/h) to 20 mGy/22h/day(0.91 mGy/h).

Aims

- 1. To investigate dose and dose-rate-effects on chromosome aberrations in mice exposed to wide ranges of dose (100 -8000 mGy) and low dose rate (LDR)(1 mGy/22h/day) to medium dose rate (MDR)[400 mGy/22h/day(18.2 mGy/h)].**
- 2. To estimate dose and dose rate effectiveness factor (DDREF) at low dose range.**

Materials and Methods

1. SPF female mice were continuously irradiated from 8 weeks of age with ^{137}Cs γ -rays at different dose rates. 3-13 mice were used for a dose point. —①
2. Spleen cells were cultured for 48h with ConA, LPS and 2 ME to obtain chromosome metaphases.
3. Unstable-type chromosome aberrations such as dicentric and ring (Dic + Rc) and Cu (Dic+Rc+ace+Frag)) were observed by Giemsa staining, and dicentrics by centromere FISH (Dic by FISH). Also, translocations were scored by M-FISH. 500-1000 metaphases/mouse were analyzed. —②
4. Multiple linear regression analysis was used on the linear quadratic and linear models adjusting for age-related differences to obtain regression coefficients $b_j(j=1-8)$ and $a_j(j=1-7)$ with weighting observed cell number. Response variable (y) is number of chromosome aberrations and explanatory variables are accumulated dose (D) and age (T). Regression coefficients, b1 and b2 are common in all dose rates in each chromosome aberration (Dic+Rc, Cu, Dic by FISH).

Regression models are expressed as

(1) Linear quadratic model, ≤ 8000 mGy

non exposed: $y=b_1+b_2T$,

1 m Gy/22h/ day: $y=b_1+b_2T+ b_3D+ a_3D^2$,

20 mGy/22h /day: $y=b_1+b_2T+b_4D+a_4D^2$,

200 mGy/22h/day: $b_1+ b_2T+b_5D+a_5D^2$,

400 mGy/22h/day: $y=b_1+b_2T+b_6D+a_6D^2$,

890 mGy / min: $y=b_1+b_2T +b_7D+ a_7D^2$,

(2) Linear model, ≤ 8000 mGy

non exposed: $y=b_1+b_2T$,

1 mGy/22h/ day: $y=b_1+b_2T+ b_3D$,

20 mGy/22h /day: $y=b_1+b_2T+b_4D$,

200 mGy/22h/day: $y=b_1+ b_2T+b_5D$,

400 mGy/22h/day: $y=b_1+b_2T+b_6D$,

890 mGy / min: $y=b_1+b_2T +b_7D+ b_8D^2$,

(3) Linear model , ≤ 1000 mGy

non exposed: $y=b_1+b_2T$,

1 mGy/22h/day: $y=b_1+b_2T+b_3D$,

20 mGy/ 22h/day: $y=b_1+b_2T+b_4D$,

, where D is accumulated dose in mGy and T is

age(in days) minus 56.

① Fig. 1 Irradiation procedure and dose rates and accumulated doses

Irradiation started from 8 weeks old age (56 days)

① **890 mGy/min** 0, 250 500, 1000, 2000, 3000 mGy - HDR

② **400 mGy/22h/day (200 mGy22h//day)** - MDR

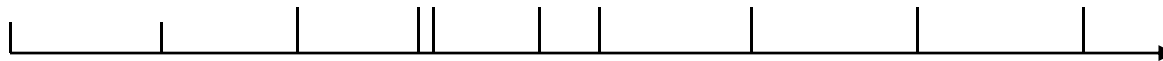


0 (200) 400 800 (1000) 1200 2000 4000 8000 mGy

• 1 2 3 4 10 20 days

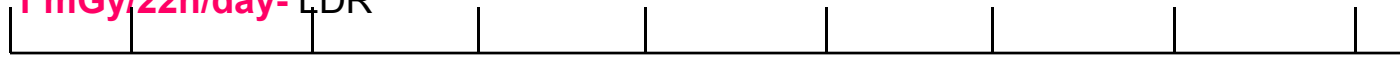
• (1) (2) (4) (5) (10) (20) (40) days

③ **20 mGy22h//day-** LDR



0 250 500 750 1000 1200 2000 4000 8000 mGy
12.5 25 33 50 60 100 200 400 days

④ **1 mGy/22h/day-** LDR

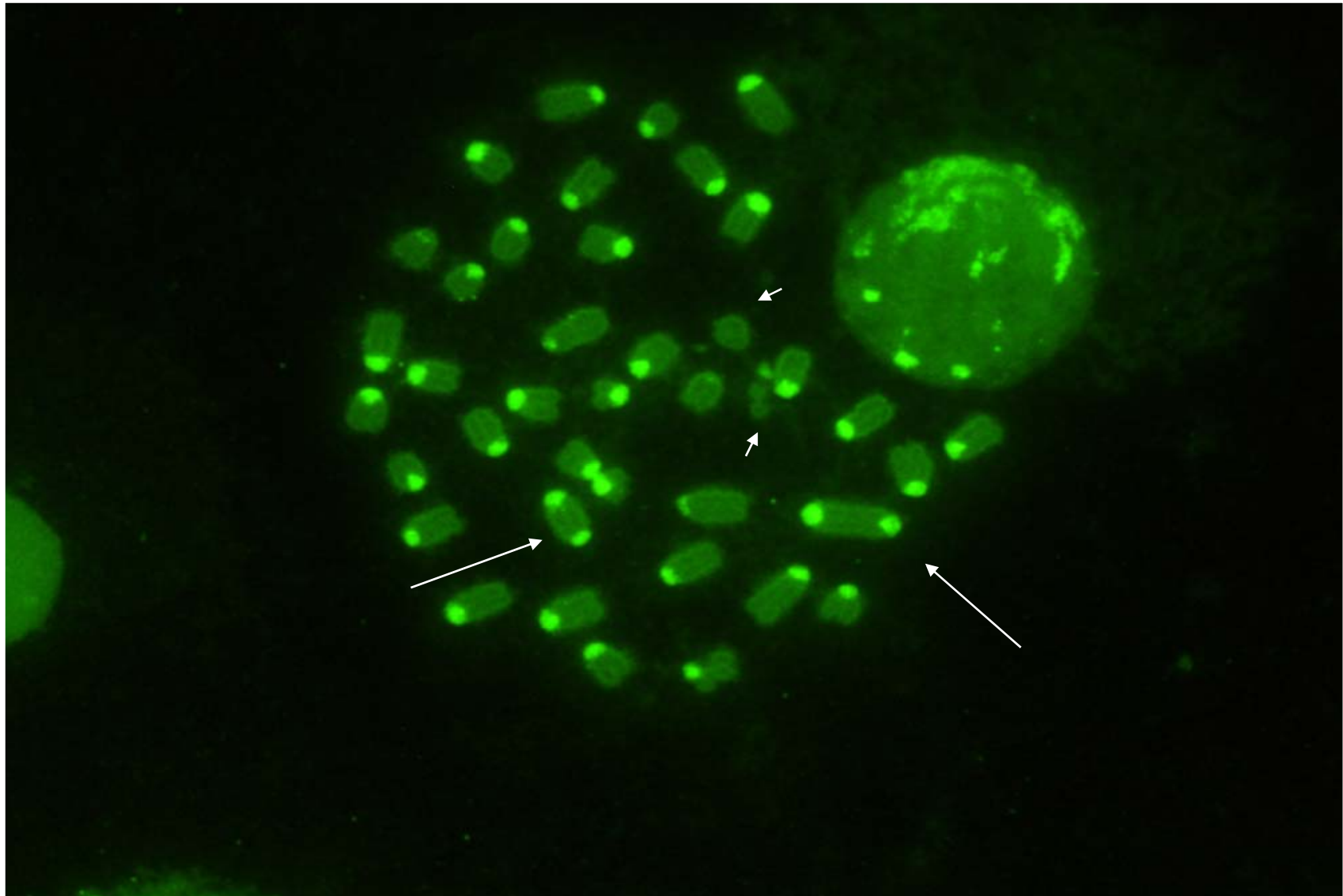


0 125 187.5 250 312.5 375 412.5 500 615 mGy
125 188 250 313 375 413 500 615 days

⑤ **Non-irradiated** control mice
mice of same age at the time of each accumulated dose

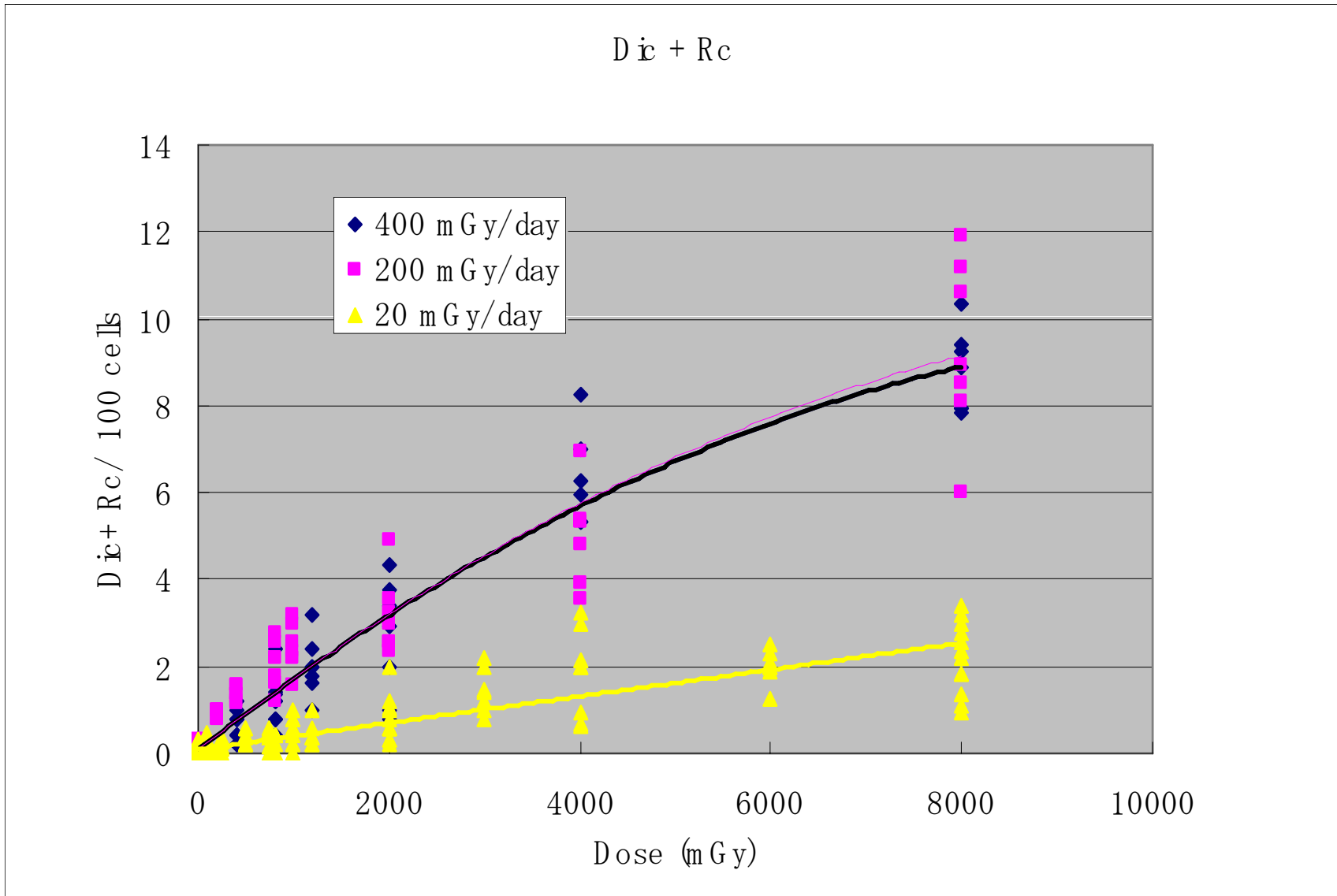


② Fig.2 Dicentric chromosomes (long arrow) and fragments (short arrow) detected by centromere FISH

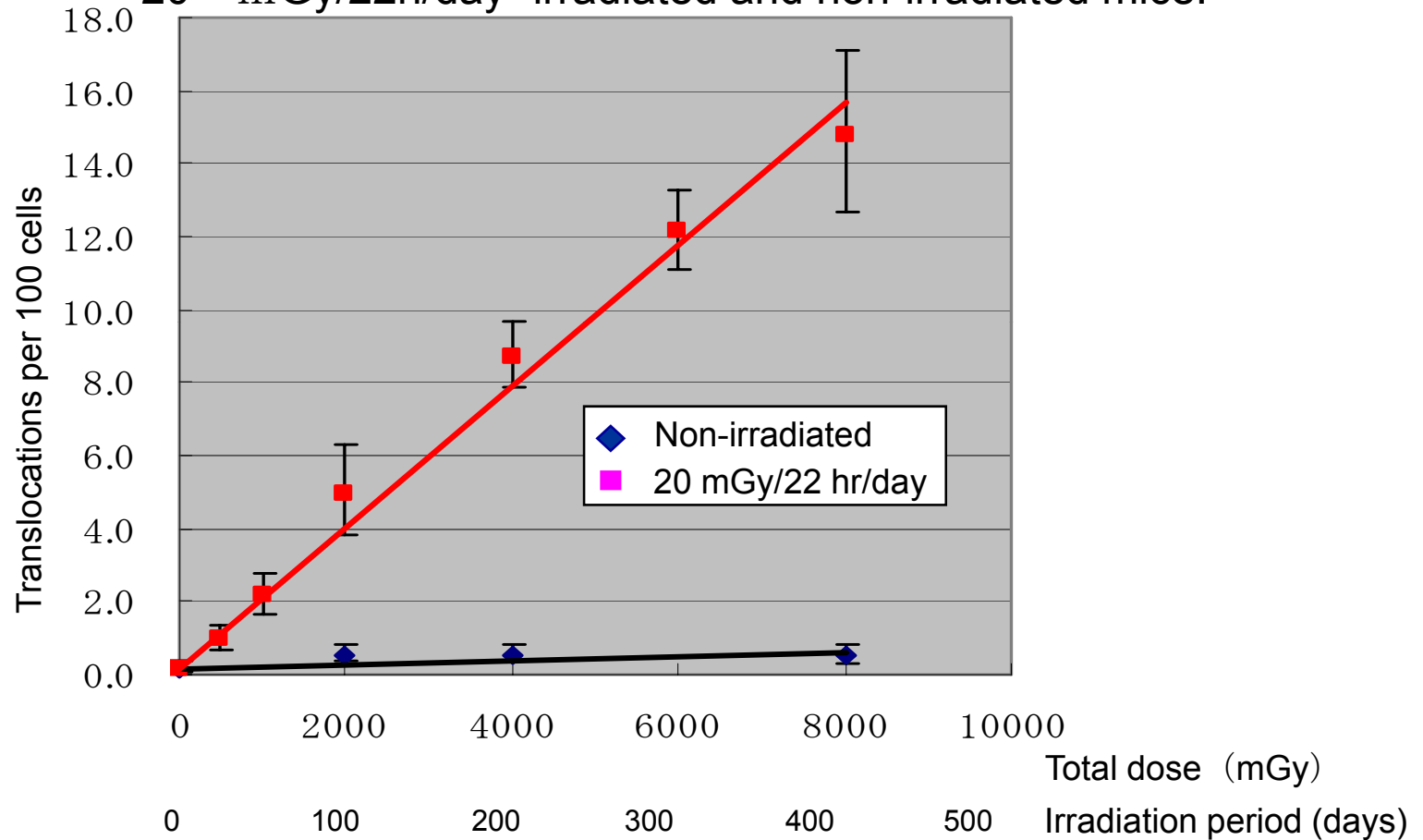


③

Fig.3a



④ Fig. 3 b Changes of translocation rates in splenocytes from 20 mGy/22h/day -irradiated and non-irradiated mice.

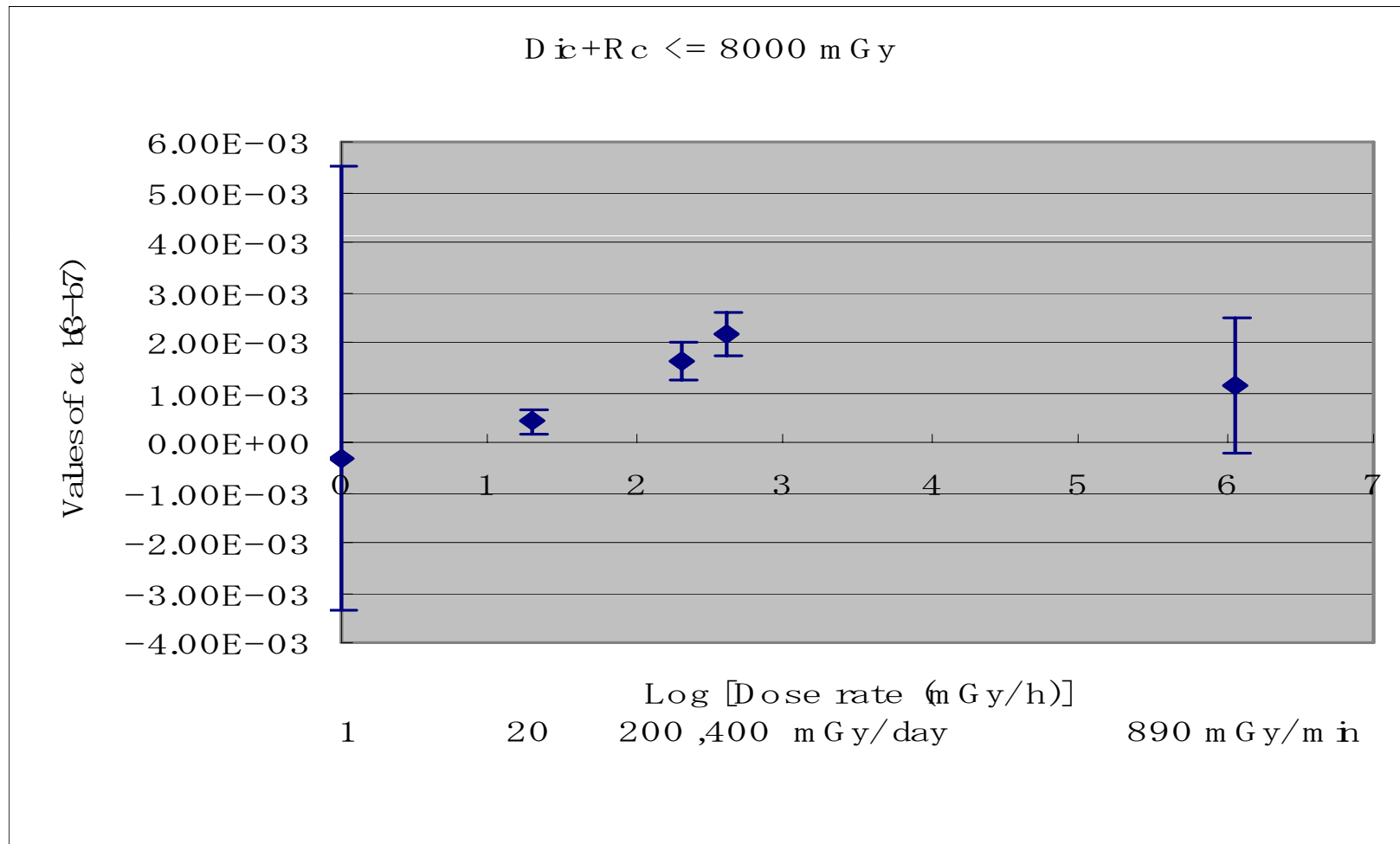


Chronological changes of translocation rates in splenocytes from LDR-irradiated and non-irradiated mice.

*X axis is the total dose (mGy) or irradiation period in days, and Y axis is chromosome aberration rates (number of aberrations per 100 metaphases).

Horizontal bar around each value point shows 95% confidence intervals.

⑥ Fig.5a Reduction of regression coefficients (linear term) with decrease of dose rate from 400 mGy/22h/day to 20 mGy/22h/day (linear quadratic model). Bars show 95%C.I.



⑧ Table 1 Reduction of regression coefficients (linear term) from MDR to LDR

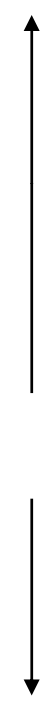
(1) Linear quadratic model - Dic+ Rc <= 8000 mGy

Dose Rate	$\alpha(b_{3,4,5,6,7}) \pm S.E.$	95% C.I. p<0.05	
890 mGy/min	$1.57 \times 10^{-3} \pm 6.02 \times 10^{-4}$		
400 mGy/22h/day	$2.18 \times 10^{-3} \pm 2.15 \times 10^{-4}$	*	
200 mGy/22h/day	$1.61 \times 10^{-3} \pm 1.91 \times 10^{-4}$		*
20 mGy/22h/day	$4.14 \times 10^{-4} \pm 1.26 \times 10^{-4}$	*	*
1 mGy/22h/day	$-3.13 \times 10^{-4} \pm 1.53 \times 10^{-3}$		

Impact of dose range

(2) Linear model- Dic by FISH <=1000 mGy

Dose Rate	$\alpha(b_{3,4}) \pm S.E.$	95% C.I. p<0.05	
20 mGy/22h/day	* $6.15 \times 10^{-4} \pm 5.70 \times 10^{-5}$	*	
1 mGy/22h/day	* $2.76 \times 10^{-4} \pm 1.07 \times 10^{-4}$	*	



⑨

Table 2

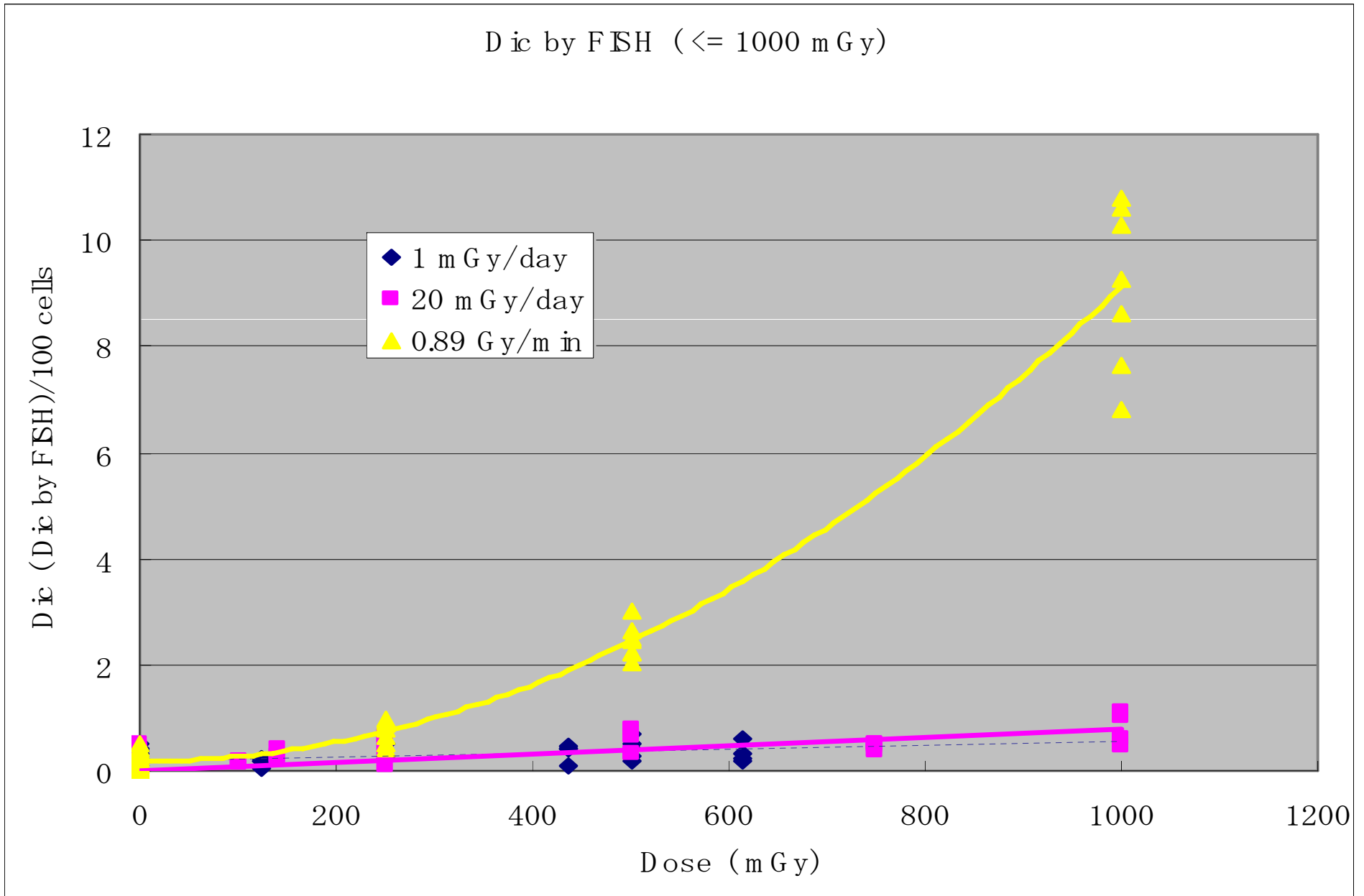
DDREF obtained for Dic by FISH and Dic + Rc using linear model

• Dose (mGy)	Dic by FISH	Dic + Rc
• 1000	17.8	24.5
• 500	10.4	13.8
• 200	6.0	7.3
• 100	4.5	5.2

- DDREF = HDR(890 mGy/min)/LDR(20 mGy/22h /day) = $(\beta_7 D^2 + \alpha_7 D) / \alpha_4 D$. We could not use $(\beta_7 / \alpha_4) D + 1$, because α_7 and α_4 were not equal.

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Fig.6



⑪ Discrepant from the current formula to obtain DDREF

< Chromosome breakage-reunion hypothesis >

HDR irradiation $Y = \beta D^2 + \alpha_1 D + c$

Y: chromosome aberration rate

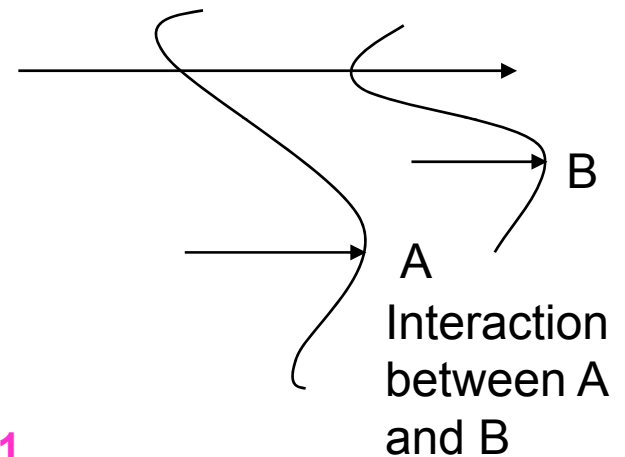
(Number of aberrations/100 cells)

D: Dose (mGy)

LDR irradiation $Y = \alpha_2 D + c$

α coefficient: independent on dose rate

β coefficient: dependent on dose rate



• ICRP(1990), BEIR VII Report (2006) et al.

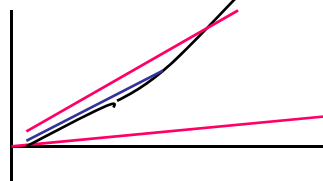
DDREF= ratio of (HDR/LDR)= $(\beta D^2 + \alpha_1 D) / \alpha_2 D = D (\beta/\alpha) + 1$

On the assumption that $\alpha_1 = \alpha_2$

→ **However, in our results, values of α coefficient significantly decreased with reduction of dose rate.**

• NCRP(1980) DREF= ratio of HDR $(\alpha_1 D + c)$ /LDR $(\alpha_2 D + c)$: Dic+Rc ,Dic by FISH

$\alpha_1/\alpha_2 = 55.2$ and 36.2



Conclusions

- 1. Dicentric and ring chromosomes, and translocations were increased almost linear with accumulated dose at 20 mGy/22h/day. — ③,④,⑤**
- 2. Dose-rate effects were clearly found between the dose rate ranges of 400 mGy/22h/day and 1mGy /22h/day. — ③,⑤,⑥,⑦,⑧**
- 3. The values of regression coefficients (linear term) were decreased with reduction of dose rate, which is discrepant from current formula recommended by NCRP(1980), ICRP(1991) and BEIR(2006) to estimate DDREF. — ⑥,⑦,⑧,⑪**
- 4. DDREF for dicentric chromosomes decreased from 17.8 to 4.5 with reduction of accumulated dose, and also DDREF varied**

References

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