CYTOGENETICAL EVALUATION OF A NEW ANIMAL MODEL FOR RADIOBIOLOGICAL STUDIES

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SUMMARY
The response of a New World monkey species (Cebus apella paraguayanus) lymphocytes to various doses of 60 Co gamma-rays has been studied using dicentrics + rings frequency in first mitosis and compared to that of man . Results have shown that differences between both species are no significant.
The distribution of 200 breakpoints in G-banded metaphases has been scored showing an excess of breaks in chromosomes 1,11,12 and 16.Terminal heterochromatic blocks differ from intercalar heterochromatin in the response to gamma radiation being the former more affected.

INTRODUCTION

Frequency of accidental whole-body overexposures is low even though they may result in lethal effects. For that reason, present knowledge for diagnosis and treatment of such overexposures is scanty and it is necessary to extrapolate qualitative and quantitative data from animal models used to reproduce accidental overexposure conditions. To extrapolate with confidence, an adequate animal model should have a close phylogenetical relationship and show radiosensitivity similar to that of man.

Among New World Monkeys, Cebus apella paraguayanus (Fisher, 1829) is one of the most widely spread species in South America and frequently used in biomedical research. This species has shown a successful breeding and reproduction rate in captivity. Its karyotype has been standardized (1) and the amounts and variations of constitutive heterochromatin have been characterized (2). Among Platyrrhini, this species is the least distant to man, with about thirty five rearrangements separating both karyotypes (3). However, previous studies have shown that phylogenetical proximity does not necessarily imply a similar radiosensitivity because even the higher primates such as gorilla and chimpanzee (4) don't represent good models, at least for quantitative estimation of cytogenetical hazards, because chromsomal radiosensitivity is not related to chromosome number, chromosome arm number or similarities in the karyotypes (5) even if only first mitoses are considered.

Unstable chromosome aberrations frequency in short-term lymphocytes cultures is a useful tool to make direct analysis of the effects of ionizing radiation on human and other species genetic material using in-vitro irradiations or samples from in-vivo ovesexposures.
In order to stablish if Cebus apella paraguayanus has a chromosomal radosensitivity similar to that of man we have estimated the frequency of unstable chromosomal aberrations (dicentrics+rings/cell) in 48 hours lymphocytes cultures of

both species, using simultaneously in-vitro irradiated human and primate blood samples with different doses of 60 Co gamma-radiation, from 0.5 Gy to dosimeter saturation point.
Distribution of the breakpoints among the chromosomes involved in the production of dicentrics and rings has been scored in G-banded metaphases.

MATERIAL AND METHODS
Three human and three Cebus apella paraguayanus whole blood samples have been irradiated by gamma-rays at high dose-rate from a 60 Co source,pairing a human and a monkey sample in each irradiation at 0.5 , 1 , 2 , 3 , 5 and 6 Gy doses.
Whole-blood cultures have been immediately set-up using RPMI 1640 medium with 1-2 % PHA and supplemented with FCS (15 %).
Cells have been harvested after 48 hours. At this time practically 99 % and more than 95 % of human and monkey (own data) lymphocytes respectively are in first division.
Irradiations at 7 Gy in both species indicate dosimeter saturation (y:2 dic+r/cell). For that reason the higher dose considered in data analysis has been 6 Gy.
A blood sample of Cebus apella has been irradiated at 2 Gy and 300 well spread metaphases from 48 hours cultures were used to localize breakpoints on G-banded chromosomes following the procedure of Seabright, 1971 (6).

RESULTS
Human results have been fitted to a linear quadratic dose-effect model,using least squares weighted method (weight: 1/y) obtaining values of 2.613 E-02±0.01 and 5.074E-02±0.004 for α and β coefficients respectively (chi-square: 2.35 E-02;DF:4). (TABLE I)
Cebus apella results have been fitted to a quadratic dose-effect model with β coefficient: 5.575 E-02 ± 0.01 (chi-square: 2.25 E-02; DF:5) (Fig. I).
Background levels have been descounted from y values in each dose and in both species. The two way analysis of variance indicates that there is no significant difference between species or for interaction dose-species ($p > 0.05$).

A total of 100 two-breaks chromosome aberrations (dicentrics + rings) were scored in 300 G-banded karyotypes prepared from gamma irradiated at 2 Gy cells of 48 hs culture. The distribution of the observed number of breakpoints per chromosome and comparison with the expected numbers is presented in Table II. The expected numbers has been calculated assuming that break distribution is proportional to chromosome corrected length in 54,XY cells, taking into account C-bands heteromorphisms for 11 and 19 chromosome pairs. This specimen shows the following heterochromatic variations: Pair 11:one chromosome (11 L) with a large-size terminal block (85% of the chromosome length) and the other (11M) with a medium-size block (75% of the chromosome length). Pair 19: one chromosome (19H) with an intercalar heterochromatic block (40% of the chromosome length) and the other without it.
The differences between the observed and expected number of breakpoints per chromosome, determined by chi-square analysis,

are shown in Fig. II. As can be seen, chromosomes 1 , 12 and 16 show significant excess of breakpoints ($p < 0.01$). A trend toward a deficiency of breaks has been observed in chromosome 4($p < 0.1$)and to an excess of breaks in chromosome 11 L ($p<0.1$) and 11 M ($p < 0.05$).

DISCUSSION

Even though each species fits to different models the results of analysis of variance indicate that differences are no significant. Under the light of this data this species could be considered a model in terms of cytogenetical evaluation, at least in the range from 0.5Gy to 6Gy. However, being ratio α/β aproximately 0.5 Gy for man, the authors think this model deserves further studies under this low dose, to focalize the precise behavior of monkey chromosomes in the zone where it is postulated that dicentrics are produced principally by one track.

Respecting distribution of breakpoints, the results have indicated that chromosomes 1 , 12 and 16 have been more frequently involved in radiation induced aberrations but they have shown random association with the second chromosome which takes part in each aberration. Interestingly, conspicuous terminal heterochromatic blocks in chromosomes 11 have shown more breaks than expected resembling chimpanzee terminal heterochromatin behavior (7), though this species seems to be more radiosensitive than man, contrary to Cebus apella paraguayanus.However, in the case of chromosome 19 H, clustered breaks in euchromatin but none in the intercalar heterochromatic block has been observed.If the amount and position of heterochromatin play some role and must be taken into account when chromosome radiosensitivity of a particular species is evaluated, requires more information.

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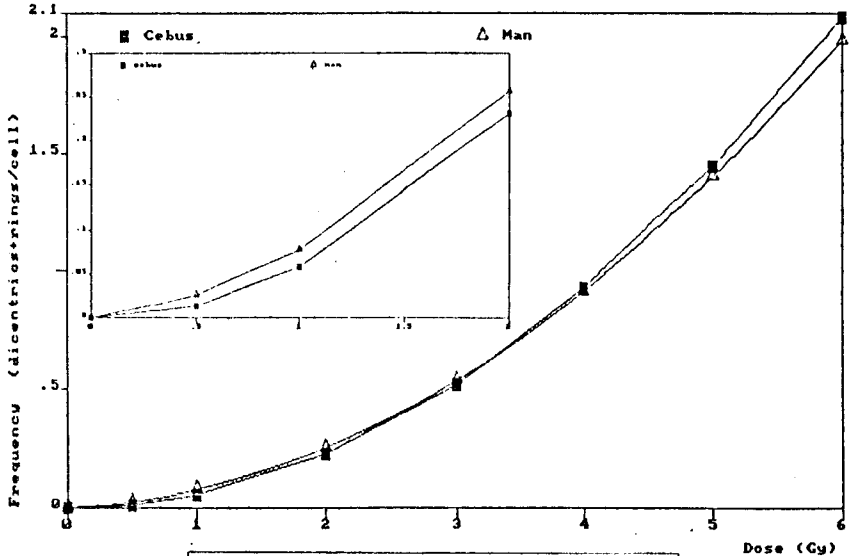
TABLE I

DOSE (Gy)	MAN			CEBUS		
	Cells scored	Dic+rings	Frequency	Cells scored	Dic+rings	Frequency
0.5	501	18	0.035	500	14	0.028
	500	13	0.026	500	20	0.040
	500	10	0.020	500	18	0.036
1	300	18	0.060	311	7	0.022
	352	26	0.073	500	30	0.060
	315	21	0.066	500	22	0.044
2	203	56	0.275	200	46	0.230
	250	56	0.224	245	45	0.306
	200	60	0.300	200	82	0.410
3	200	116	0.580	200	86	0.430
	200	123	0.615	200	108	0.540
	200	100	0.500	200	98	0.490
5	100	142	1.420	96	121	1.260
	100	133	1.330	60	95	1.583
	100	152	1.520	75	110	1.466
6	62	125	2.016	50	108	2.160
	100	190	1.920	95	180	1.894
	80	148	1.850	50	118	2.360

TABLA II Distribution of breaks per chromosome

Chromosome	Observed	Expected
1	23	12
2	7	11.04
3	10	9.98
4	3	9.28
5	6	8.70
6	9	8.36
7	5	7.16
8	4	6.20
9	2	4.48
10	3	4.48
11 L	13	7.08
11 M	10	4.80
12	19	9.84
13	8	8.84
14	5	8.52
15	4	8.32
16	16	7.60
17	11	7.50
18	6	7.00
19	4	2.90
19 H	5	4.94
20	5	5.74
21	4	4.76
22	2	4.62
23	2	4.48
24	3	3.84
25	2	3.04
26	1	3.04
X	6	8.92
Y	1	2.64

Fig.I: Gamma radiation dose-response relationship



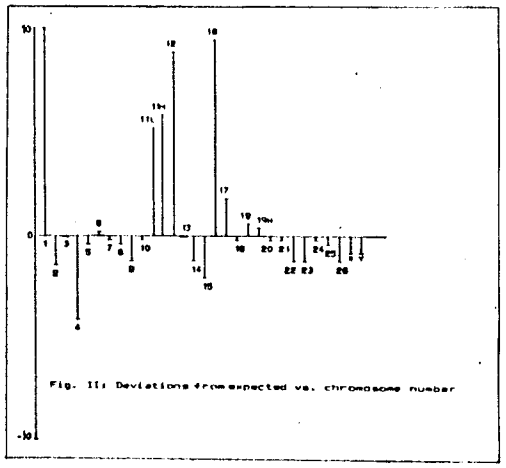


Fig. II: Deviations from expected vs. chromosome number