Clarithromycin Genotoxicity in Mice

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Summary The effect of clarithromycin on the induction of chromosome aberrations in mice bone-marrow, splenocyte cells and spermatocyte cells was investigated. Male Swiss mice were orally treated by gavage once with doses 50, 100, 200 and 300 mg kg⁻¹ b.wt. A repeated daily dose of 50 mg kg⁻¹ b.wt. was given for successive days. Clarithromycin induced chromosome aberrations in both bone-marrow and splenocyte cells. The percentage of chromosome aberrations was found to be statistically significant after single and repeated treatments.

The percentage of chromosome aberrations in diakinesis–metaphase I spermatocytes increased in a dose dependent manner and was found to be statistically significant after 2 higher and repeated doses.

The high doses of the antibiotic caused a significant increase in the frequency of sister chromatid exchanges in mice bone-marrow. Its highest values were $9.99 \pm 0.48/\text{cell}$ after treatment with high dose (300 mg kg⁻¹ b.wt.) compared with $4.24 \pm 0.44/\text{cell}$ in the non treated mice.

Key words Genotoxicity, Clarithromycin, Chromosome aberrations, Mice.

The newer macrolides have added effectiveness against gram-negative bacteria and anaerobes. Macrolides are of tremendous value for their unique ability to treat atypical pathogens seen in community acquired pneumonias (Laurence *et al.* 1997).

Clarithromycin is a newer macrolide antibiotic which appears to have significant increase potency against many gram positive and negative aerobic and anaerobic organisms. It is indicated in the treatment of infections caused by microorganisms sensitive to clarithromycin *e.g.* pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, bacterial pneumonia and atypical pneumonia, uncomplicated skin and skin structure infection (Delgado and Remers 1998).

The present work aims to shed some light on a mutagenic potential of clarithromycin in somatic and germ cells in the mice.

Materials and methods

Animals

Mature male Swiss mice, aged 9–12 weeks and weighted 25–30 gm obtained from a close random-bred colony at National Research Centre, were used.

Chemical and treatment

Clarithromycin was purchased from Sigma pharmaceutical industries S.A.E. Egypt. Clarithromycin (dissolved in distilled water) was administered by gavage at 4 different doses: 50, 100, 200 and 300 mg kg⁻¹ b.wt. A dose of 50 mg kg⁻¹ b.wt. is the therapeutic dose for humans (Delgado and Remers 1998).

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Table 1. Chromosome aberrations in mice bone-marrow after oral treatment by gavage with different doses of clarithromycin

Treatment	Mice killed after (h) [†]	% of chromosome aberrations±S.E.		No. of metaphases with						
		With gaps	Without gaps	Gaps	Frag and/or break	Del.	Frag.+ Del	Frag. + Gaps	Robertson trans	Polyploidy
I. Control (non-treated)										
II. Positive control i.p. injection with mitomycin C 1 mg kg ⁻¹ b.wt.	24	3.6±0.40	2.6±0.71	5	6	3	2	2	—	—
		22.6±0.79**	15.6±0.86**	35	47	20	3	2	2	4
III. Single treatment (clarithromycin)										
50 mg kg ⁻¹ b.wt.	24	6.2±0.74	4.4±0.68	9	11	4	4	3	—	—
100 mg kg ⁻¹ b.wt.	24	7.0±0.71*	5.0±0.95	10	13	5	3	2	1	1
200 mg kg ⁻¹ b.wt.	24	9.4±0.93**	7.2±0.66**	11	15	6	6	6	1	2
300 mg kg ⁻¹ b.wt.	24	10.6±0.93**	7.6±0.93**	15	17	5	7	4	2	3
IV. Repeated treatment clarithromycin 50 mg kg ⁻¹ b.wt. for 14 successive days	24	8.2±0.86**	5.6±0.66*	11	12	5	5	4	1	1

[†]Total number of scored metaphases was 500/group. * Significant at 0.05 level (*t*-test). ** Significant at 0.01 level (*t*-test).

Table 2. Chromosome aberrations in mice splenocyte cells after oral treatment by gavage with different doses of clarithromycin

Treatment	Mice killed after (h) [†]	% of chromosome aberrations ± S.E.		No. of metaphases with						
		With gaps	Without gaps	Gaps	Frag and/or Break	Del	Frag. + Del	Frag. + Gaps	Robertson trans	Polyploidy
I. Control (non-treated)										
II. Positive control i.p. injection with mitomycin C 1 mg/kg ⁻¹ b.wt.	24	4.4 ± 0.48	3.2 ± 0.41	6	8	4	2	2	—	—
		24.4 ± 0.78**	16.8 ± 0.57**	38	50	22	2	2	3	5
III. Single treatment (clarithromycin)										
50 mg kg ⁻¹ b.wt.	24	7.2 ± 0.86	5.0 ± 0.97	9	10	8	4	3	—	—
100 mg kg ⁻¹ b.wt.	24	8.4 ± 0.93**	6.6 ± 1.12*	9	11	7	9	4	2	—
200 mg kg ⁻¹ b.wt.	24	10.0 ± 0.71**	6.6 ± 0.58*	13	14	6	5	5	1	2
300 mg kg ⁻¹ b.wt.	24	11.4 ± 0.93**	8.8 ± 0.84**	11	13	8	8	11	2	2
IV. Repeated treatment clarithromycin 50 mg kg ⁻¹ b.wt. for 14 successive days	24	9.0 ± 0.71**	7.6 ± 0.14**	10	16	9	5	5	1	2

[†]Total number of scored metaphases was 500/group. * Significant at 0.05 level (*t*-test). ** Significant at 0.01 level (*t*-test).

Treatment and cytological preparation

Chromosome aberration in somatic cells. For bone-marrow and splenocyte cells, a group of mice (5/dose) were orally treated with a single dose of 50, 100, 200 and 300 mg kg⁻¹ b.wt. For repeated treatment, mice were orally treated daily for 14 successive days with 50 mg clarithromycin kg⁻¹ b.wt., samples were taken 24 h after post treatment.

Chromosomes from bone-marrow cells were prepared following the method of Yosida and Amano (1965) and from splenocyte cells were prepared according to Amer *et al.* (1993). Slides were stained with phosphate buffer Giemsa, 100 well spreaded metaphases were analyzed per animal.

Chromosome aberrations in germ cells. Spermatocyte metaphases at diakinesis metaphase I was prepared according to Evans *et al.* (1964). Slides were stained with 7% Giemsa stain in phosphate buffer pH 6.8. At least 100 well spread metaphases were analyzed for each animal, scoring different types of aberrations.

Sister chromatid exchanges (SCEs). For SCEs random-bred male swiss mice aged 3–4 months were used and method described by McFee *et al.* (1983) was followed. A subcutaneous implantation of 5-Bromodeoxyuridine (BrdU) tablet (55 mg) was initially made in each mouse and 30 min later the test compound was administered by gavage. After 21 h, the animals received an intraperitoneal injection of colchicine (10 mg kg⁻¹ b.wt.). Bone-marrow cells were fixed 24 h later and stained with fluorescence plus Giemsa method of Perry and Wolff (1974). Five animals were taken for each treatment and 40 well spread metaphases per animal were examined microscopically for SCEs.

In all experiments a negative control received only distilled water and mitomycin C at a dose 1 mg kg⁻¹ b.wt. (*i.p.*) was used as a positive control.

Statistical analysis. The significance of experimental versus control data was calculated using the *t*-test.

Results

Chromosome aberrations

Somatic cells. The different doses of clarithromycin induced an increase in the percentage of chromosome aberrations in both mice bone-marrow and splenocyte cells after a single and repeated oral treatments. This increase was statistically significant after oral treatment with the higher single doses and repeated dose for 14 consecutive days (Tables 1, 2).

The types of aberrations observed in both mice bone-marrow and splenocyte cells were the same. Main type of aberrations was chromatid gaps, chromatid deletions, fragments and/or breaks. Robertsonian translocations and numerical chromosome aberrations in the form tetraploid cells were present in a few number (Tables 1, 2 and Fig. 1a).

Germ cells. Table 3 presents the frequencies and distributions of chromosome aberrations in spermatocyte cells after a single and repeated doses of clarithromycin. The percentage of aberrations was dose and time dependent.

The clarithromycin induced abnormal chromosome associations including X, Y and autosomal univalents as well as reciprocal translocations as chain IV (Table 3, Fig. 1-b).

Sister chromatid exchanges (SCEs)

Clarithromycin induced dose dependent increase in SCEs frequency in mice bone-marrow cells. The frequency of SCEs was found to be statistically significant after oral treatment with the higher doses 100, 200 and 300 mg kg⁻¹ b.wt. (Table 4, Fig. 1-c, d).

Fig. 1. Metaphases from mice treated with different doses (100, 200, 300 mg kg⁻¹ b.wt.) of clarithromycin. a) Chromatid break in mice spleenocyte cells. b) X-Y univalent in mice spermatocyte. c, d) Metaphases from mice bone-marrow cells showing SCEs induced after treatment with clarithromycin.

Discussion

Drugs are considered to be an important group of chemicals. Recently there is a growing concern about possible genotoxic and mutagenic potential of drugs in mammalian cells (Mailhes *et al.* 1993, Donya 2002, Ibrahim 2003).

The different doses of clarithromycin induced an increase in the percentage of chromosome aberrations in both bone-marrow and spleenocyte cells after a single and repeated oral treatment. Also, the percentage of chromosome aberrations increased significantly ($P < 0.01$) in bone-marrow and spleenocyte cells after treatment of mice by gavage with 50 mg kg⁻¹ b.wt. clarithromycin for 14 successive days. In this connection, it may be mentioned that the same effect was observed after oral treatment with Diazepam (Fahmy and Aly 1995) Cefadroxil (Aly 2001), and Cefuroxime (Ibrahim 2003).

A single oral treatment with clarithromycin (therapeutic dose, 50 mg kg⁻¹ b.wt.) was not significant in both somatic and germ cells. However, repeated treatment with the same dose (50 mg kg⁻¹ b.wt.) was highly statistically significant in all types of cells. This may be attributed to the rapidly and completely absorbed from the gastrointestinal metabolism which is saturable ($t_{1/2}$ is increased with the dose) (Laurence *et al.* 1997).

Table 3. Frequency of chromosomal aberrations in primary spermatocytes of male mice induced after oral treatment with different doses of clarithromycin

Treatment	Mice killed after (h) [†]	% of chromosome aberrations $\pm$ S.E.	No. of metaphases with					
			X-Y univalent	Autosomal univalent	X-Y + A.u.	Frag. and/or Break	Chain	Treploidy
I. Control (non-treated)		3.4 $\pm$ 0.67	8	6	3	—	—	—
II. Positive control i.p. injection with mitomycin C 1 mg kg ⁻¹ b.wt.	24	21.0 $\pm$ 0.76**	47	38	10	2	3	5
III. Single treatment (clarithromycin)								
50 mg kg ⁻¹ b.wt.	24	5.2 $\pm$ 0.73	11	9	4	1	—	—
100 mg kg ⁻¹ b.wt.	24	6.0 $\pm$ 0.71*	16	15	1	2	2	5
200 mg kg ⁻¹ b.wt.	24	8.2 $\pm$ 0.86**	9	8	4	5	4	7
300 mg kg ⁻¹ b.wt.	24	9.8 $\pm$ 0.86**	18	13	3	7	4	4
IV. Repeated treatment clarithromycin 50 mg kg ⁻¹ b.wt. for 14 successive days	24	7.4 $\pm$ 0.93*	15	7	6	2	3	3

[†] Total number of scored metaphases was 500/group. * Significant at 0.05 level (*t*-test). ** Significant at 0.01 level (*t*-test).

Table 4. Frequency of sister chromatid exchanges (SCEs) in mice bone-marrow cells after oral treatment by gavage with different doses of clarithromycin

Treatments	Metaphases No.	SCEs No.	SCEs/cell Mean $\pm$ S.E.
I. Control (non-treated)	200	847	4.24 $\pm$ 0.44
II. Positive control i.p. injection with mitomycin C 1 mg kg ⁻¹ b.wt.	200	2615	13.07 $\pm$ 0.58**
III. Single treatment (clarithromycin)			
50 mg kg ⁻¹ b.wt.	200	1137	5.69 $\pm$ 0.15
100 mg kg ⁻¹ b.wt.	200	1457	7.29 $\pm$ 0.24*
200 mg kg ⁻¹ b.wt.	200	1768	8.84 $\pm$ 0.69**
300 mg kg ⁻¹ b.wt.	200	1997	9.99 $\pm$ 0.48**

* Significant at 0.05 level (*t*-test). ** Significant at 0.01 level (*t*-test).

In this study, the percentage of chromosome aberration in diakinesis–metaphase I increased by increasing both the dose and the time of treatment. Univalents were the most common types of abnormalities in mouse spermatocyte cells after treatment with clarithromycin. The number of sex univalent aberration type was higher than the autosomal univalent aberration type. Numerous mutagens were shown to increase the frequency of chromosomal univalency in mouse spermatocytes (Allen *et al.* 1986). It should be noticed that X and Y were more often separated than autosomes. This phenomenon has been observed in spermatocytes of mice treated with other chemical agents, *e.g.* uranyl fluoride (Hu and Zhu 1990).

SCEs have proved to be a high sensitive indicator for assessing suspected mutagens or carcinogens. SCEs can be precisely scored and readily induced in mammalian cells by various known mutagens and carcinogens at concentration far below cytotoxic and clastogenic levels (Latt 1974, Perry and Evans 1975).

A single oral treatment with the higher doses of clarithromycin caused a significant increase in SCEs frequency. The frequency of SCEs per cell was found to be 9.99 $\pm$ 0.48/cell ($P < 0.01$) after

oral treatment with $300 \text{ mg kg}^{-1} \text{ b.wt.}$ compared with $4.24 \pm 0.44/\text{cell}$ for the control *i.e.* higher than the two fold of the control value. This indicate that clarithromycin is a strong inducer of SCEs in mice bone-marrow cells. This has been described for other drugs Rifampicin (Aboul Ela 1995) and Paracetamol (Farghaly 2003).

The present results indicate that clarithromycin has cytogenetic effect in the different kinds of cells analyzed.

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