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The cytogenetic effect of the new asthma and allergy drug montelukast on albino mice: chromosomal studies

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ABSTRACT

Montelukast or Singulair has recently been introduced to treat asthma. Because it is apparently free of serious side-effects, the present work aimed to investigate its effects on chromosomes of bone marrow cells of mice. The percentage of structural chromosomal aberrations was highly elevated due to treatment with Montelukast. The aberrations increased successively with increasing the time and the dose of the therapy. Numerical chromosomal aberrations were also increased, and interstitial deletions of certain bands were detected in G/T-banded karyotypes of the treated samples. Montelukast appears to have potential genotoxicity in the somatic cells of mice *in vivo.*

KEYWORDS: Montelukast, chromosome aberrations, mice, bone marrow cells, G- banded karyotypes.

INTRODUCTION

Montelukast or Singulair is an anti-asthma drug belonging to the leukotriene antagonist family. It has been available in the United States since March 1998 and has received excellent acceptance by physicians, parents and patients (Kemp 2000).Many studies indicate that Montelukast demonstrates both anti-inflammatory and bronchodilatory properties (Lee *et al.* 2005), it exhibits additive effects when given with inhalent corticosteroids (Stey 2000).

The pharmacology, pharmacokinetics, clinical efficacy and adverse effects of Montelukast, its therapeutic role as a long-term medication and difficulties associated with the management of asthma have been reviewed by many specialists (e.g. Blake 1999; Jarvis & Markham 2000). Blake (1999) used all the available published reports of controlled clinical trials of Montelukast in adults and children with asthma to reach three main points. (1) It may be considered for use as first-line therapy in patients with mild persistent asthma, or for additional control in patients treated with inhaled corticosteroids. (2) It may be used in aspirin-sensitive asthmatic patients. (3) Chronic treatment with Montelukast can provide additional control of symptoms during exercise, but inhaled beta-2 agonists remain the first-line therapy prophylaxis and treatment.

The most common adverse effects of Montelukast in pediatric studies were headache, asthma, and respiratory tract infection at rates not significantly different from those with placebo. Montelukast has been associated with Churg-Strauss syndrome in a very small number of adults, described as a rare allergic granulomatous eosinophilic vasculitis characterized by late-onset asthma and upper air-ways disease including allergic rhinitis, sinusitis and systemic vasculitis (Wechsler *et al*. 1999; Hemelaers *et al.* 2006). The lack of major adverse effects, the palatability of the oral route administration and the once-daily regimen combine to make Montelukast a generally well-tolerated medication in children (Price 2000; Alsarra *et al.* 2005).

Due to the importance of Montelukast , and because it is free of serious side effects, it is desirable to assess its genotoxic effects. Here the bone marrow cells of mice were used

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as a model system. Chromosomal aberration analysis was used as a genotoxic endpoint. Reports concerning the cytogenetic effects of Montelukast on humans or animals are not available, so the present investigation seems to be the first report dealing with this problem.

MATERIALS AND METHODS

Four-weeks old Swiss albino mice (*Mus musculus*) were used in this study, supplied by Abbasia Farm of the Egyptian Organization for Vaccine and Biological Preparations. 60 mice were divided into 10 experimental groups each of 6 animals and allowed to acclimatize for at least 7 days prior to the study. They were fed with standard laboratory chaw and tap water.

The Montelukast was produced by Global Napi Pharmaceuticals (Egypt) in the form of film- coated chewable tablets of 5 and 10 mg. The human therapeutic dose is 5mg/d for children and 10 mg/d for adults (Storms *et al. 2001*). These doses were adjusted for mice according to Paget & Barnes (1964). Such that the dose for a 30 mg mouse = dose for human x 0.0026. By calculation the adjusted doses become 0.013mg/d and 0.026 mg/d. These two doses were given to groups of mice (4 groups of 6 mice per dose). Montelukast was dissolved in sterile distilled water just before use. The dissolved drug was administered to the animal via oral gavage for 7 consecutive days.

The mice were sacrificed 1, 2, 3 and 8 days post-treatment (n=6 in each case). In addition, another group of mice (6 animals) were administered distilled water as a negative control, and a positive control used mice receiving a single dose of 5 mg cyclophosphamide, a known cytotoxic and clastogenic agent. Metaphase chromosomes were prepared according to Rickart *et al. (*1989). Some of the prepared slides were stained for 15 minutes in 6 % Giemsa in phosphate buffer (pH 6.8). These slides were used for screening chromosomal aberrations. The other slides were subjected to the Trypsin-Giemsa banding technique, for banding analysis as recommended by Seabright (1971).

For each animal 50 metaphases were analyzed for chromosomal aberrations using the light microscope at 1000× magnification. Structural chromosomal aberrations, (chromatid and chromosomal breaks, chromatid and chromosomal gaps, deletions, fragments, chromatid exchanges, centric fusion and pulverization) were scored according to Savage (1975), and numerical chromosomal aberrations were also counted. In addition, mouse chromosomes were individually identified through the use of the Trypsin-Giemsa banding technique. Karyotypes were prepared according to the system suggested by Wurster (1972). G-band patterns of each mouse in the control and treated groups were analyzed in at least 3 karyotypes.

Statistical analysis was carried out according to one-way ANOVA to determine the test of significance. A 2-way ANOVA test was used, to test for significant differences amongst the different doses and sampling times.

RESULTS

Data of the resulting structural chromosomal aberrations (Fig. 1) following administration of Montelukast are given in Table I. At the 5 mg/d dose deletions, dicentric chromosomes, chromosomal gaps and centric fusion represent the most predominant types of aberrations. The highest readings of deletions, chromosomal gaps and centric fusion were recorded 3 days after treatment, whereas dicentric chromosomes, centric separation and chromatid exchanges were highest after 8 days. Breaks, rings and acentric fragments were also noted but at lower frequencies than other types of chromosomal damage. At the 10mg/d treatment, it is obvious that the most frequent types of aberrations are deletions and centric

separations. In addition, several metaphases with more than one sign of chromosomal aberrations were repeatedly detected. Chromosomal aberrations were highly elevated due to the treatment with Montelukast, starting from 24 h post-treatment and increasing successively with time of the therapy to reach a maximum after 8 days. The higher dose produced greater damage. The mean of chromosomal aberrations detected after 24 h of treatment with 5 or 10 mg increased significantly (1-way ANOVA: P<0.001).

Table I: The frequency of structural chromosomal damage after treatment with Montelukast.

Treatment	Time in days	Types of structural aberrations									Total	Mean ± S.D.	%
		Del.	Ac. F.	Cht. exch.	C. F.	C. S.	Dic.	Chrsal g.	B.	R.			
–ve control dist. Water	1	12	–	3	13	19	8	8	2	–	46	7.66 ± 4.22	15.33
+ve control 5 mg/kg Cp	1	52	39	27	20	42	24	92	39	7	300	50 ± 2.83	100
	1	60	12	15	32	56	38	32	7	10	262	43.67± 1.21**	87.33
5 mg/d MK	2	67	2	26	38	61	61	53	10	5	323	53.83± 1.94	107.7
	3	80	19	6	53	36	47	78	16	34	369	61.50± 2.45	123
	8	75	11	30	80	73	84	60	30	6	449	74.81± 1.48	149.4
	1	119	5	20	102	96	30	31	3	10	416	69.33± 3.27**	138.7
10 mg/d MK	2	103	4	42	56	55	64	80	1	7	423	70.50± 1.87	141.00
	3	131	2	58	52	73	67	60	12	7	470	78.30 ± 2.16	156.70
	8	164	2	57	48	78	82	90	4	9	538	89.60± 1.86	179.33

Del. = deletion, Ac.F. = acentric fragment, Cht.Exch. = chromatid exchange, C.F. =, centric fusion, C.S. = centric separation, Dic. = dicentric, Chrsal g = Chromosomal gap, B. = break, R. = ring.
NB: C.S. is not involved in the calculation. Cp = Cyclophosphamide.
** highly significant as detected by a-one-way ANOVA test (F=324.175, 468.124, P<0.001).

Using 2-way ANOVA showed that the differences between 5mg and 10 mg doses were highly significant, and changes due to time were also highly significant, (Table II). The effects of Montelukast therefore occurred in a dose- and time- dependent manner (Fig. 2). The changes between doses were larger than those due to time. The interaction was highly significant also, probably because changes with time had a steeper slope for the lower dose (Fig. 2).

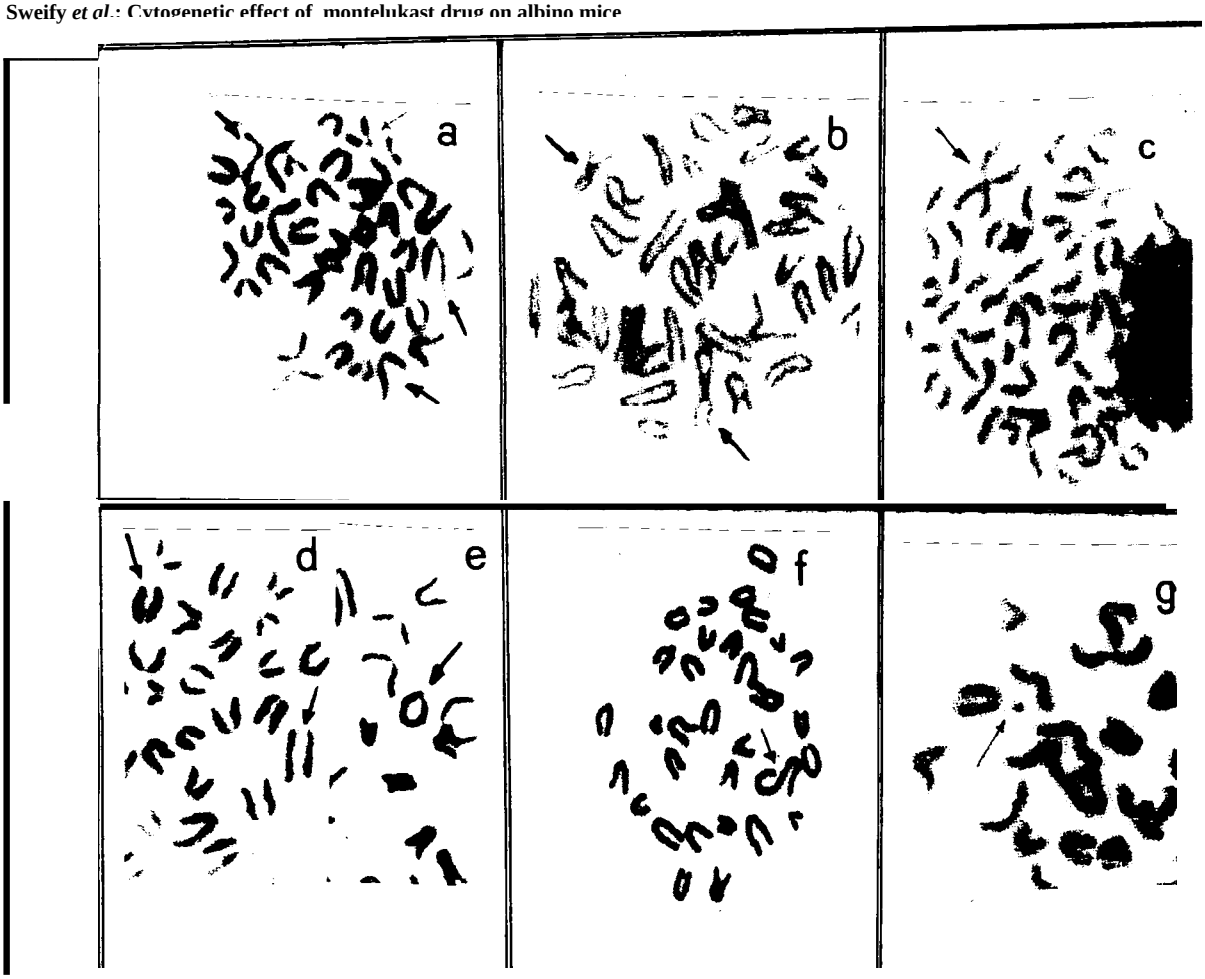

Fig. 1: Metaphases prepared from mice treated with Montelukast having a: deletion - b: dicentric - c : centric fusion - d: centric separation. e: ring - f: chromatid exchange - g: acentric fragment.

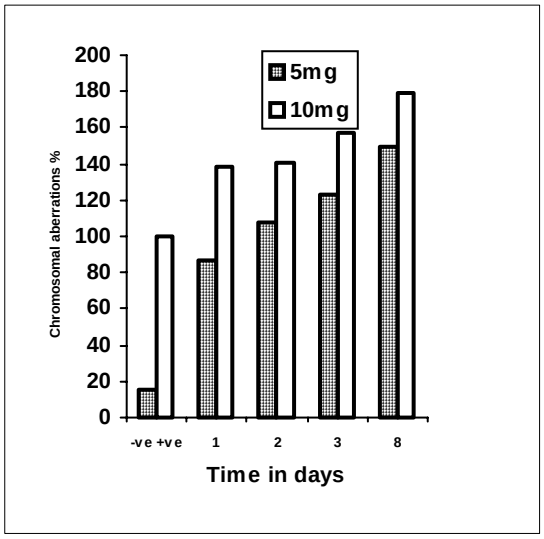

Fig. 2: The percentage of structural chromosomal aberrations following treatment with 5&10mg Montelukast & the accompanied controls

Table II: Two-way ANOVA test to observe significant differences amongst the various Treatment and sampling times

Sources of variation	D f	SS	Ms	F-ratio	Prob
Dose	1	4107.00	4107.00	944.138	<<0.001
Duration	3	4472.083	1490.694	342.688	<<0.001
Interaction	3	212.883	70.944	16.309	<<0.001
Error	40	174.000	4.350		
Total	47	8965.917			

Numerical aberrations were shown in the form of hypodiploidy and polyploidy (Table III, Fig. 3). Generally hypodiploidy was elevated throughout the time of the experiment. After 24 h, the difference was non- significant as calculated by 1-way ANOVA 2-way ANOVA showed that the changes due to doses were highly significant ($P<0.001$) and changes due to time were significant ($P<0.05$). The interaction was found to be non-significant ($P>0.5$). Polyploidy showed its highest scores 24 h post-treatment with the two tested doses of the drug (1-way ANOVA), followed by gradual decrease with time (Figs. 4 and 5). A 2-way ANOVA, revealed highly significant differences due to concentration of the drug and significant differences due to time. The interaction was also significant.

Table III: The frequency of numerical chromosomal aberrations in *Mus musculus* following treatment with Montelukast.

.		Types of aberrations / 300 cells					
Treatment	Time in	Hypodiploidy			Polyploidy		
	days	Total	Mean & %	S.D.	Total	Mean & %	S.D.
–ve control (distilled water)	1	22	3.66 7.33	1.86	7	1.16 2.33	1.16
+ve control 5 mg./kg Cp. (Cyclophos phamide).	1	21	3.5 7	1.37	8	1.3 2.66	1.03
	1	21	3.5 * 7	1.04	28	4.66 ** 9.33	1.75
5 mg/d MK (Montelukast).	2	42	4 14	2.36	22	3.6 7.33	1.86
	3	53	8.83 17	2.92	17	2.83 5.66	1.83
	8	34	5.66 11	2.16	11	1.83 3.66	1.83
	1	25	4.16 * 8.33	0.15	35	6 ** 11.66	1.78
10 mg/d MK (Montelukast)	2	32	5.33 10.66	1.03	21	3.5 7	0.83
	3	36	6 12	1.78	15	2.5 5	1.87
	8	23	3.83 6.77	1.72	14	2.16 5	1.94

* A-one-way ANOVA showed non-significant differences, ($F=0.026, 0.365$, $P > 0.5$).

** A-one-way ANOVA showed V. significant differences, ($F=12.758, 24{,}053$, $P < 0.001$).

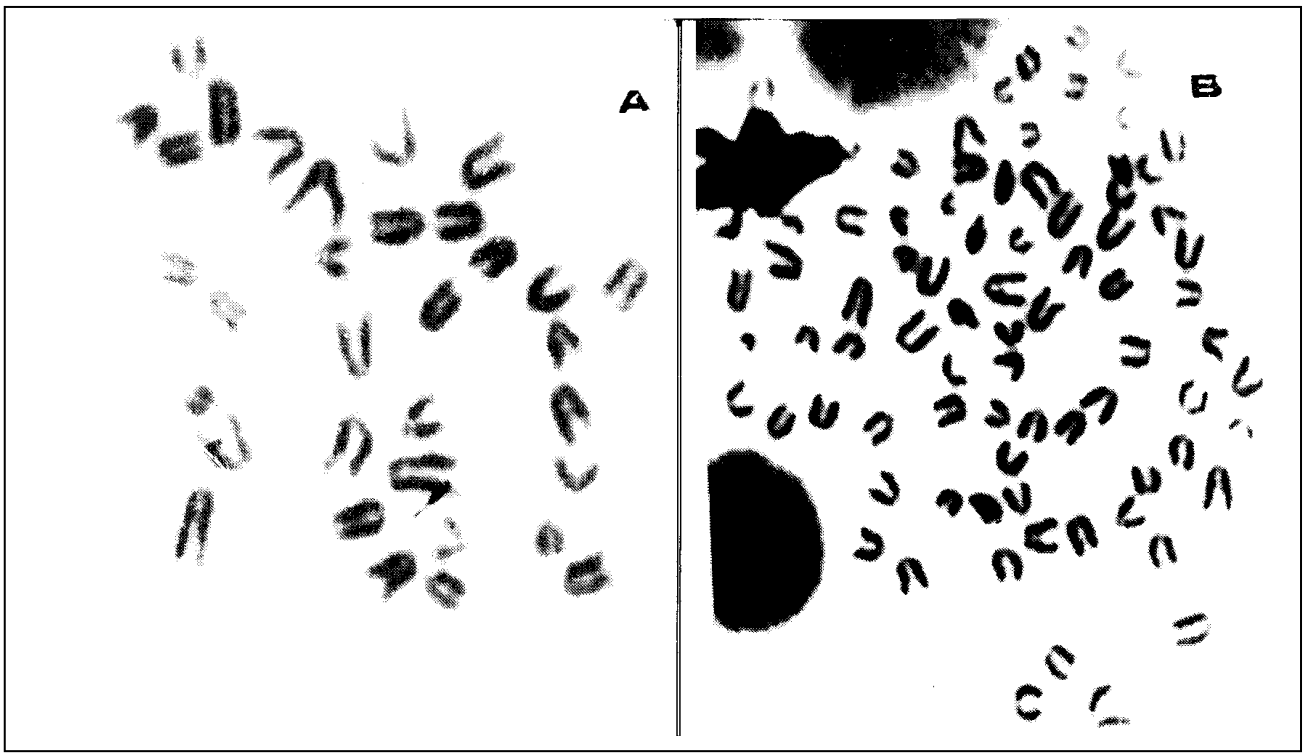

Fig. 3: A: Hypodiploid cell contains 33 chromosomes. B: Polyploid cell as a result of MK treatment.

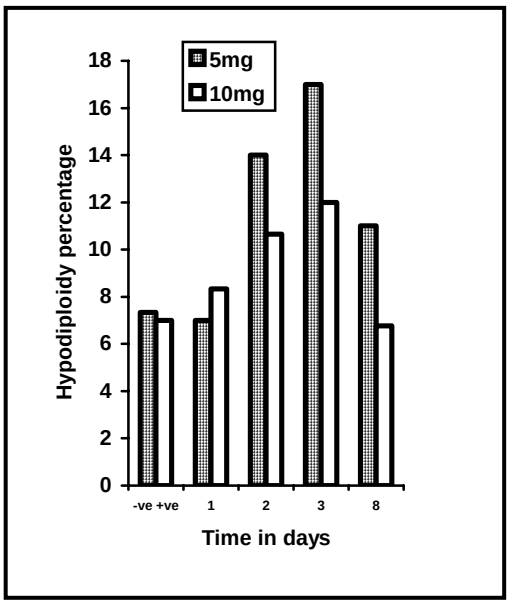

Fig. 4: The percentage of hypodiploidy in *Mus musculus* cells following treatment with 5 and 10mg MK

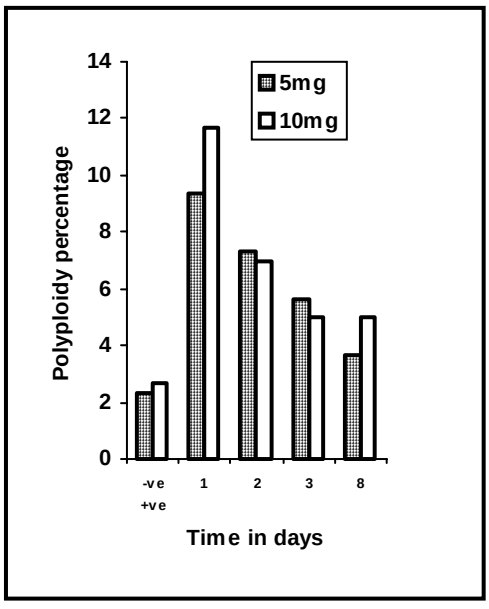

Fig. 5: The percentage of polyploidy in *Mus musculus* cells following treatment with 5 and 10mg MK

Due to the high percentage of chromosomal aberrations detected in samples after 8 days of the treatment, the cytogenetic analysis of G-banded chromosomes was only carried out in such samples. 18 banded karyotypes from mice treated with 5 or 10 mg Montelukast were

studied (Table IV). Interstitial deletions of certain bands were strictly determined in some chromosomes. In samples prepared after 5 mg therapy, the affected chromosomes were numbers 1, 3 and 6 (Fig. 6), whereas numbers 3, 4, 8, 12, 14, 16 and 18 were affected in mice treated with 10 mg. (Fig. 7).

Table IV: Data of interstitial deletions recorded in chromosomes of mice 8 days post-treatment with Montelukast.

		Interstitial deletions in chromosome no.								
Treatment	Results	1	3	4	6	8	12	14	16	18
5 mg/d	Total no.	7	6	–	8	m–	–	–	–	–
	%	38.89	33.33	–	44.44	–	–	–	–	–
10 mg/d	Total no.	–	7	9	–	3	5	6	6	4
	%	–	38.89	50	–	16.67	27.78	33.33	33.33	22.22

Number of animals/dose = 6; Number of karyotypes examined/dose =18.

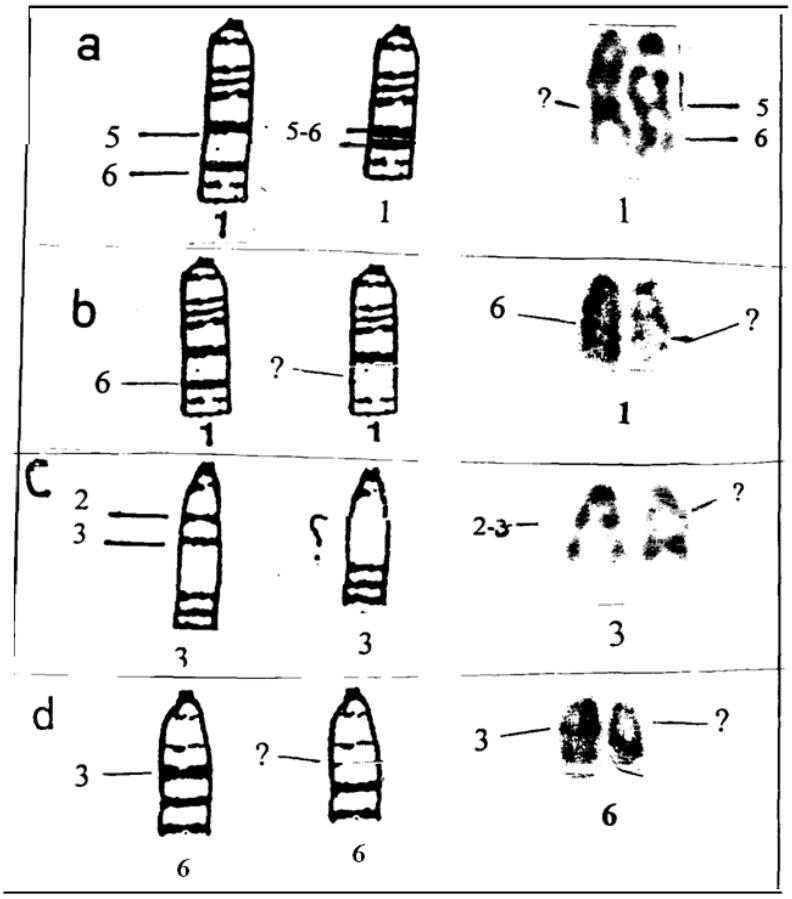

Fig. 6: Diagrammatic representation and partial karyotypes show interstitial deletions in chromosomes no, 1, 3 and 6. a: del of interband which lies between bands 5 & 6. b: del (1 q6) - c: del (3q2.3) - d: del (6q3).

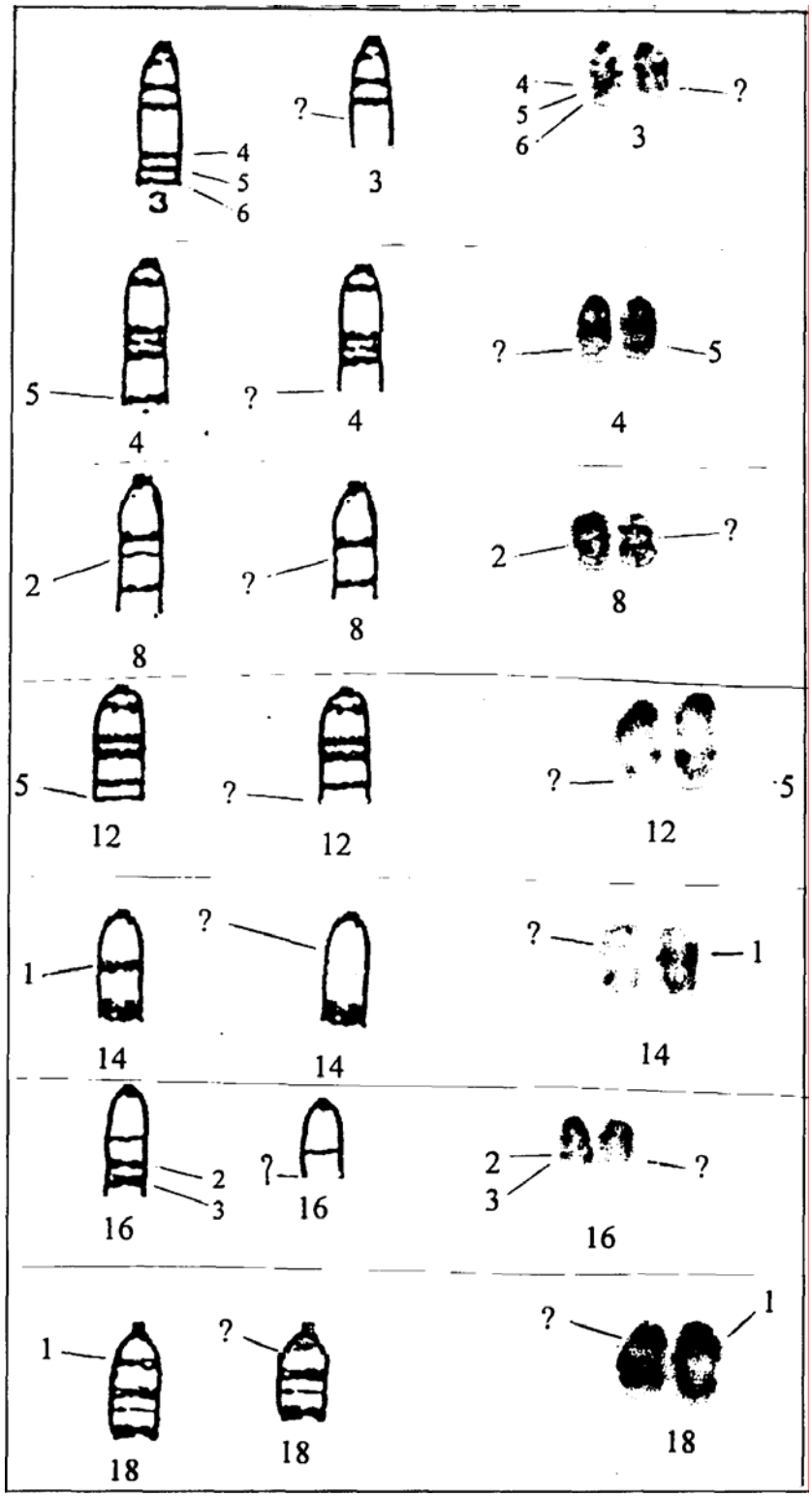

Fig. 7: Partial karyotypes and idiograms of normal and abnormal chromosomes no.3,4,8,12,14,16 & 18 prepared from samples treated with 10 mg MK 8 days post-treatment.
*Notice, del (3q 4.5.6, 4q5, 8q2, 12q5, 14q1, 16q2.3 and 18q1).

DISCUSSION

Chromosomal aberration assays are considered to be very sensitive end points for recognizing the genotoxic effects induced by chemicals (WHO, 1985). The present data showed very significant increases in the frequency of chromosomal damage in mice treated with Montelukast at all doses and all durations tested. The use of different sampling durations has been considered important (Hayashi & Sofuni 1994), and therefore the present analysis was carried out at four different times. Elevation of chromosomal damage was observed after only 24 h. This result is consistent with that of Mueller &Young (1998), who proposed that most chemicals gave a positive response after 24 h, roughly equivalent to the normal length of the cell cycle. In the present work, the percentage of chromosomal aberrations increased successively by increasing the duration of the therapy, with the maximum value after 8 days. This continuity in the elevation of damage indicated the prolonged action of the drug or its metabolites on mice bone- marrow cells. Styles *et al.* (1994) also demonstrated the prolonged action of tamoxifen metabolites on bone-marrow cells of mice. In mice treated with 10 mg/d Montelukast, the yield of chromosomal aberrations was very significantly higher than that induced by the lower dose, and therefore we conclude that the increase in structural chromosomal aberrations is time- and dose-related. Furthermore, the induced abnormalities were mainly of the chromatid type, suggesting damage at late S or G_2 phase (Sarker *et al.* 1993). Sorsa *et al.* (1992) stated that most chemical clastogens induce only chromatid aberrations, because the aberrations result from DNA synthesis on a damaged DNA template in the S period of the cell cycle.

At a dose of 5 mg and after 3 days, the most abundant types of structural chromosomal aberrations were stable (deletion and centric fusion), whereas dicentric, centric separation and chromatid exchange (unstable types) were the most predominant types after 8 days. At the 10 mg dose, deletion and centric separation (stable and unstable) were frequently observed. These observations suggest the greater potential of higher doses and longer duration of the drug. In addition, Montelukast enhanced the formation of numerical chromosomal aberrations (hypodiploidy or polyploidy). As recommended by Hoffmann (1996), it is useful to separate the induction of structural chromosomal aberration from the induction of numerical chromosomal aberration, because the principle target for the induction of the former is DNA, whereas the targets of the latter are often components of mitotic or meiotic apparatus such as spindle fibers (Ilies, 2002)**.** Agents that damage polymerization of tubulin, the principal protein of spindle fibers, cause hyperdiploidy; the chromosomes duplicate normally in interphase, but the chromatids do not separate into the daughter cells (Vogel & Motulsky 1997). Agents that damage kinetochores can cause aneuploidy, involving the gain or loss of one or a few chromosomes (Kingston 1998).

In samples prepared from animals treated with 5 mg, interstitial deletions were observed in chromosome 1, 3 and 6 in 7, 6 and 8 karyotypes respectively. Many affected chromosomes were identified in samples treated with 10 mg, with interstitial deletions found in 3q3q4, q6, 4q1q5, 8q2, 12q4q5, 14q1, 16q2 and 18q1. The analysis of banded chromosomes is more labor-intensive than standard cytogenetic analysis (Hoffmann, 1996) but gives more detail. Although the number of the examined banded karyotypes was limited, interstitial deletions were determined in several banded chromosomes, suggesting that such bands may be found near a locus responsible for regulating leukotriene receptors. Therefore, treatment with Montelukast (a leukotriene receptor antagonist) may affect such regions and lead to the absence of the mentioned bands.

Literature relating to the pharmacologic effects and therapeutic uses of Montelukast is abundant, but data concerning mutagenic assays are lacking from animal models or humans. Asthma is known to be a complex polygenic disorder with marked gene-environment

interaction. Several genes related to bronchial hyper-responsiveness (BHR) and atopy have been identified. Postma *et al.* (1995) found that a gene governing BHR is located near a major locus that regulates serum immunoglobulin (IgE) levels on chromosome 5q. Sandford *et al.* (1996) found that a cluster of cytokine gene on chromosome 5q31q33 are linked to atopy and BHR. Furthermore, genome scans have identified other regions of interest on chromosomes 2q, 4, 5p, 6, 7, 11p, 13, 16, 17, 19q and 21q (Abramson & Harrap 1998). The present results indicate genotoxic potential of the drug in somatic cells of mouse *in vivo*. Further investigations should be conducted to substantiate the mutagenic effect of Montelukast on human cells.

REFERENCES

Abramson MJ & Harrap SB (1998) The new asthma genetics and its implications for public health. *Public Health Res.* 26 (2): 127-144.

Alsarra I, Khalil NY, Sultan M Al-Ashban R & Belal F (2005) Spectrofluorometric determination of montelukast in dosage forms and spiked human plasma. *Phamazie.* 60(11): 823-826.

Blake KV (1999) Montelukast : data from clinical trials in the management of asthma. *Ann. Pharmacother*. 33 (12) : 1299-1313.

Hayashi M & Sofuni T (1994) The micronucleus assay with rodent peripheral blood and acridine orange supravital staining. *Chromosomal Alterations* 203-213.

Hemelaers I, Henket M, Sele J, Bureau F & Louis R (2006) Cysteinyl-leukotrienes contribute to sputum eosinophil chemotactic activity in asthmatics. *Allergy* 61(1): 136-139.

Hoffmann GR (1996) Genetic Toxicology in Casarett & Doull: *Toxicology : The basic science of poisons.* Klaassen ed. McGraw-Hill New York, 5th ed. pp. 269-300.

Ilies RK (2002) Cell and molecular biology, and genetic disorder. in P. Kumar & MWB Clark (eds) *Clinical Medicine* 5th edn. Saunders, U.K. London, p. 162.

Jarvis B & Markham A (2000) Montelukast : a review of its therapeutic potential in persistent asthma. *Drugs* 59 (4) : 891-928.

Kemp JP (2000) Role of leukotriene receptor antagonist in pediatric asthma . *Pediatr. Pulmonol.* 30 (2): 177-182.

Kingston HM (1998) *ABC of Clinical Genetics.* Revised Second Edition. BMJ Publishing group p. 22.

Lee MY, Lai YS, Yang KD, Chen CJ & Hung CH. (2005) Effect of Montelukast on symptoms and eNO in children with mild to moderate asthma. *Pediatr Int.* 47(6): 622-626.

Mueller RF & Young ID (1998) *Emery's elements of medical genetics*. Tenth Edition, Churchill-Livingstone, p. 44.

Paget GE & Barnes JM (1964) Evaluation of drug activities. In P*harmacometries* 1st ed. Laurence, Acad. Press, London and New York.

Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CIM, Meyers DA & Levitt RC (1995) Genetic susceptibility to asthma-bronchial hyper-responsiveness coinherited with a major gene for atopy. *N. Engl. J. Med.* 333 (14): 894-900.

Price D (2000) Tolerability of Montelukast. *Drugs* 59 (1): 35-45.

Rickart EA, Heaney LR & Rosenfled MJ (1989) Chromosomes of ten species of Philippine fruit bats *Chirpotera Pteropodidae* . *Proc. Biol. Soc. Wash.* 102 (2): 520-531.

Sandford A, Weir T & Pare P (1996) The genetics of asthma. *Am. J. Respir. Crit. Care Med.* 153: 1749-1765.

Sarker D, Sharma A & Talukder G (1993) Differential protection of chlorophyllin against clastogenic effects of chromium and chlordane in mouse bone marrow *in vivo*. *Mut. Res.* 301: 33-38.

Savage JRK (1975) Classification and relationship of induced chromosomal structural changes 3. *J. Med. Genet.* 12 : 103-122.

Seabright M (1971) A rapid banding technique for human chromosomes. *Lancet II*: 971-972.

Sorsa M, Wilbourn J & Vainio H (1992) Human cytogenetic damage as a predictor of cancer risk. in Vainio H, Mogea BN, McGregor DB & McMichael AJ (eds): Mechanisms of carcinogenesis in *Risk Identification*, Lyons: International Agency for Research on Cancer. pp. 543-554.

Stey C (2000) Leukotriene antagonists - do they have a value ? *Schweiz. Rundsch. Med. Prax.* 89 (14): 586-590.

Storms W, Michele TM, Knorr B, Noonan G, Shapiro G, Zhang J, Shingo S & Reiss TF (2001) Clinical safety and tolerability of Montelukast, a leukotriene receptor antagonist, in controlled clinical trials in patients aged > or = 6 years . *Clin Exp. Allergy* 31(1): 77-87.

Styles JA, Davies A, Lim CK, deMattis F & Smith LL (1994) Genotoxicity of tamoxifen, tamoxifen epoxide and toremifene in human lymphoblastoid cells containing human cytochrome p450S. *Carcinogenesis* 15: 5-9.

Vogel F & Motwcky AG (1997) *Human genetics: problems and approaches*.. 3rd ed. Springer-Verlag, Berlin. p115

Wechsler ME, Pauwels R & Drazen JM (1999) Leukotriene modifiers and Churg-Strauss syndrome: adverse effect or response to corticosteroid withdrawal . *Drug Saf*. 21(4): 241-251.

Winer BJ, Brown DR & Michels KM (1991) *Statistical principles in experimental design* 3rd ed. McGraw-Hill Inc., New York. pp 172-197

World Health Organization (1985) Environmental health criteria 51: guide to short-term tests for detecting mutagenic and carcinogenic chemicals. WHO, Geneva, pp.114.

Wurster DH (1972) Mouse chromosomes identified by Trypsin-Giemsa (T-G) banding . *Cytogenetics* 11: 379-387.

الملخص العربى

التأثير الوراثى الخلوى لعقـار الربـو والحساسـية الجديد (مونت ليوكاست) على الفئران البيضاء: دراسات كروموسومية

كريمة محمد سويفى – إيمان عبد المنعم درويش – سحر عبد الرازق مسلم
كلية البنات للأداب والعلوم والتربية جامعة عين شمس - القاهرة

العقار مونت ليوكاست صوديوم المضاد لمستقبلات الليكوترين يوصف لتهدئة أعراض أزمات الربو وكذلك لعلاج حالات الربو المزمنة والتى تشمل : منع المعاناة من الأعراض التى تظهر خلال النهار وأثناء الليل ، علاج حالات الحساسية من تناول الأسبرين ، فانه يمنع أعراض انقباض الشعب التى تظهر عقب التمرينات الرياضية البسيطة . وهو دواء مفيد لتقليل الحاجة للكورتيزون في أزمات الربو المستمرة . ونظرا لأهمية هذا الدواء ولعدم الشكوى من ظهور أية أعراض جانبية خطيرة قد تظهر أثناء الاستعمال ،يهدف البحث الحالى الى إلقاء الضوء على الضرر الذى قد يحدثه عقار مونت ليوكاست على المحتوى الوراثى الخلوى لخلايا الفئران . وقد استخدم مونت ليوكاست بجرعتين (٥ ، ١٠ مجم/يوم) لمدة ٨ أيام متتالية ، وتم تحضير كروموسومات المرحلـة الاستوائية من خلايا نخاع العظم بعد الأيام ١ ، ٢ ، ٣ ، ٨ ارتفعت نسبة التغيرات الكروموسومية في خلايا الحيوانات المعالجة وذلك في كل أزمنة التجربة وبالجرعتين المستخدمتين، بدأ الارتفاع بعد ٢٤ ساعة وبلغت أقصاها بعد اليوم الثامن وبمقارنة تأثير الجرعتين المستخدمتين تبين أن النتائج تناسبت طرديا مع تركيز الجرعة من جهة ومع زمن التجربة من جهة أخرى . و قد ارتفعت نسبة التغيرات العددية فى كل العينات وانخفضت بعد اليوم الثامن .أمكن من خلال فحص وتحليل الهيئة الكروموسومية المجهزة بتقنية الحزم ملاحظة انتقاصـات بينية فى حزم معينة لكل من الكروموسومات أرقام ١ ، ٢ ، ٣ ، ٤ ، ٦ ، ٨ ، ١٢ ، ١٤ ، ١٦ و ١٨ . مما سبق يتضح التأثير المطفر الواضح للعقار المستخدم على كروموسومات الخلايا الجسدية للفأر الأبيض لذلك يجب الحذر عند استخدامه على المستوى البشرى.