Saudi Pharmaceutical Journal (2011) 19, 215–220

King Saud University

Saudi Pharmaceutical Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Effects of Montelukast on free radical production in whole blood and isolated human polymorphonuclear neutrophils (PMNs) in asthmatic children

Muslim M. Al Saadi [a,*], Sultan Ayoub Meo [b], Ali Mustafa [c], Ahmed Shafi [d], Ali S. Al Tuwajri [b]

[a] *Department of Paediatrics, College of Medicine, King Saud University, Riyadh, Saudi Arabia*
[b] *Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia*
[c] *Department of Pharmacology, College of Medicine, King Saud University, Riyadh, Saudi Arabia*
[d] *Family and Community Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia*

Received 4 March 2011; accepted 16 June 2011
Available online 25 June 2011

KEYWORDS

Montelukast;
Antioxidant;
Free radicals production;
Bronchial asthma

Abstract Montelukast is a highly selective leukotriene-receptor antagonist (LTRA). It is widely used in the treatment of bronchial asthma, primarily as an adjunct to corticosteroids. Reactive oxygen species (ROSs) play an important role in the pathogenesis of asthma and oxidative stress contributing to the initiation and worsening of inflammatory respiratory disorders, such as asthma. Antioxidant drugs may have a role in minimizing or preventing damage in asthmatic children. The aim of this study was to assess the antioxidant effect of montelukast on the production of free radicals in the whole blood and polymorphonuclear neutrophils (PMNs) in asthmatic children. A group of 48 (38 males and 10 females), apparently healthy asthmatic children were recruited with ages ranging between 6 and 14 years. In asthmatic children, base line (premedication) and post medication free radicals activity in the whole blood and polymorphonuclear neutrophils (PMNs) was

* Corresponding author. Address: Department of Pediatrics (39) and College of Medicine & King Khalid University Hospital, King Saud University, P.O. Box 2925, Riyadh 11461, Saudi Arabia. Tel.: +966 1 4670878; fax: +966 1 14672650.
E-mail address: alsaadi@ksu.edu.sa (M.M. Al Saadi).

Peer review under responsibility of King Saud University.
doi:10.1016/j.jsps.2011.06.002

Production and hosting by Elsevier

determined by measuring chemiluminescence (CL) response through chemiluminescence luminometer. Free radical productions were significantly decreased in the whole blood, when stimulated with Phorbol Myristate Acetate ($p < 0.04$) and Opsonised Zymosan ($p < 0.05$). The free radicals were also significantly decreased in isolated polymorphonuclear neutrophils (PMNs) when stimulated with Opsonised Zymosan ($p < 0.05$) after the post medication treatment of montelukast in asthmatic children. Montelukast decreased the reactive oxygen species production, both in the whole blood as well as isolated PMNs in asthmatic children.

1. Introduction

Montelukast is highly selective antagonist of cysteinyl leukotriene (CysLT) receptors. It is commonly used in the treatment of bronchial asthma (Anonymous, 2004; Currie et al., 2005; Diamant and van der Molen, 2005; Riccioni et al., 2007; Al-Saadi, 2007). It has both anti-inflammatory as well as bronchodilator effects (Bousquet et al., 2009). Montelukast is an attractive drug, because it is available in an oral preparation form, which is easy to administer in children; and once-daily dosing is practically suitable to achieve greater compliance. It has a wide therapeutic window with low toxicity at therapeutic concentrations, appears safe and well tolerated by the patients (Cantrell and Farson-Collier, 2004). Montelukast has been found to reduce the symptoms of cough and wheeze, following bronchiolitis in infancy and has become popular in the treatment of bronchial asthma (Bisgaard et al., 2003).

The therapeutic effect of montelukast is achieved through antagonism of CysLT-mediated bronchoconstriction, increased vascular permeability and mucus secretion, following release of these mediators, mainly from monocytes/macrophages, eosinophils, mast cells and basophils. It also acts through the anti-inflammatory actions targeting type 2 helper $CD4^+$ T-lymphocytes (Peters-Golden and Henderson, 2007). Unlike corticosteroids, montelukast has been reported to modulate airway remodelling in patients with chronic asthma (Muz et al., 2006; Henderson et al. 2006).

Bronchial asthma is the most common chronic inflammatory disease of childhood and its prevalence has substantially increased worldwide, particularly in pre-school children. It is associated with significant morbidity and economic burden (Harmanci, 2007). In bronchial asthma, mast cells, eosinophils and lymphocytes are involved in the expression of an inflammatory process in response to triggering agents in susceptible individuals. It is well-known that oxygen-derived free radicals contribute to the inflammation in asthma. When PMNs are stimulated in vivo, their phoslipase is activated and leukotrienes are liberated through the lipoxygenase pathway. The leukotrienes contribute to oxidative stress and the initiation and worsening of asthma. They actually produce typical presentation of the late phase of an asthma attack. The pharmacological indications of Montelukast have been well established, but its clinical significance is still lacking in certain areas, such as its role in free radicals production. Therefore, the aim of the present study was to investigate the possible antioxidant effect of montelukast in asthmatic children, using in vitro chemiluminescence technique in the whole blood and isolated ploymorphonuclear neutrophils.

2. Subjects and methods

The present study was conducted in the Departments of Pediatrics and Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia from the year 2006–2008. The Ethics Committee, College of Medicine Research Centre (CMRC) approved the study.

2.1. Subjects

In this study, a group of 48 apparently healthy, volunteers, asthmatic children (38 males and 10 females) with ages ranging between 6 and 14 years, were recruited from the various hospitals in Riyadh. The mean duration of the disease was 4.55 ± 0.44 years. The participating children or their parents completed a questionnaire, which included anthropometric data and a consent form.

2.2. Exclusion criteria

Children with gross anemia, known history of diabetes mellitus, autoimmune diseases and malignancy were excluded. Children with current or previous history of cigarette smoking were also excluded from the study.

2.3. Methods

Blood (5–6 ml) was collected from each child by venipuncture in a disposable syringe. Blood was heparinized (10 IU/ml Fischer Scientific Co., NJ) and the tubes containing this heparanized blood were immediately kept in ice, to prevent any reactions to the time of measurement of free radicals activity by measuring the chemiluminescence (CL) response. Each specimen bottle was labeled with the subject identification code number.

2.4. Chemiluminescence assay

LKB-Wallac 125l Luminometer is a bench top, microcomputer controlled luminescence-measuring instrument capable of providing instantaneous monitoring of reactive oxygen species events. This Luminometer is attached to a display unit and printer. Disposable polystyrene cuvettes suitable for the Luminometer and micropipettes with disposable tips for dispensing 100–1000 µ1 volumes were used.

2.5. Reagents: preparation of luminol

Liminol (LKB-Wallac 1234–216) was dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 1.77 mg/ml. It

was further diluted in phosphate-buffered saline (PBS) to 17.7 μg/ml prior to use .

2.6. Preparation of phorbol myristate acetate (PMA solution)

PMA was dissolved in DMSO to give a stock solution of 2 mg/ml. The solution was stored at 20 °C until used. It was further diluted with phosphate buttered saline (PBS) to 20 μg/ml, prior to use.

2.7. Phosphate-buffered saline solution (PBS)

It was made up in distilled water according to the following composition NaCl 0.14 M, KCL 2.7 mM, Na_2HPO_2 mM, KH_2PO_4 1.5 mM, $CaCl_2$ 0.9 mM, and $MgCl_2$ 0.49 mM.

2.8. Polymorphonuclear neutrophils (PMN) separation

PMNs were separated by using Neutrophils isolation medium (NIM) obtained from Cardinal Associates Inc, Santa Fe, NM 87502. In a 15 ml tube, 5–6 ml of heparinized blood was layered over 4 ml of NIM and then centrifuged at 400*g* for 30 min, at room temperature. After centrifugation, the blood was separated into layers according to the weight of the cells. Carefully, the leukocyte-rich plasma (appears as pinkish ring) was removed with a Pasteur pipette and transferred to a 15 ml conical centrifuge tube. The tube was filled with PBS and centrifuged at 350*g* for 10 min in a Heraeus (Model GmbH, Osterode). The residual erythrocytes were lysed in 2 ml of lysing buffer (E-lyse) obtained from Cardinal Associate Inc. Santa Fe, NM 87502, which was vortexed to resuspend the pellets and then centrifuged at 250*g* for 10 min. The supernatant was discarded and the sediment was suspended in 1 Ml of 5% FCS. The cells were counted and adjusted to 5 million/ml.

2.9. Isolated PMNS assay

The cuvette contents were mixed gently and conveyed to the measurement position. The light emission was recorded in mV. The assay temperature was kept constant at 37.0 °C. Each sample was measured over a period of 30 min; the results were plotted using a computer in a graph with *Y*-axis representing the light intensity in mV and *X*-axis representing the time in minutes.

2.10. Antileukotriene (Montelukast) in vitro-study

Montelukast was purchased from Sigma Chemical Company, St. Louis MO, USA and the drug was supplied as a pure and authentic powder. To each 2 mg of the 6 μl of 1 N, sodium hydroxide was added until the mixture appeared pasty. The 100 μl of polyethylene glycol (400 MWT) were added. This paste-like material was warmed at low temperature, until a clear solution was obtained. This was made up to the required volume of 2 ml by adding PBS (pH 7.4). The PBS was added in increasing amount, to avoid precipitation of the compound. This stock solution was further diluted to the required concentration (range 1.25–60 μg/ml).

2.11. Effects of different concentrations on ROS generation from PMNS

The effect of Montelukast on the generation of ROS, as assessed by CL was determined by addition of different concentrations of the drug to isolated PMNs that were stimulated with PMA.

2.12. Effect of incubation time on ROS generation

The effect of Montelukast (5 and 10 μg) on ROS generation was tested at different time intervals: 0, 10, 30, and 60 min after incubation at 37 °C.

2.13. Reversibility of the effect of Montelukast

In order to investigate whether Montelukast would have a permanent effect on PMNs or not, two different concentrations of 30 and 60 μg were incubated for 45 min with isolated PMNs and washed thereafter several times with PBS. The CL of unwashed and washed samples in the presence of Montelukast was recorded.

2.14. Statistical analysis

The data were analyzed by using a SPSS Pc+ version 16.0 Statistical program. The descriptive statistics, median, range and inter quartile range values were used to describe the outcome variables, due to the skewed distribution of outcome variables. The comparison of pre and post values of outcome variables was carried out by applying a non parametric Wilcoxon Signed Ranks Test. A p-value of < 0.05 was considered as statistically significant.

3. Results

In this study on asthmatic children, base line (premedication) and post medication free radicals activity in the whole blood and polymorphonuclear neutrophils (PMNs) was determined

Table 1 Effects of Montelukast on free radical production in the whole blood (WB), stimulated with PMA and OPZ in asthmatic children.

Parameters	Asthmatic children premedication ($n = 48$)	Asthmatic children post medication ($n = 48$)	p-Value
PMA(CL-mV)			
Median	14.40	10.15	0.04
Range	47.30	31.69	
Interquartile range	14.20	13.15	
OPZ(CL-mV)			
Median	24	21.75	0.05
Range	100.60	213.80	
Interquartile range	39.92	22.25	

CL = Chemiluminescence response, PMNs = Polymorphonuclear Neutrophils; OPZ = Opsonised Zymosan; PMA = Phorbol Myristate acetate. PMN concentration = 5×10^6 cells/ml; OPZ concentration = 1.25 mg/ml; Luminol concentration = 10^{-4} M. Values are expressed as median, range, and interquartile range.

Table 2 Effects of Montelukast on free radical production in isolated polymorphonuclear neutrophils PMNs, stimulated with PMA and OPZ in asthmatic children.

Parameters	Asthmatic children premedication ($n = 48$)	Asthmatic children post medication ($n = 48$)	p-Value
PMA (CL-mV)			
Median	398.55	245.35	0.08
Range	1494	1363	
Interquartile range	280.80	244.30	
OPZ (CL-mV)			
Median	1517.45	1171	0.04
Range	3551.70	4002.60	
Interquartile range	1144.37	1913.20	

CL = Chemiluminescence response, PMNs = Polymorphonuclear Neutrophils; OPZ = Opsonised Zymosan; PMA = Phorbol Myristate acetate. PMN concentration = 5×10^6 cells/ml; OPZ concentration = 1.25 mg/ml; Luminol concentration = 10^{-4} M. Values are expressed as median, range, and interquartile range.

by measuring chemiluminescence (CL) response through chemiluminescence luminometer.

Table 1 shows the effects of Montelukast on free radical production in the whole blood, (WB) stimulated with phorbol myristate acetate (PMA) and opsonised zymosan (OPZ) in asthmatic children. The median, range, and Interquartile range values were obtained from the whole blood in asthmatic children. There was a significant decline in free radical production in the whole blood (WB) stimulated with phorbol myristate acetate (PMA) ($p < 0.04$) and opsonised zymosan (OPZ) ($p < 0.05$) in asthmatic children before and after the treatment with montelukast.

Table 2 demonstrates the effects of montelukast on free radical production in isolated Polymorphonuclear neutrophils (PMNs), stimulated with phorbol myristate acetate (PMA) and opsonised zymosan (OPZ) in asthmatic children. The median, range, and interquartile range values were measured before and after the medication of montelukast. There was a significant decrease in free radical productions in isolated PMNs when stimulated with OPZ ($p < 0.043$) in asthmatic children. However, no significant difference was achieved when stimulated with PMA ($p = 0.08$).

4. Discussion

Montelukast is a selective and orally active leukotriene receptor antagonist, that inhibits the cysteinyl leukotriene $CysLT_1$ receptor. It appears to be useful for the preschool age children, when there is coexisting allergic rhinitis (Van Adelsberg et al., 2003), and may also reduce the symptoms, when used as a short course in intermittent asthma. It has a very narrow spectrum of action in comparison to the broad anti-inflammatory action of the commonly used inhaled corticosteroids. It blocks the action of cysteinyl leukotrienes, which catalyze the inflammatory cascade from eosinophils, mast cells and alveolar macrophages (Bisgaard et al., 2005). This reduces the main characteristics of asthma, such as airflow obstruction, mucus hypersecretion, mucosal oedema and desquamation, bronchoconstriction, bronchial hyperresponsiveness and eosinophil accumulation (Smith, 2007). Many drugs are used in the treatment of asthma in children, but in the present study, the objective was to find out a suitable drug which can also reduce the reactive oxygen species (ROS) production in asthmatic children. This is because the ROS play an important role in the pathogenesis of asthma and oxidative stress contributes to the initiation and worsening of inflammatory respiratory disorders, which include asthma. According to an earlier study, antioxidant drugs may have a role in reducing or preventing damage in asthmatic patients (Al-Zamil et al., 2005).

The free radicals and reactive oxygen molecules are generated by activated neutrophils, monocytes and other cells during inflammatory processes. In acute inflammation, activated polymorphonuclear leukocytes release lysosomal hydrolytic enzymes, lipid mediators, and reactive oxygen species that may damage the surrounding viable tissues (Noiri et al., 2000). Tugtepe et al. (2007) demonstrated that montelukast attenuated neutrophil recruitment and promoted the resolution of inflammation by antagonizing the effects of leukotrienes, which are potent stimuli for leukocyte infiltration. It has been shown that montelukast acts through the inhibition of neutrophils in several organs targeted by various inflammatory challenges (Sener et al., 2006). The results of the present study suggest inhibitory effect of the montelukast on reactive oxygen species (ROS) production in the whole blood (WB) and isolated PMNs, when stimulated with phorbol myristate acetate (PMA) and opsonised zymosan (OPZ) in asthmatic children.

Biber et al. (2009) reported that montelukast has beneficial effects against the traumatic brain injury induced oxidative stress in Sprague Dawley rats, suggesting that montelukast, not only decreases the reactive oxygen species in respiratory system, but it is also effective against the traumatic brain injury induced oxidative stress.

Anderson et al. (2009) demonstrated the effects of montelukast on the generation of ROS, using the lucigenin and luminal enhanced CL procedures. They showed that, treatment of the cells with montelukast resulted in a dose-related inhibition of the generation of ROS, which was evident in both procedures and which achieved statistical significance at the concentrations of 0.5 μmol/L (lucigenin, $P < 0.001$) or 1 $\mu mol/L^{-1}$ (luminol, $P < 0.01$). Moreover, they found a maximal inhibition at 2 $\mu mol/L^{-1}$ montelukast, resulting in 70% and 60% inhibition of the generation of ROS with lucigenin and luminal enhanced CL procedures, respectively.

In the present study, significant inhibition of ROS production was observed at the lowest concentration of the drug used without incubation. In this study, montelukast was associated with more inhibition of PMA-stimulated ROS production in the whole blood compared with that induced by OPZ as shown in Table 1. Montelukast was also associated with inhibition of OPZ-stimulated ROS production in isolated PMNs with that induced by PMA. This finding agrees with the earlier reports which concluded that, depending on the receptor or combination of receptors, activated different signal transduction pathways might be involved in respiratory burst (Della Bianca et al., 1986). In addition, Raulf and Konig (1988) found that stimulation of human PMNs with OPZ resulted in time and dose dependent release of LTB4 and LTC4. On the other

hand, priming of PMNs with PMA has been shown to increase 5-1ipoxygenase kinase activity by translocation of 5-1ipoxygenase to the nucleus and by increasing its capacity for phosphorylation. Therefore, signal transduction pathways involved in the stimulation of PMA receptors will result in high 5-1ipoxygenase activity (Nagarajan et al., 2000). In the present study, free radical productions were significantly decreased in the whole blood, when stimulated with PMA ($p < 0.04$), OPZ ($p < 0.05$), and in isolated PMNs when stimulated with OPZ ($p < 0.05$) after post medication treatment of montelukast in asthmatic children. However, no statistically significant inhibition of free radical productions was observed in isolated PMNS when stimulated with PMA, after post medication treatment of montelukast in asthmatic children. This difference could be due to the increase in complement receptor type 3, which is involved in clearing OPZ on PMNs in asthmatic patients. Based on the findings of the present study, we speculate that the activation of PMNs in asthmatic children results in increased generation of LTs, which in turn, acts on PMN cys LT1R to stimulate the release of ROS. The results of this study also suggest that montelukast interferes with this autocrine activation of PMNs by blocking the cys LT receptors, thereby inhibiting ROS production.

The results of this study suggest that LT receptor antagonists represent a novel therapeutic approach with dual beneficial effects, as an antioxidant and an anti-inflammatory drug in the management of pediatric asthma. Furthermore, our findings might explain the mechanism of action of cys LTs in producing tissue inflammation, suggesting that LT receptor antagonists in addition to their use as anti-inflammatory drugs may also be useful as antioxidants.

The present study had some limitations, including the small sample size of participating asthmatic children, and the gender disproportion in the study subjects (38 males vs 10 males). The severity of asthma was not considered in this study. It is possible that patients with different severity of asthma react differently to montelukast. Furthermore, this was an in vitro study, hence it is suggested that studies with larger sample size are needed for further in depth evaluation of the role of montelukast in asthmatic children.

5. Conclusions

Reactive oxygen species (ROSs) play an important role in the pathogenesis of asthma, and oxidative stress contributes to the initiation and worsening of inflammatory respiratory disorders. Montelukast was found to decrease the reactive oxygen species production, both in the whole blood as well as the isolated PMNs, when stimulated with PMA and OPZ in asthmatic children. It is therefore suggested that montelukast has an additional role for the inhibition of ROS production in asthmatic children.

6. Conflict of interest

No conflict of interest.

Acknowledgements

This work was supported by the College of Medicine Research Centre (CMRC), King Saud University, Riyadh, KSA (Grant No. 06-526). We also thank Mr. Casimero Victoria, Neutrophil Function Laboratory, Department of Physiology, College of Medicine, King Saud University for technical assistance.

References

Al-Saadi, M.M., 2007. The clinical utility of montelukast in paediatric respiratory diseases. Pak. J. Med. Sci. 23 (5), 962–969.

Al-Zamil, Hana A., Ai-Twaijiri, Ali S., Al-Mobeireek., Abdulla F., Mustafa, Ali A. 2005. Effects of zafirlukast on the function of human polymorphonuclear neutrophil leukocytes in asthmatic patients: a prospective, controlled, in vitro study. Curr. Ther. Res. 66(4), 279–293.

Anderson, R., Theron, A.J., Gravett, C.M., Steel, H.C., Tintinger, G.R., Feldman, C., 2009. Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism. Br. J. Pharmacol. 156 (1), 105–115.

Anonymous. 2004. Drug Review. Asthma. Montelukast, drugs in context. Part E. Respir. Med. Infect. 1, 1–40.

Biber, N., Toklu, H.Z., Solakoglu, S., Gultomruk, M., Hakan, T., Berkman, Z., Dulger, F.G., 2009. Cysteinyl-leukotriene receptor antagonist montelukast decreases blood-brain barrier permeability but does not prevent oedema formation in traumatic brain injury. Brain Inj. 23 (6), 577–584.

Bisgaard, H., Zielen, S., Garcia-Garcia, M.L., et al., 2005. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. Am. J. Resp. Crit. Care Med. 171, 315–322.

Bisgaard, H.Study Group on Montelukast and Respiratory Syncytial Virus, 2003. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. Am. J. Respir. Crit. Care Med. 167, 379–383.

Bousquet, J., Demoly, P., Humbert, M., 2009. Montelukast in guidelines and beyond. Adv. Ther. 26 (6), 575–587.

Cantrell, F.L., Farson-Collier, M., 2004. The benign clinical course following a large pediatric montelukast ingestion. J. Toxicol. Clin. Toxicol. 42 (3), 333–334.

Currie, G.P., Srivastava, P., Dempsey, O.J., Lee, D.K.C., 2005. Therapeutic modulation of allergic airways disease with leukotriene receptor antagonists. Q. J. Med. 98, 171–182.

Della Bianca, Grzeskowiak, M., Cassatella, M.A., Zeni, L., Rossi, F., 1986. Phorbol 12-myristate 13-acetate potentiates the respiratory burst while inhibits phosphoinositide hydrolysis and calcium mobilization by formyl-methionyl-leucyl-phenylalanine in human neutrophils. Biochem. Biophys. Res. Commun. 135 (2), 556–565.

Diamant, Z., van der Molen, T., 2005. Treating asthma: is there a place for leukotriene receptor antagonists? Respir. Med. 99, 655–662.

Harmanci, K., 2007. Montelukast: its role in the treatment of childhood asthma. Ther. Clin. Risk Manag. 3 (5), 885–892.

Henderson Jr., W.R., Chiang, G.K., Tien, Y.T., Chi, E.Y., 2006. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. Am. J. Respir. Crit. Care Med. 173, 718–728.

Muz, M.H., Deveci, F., Bulut, Y., Illhan, N., Yekeler, H., Turgut, T., 2006. The effects of low dose leukotriene receptor antagonist therapy on airway remodeling and cysteinyl leukotriene expression in a mouse asthma model. Exp. Mol. Med. 38, 109–118.

Nagarajan, S., Venkiteswaran, K., Anderson, M., Sayed, U., Zhu, C., Selvaraj, P., 2000. Cell-specific, activation-dependent regulation of Neutrophils CD32A ligand-binding functions. Blood 95 (3), 1069–1077.

Noiri, E., Yokomizo, T., Nakao, A., Izumi, T., Fujita, T., Kimura, S., Shimizu, T., 2000. An in ivo approach showing the chemotactic activity of leukotriene B(4) in acute renal ischemic–reperfusion injury. Proc. Natl. Acad. Sci. 97, 823–828.

Peters-Golden, M., Henderson, W.R., 2007. Leukotrienes. N. Engl. J. Med. 357, 1841–1854.

Raulf, Konig, 1988. Modulation of leukotriene release from human polymorphonuclear leucocytes by PMA and arachidonic acid. Immunology 64, 51–59.

Riccioni, G., Bucciarelli, T., Mancini, B., Di Ilio, C., D'Orazio, N., 2007. Antileukotriene drugs: clinical application, effectiveness and safety. Curr. Med. Chem. 14, 1966–1977.

Sener, G., Sehirli, O., Velioglu-Ogunc, A., Cetinel, S., Gedik, N., Caner, M., Sakarcan, A., Yegen, B.C., 2006. Montelukast protects against renal ischemia/reperfusion injury in rats. Pharmacol. Res. 54, 65–71.

Smith, M.B.H., 2007. Is montelukast effective and well tolerated in the management of asthma in young children? Part B: Clinical commentary. Paediatr. Child Health 12 (4), 309–310.

Tugtepe, H., Sener, G., Cetinel, S., Velioğlu-Oğünç, A., Yeğen, B.C., 2007. Oxidative renal damage in pyelonephritic rats is ameliorated by montelukast, a selective leukotriene CysLT1 receptor antagonist. Eur. J. Pharmacol. 557 (1), 69–75.

Van Adelsberg, J., Philip, G., Pedinoff, A.J., et al. Montelukast Fall Rhinitis Study Group. Montelukast improves symptoms of seasonal allergic rhinitis over a 4-week treatment period. Allergy 58, 1268–1276.