Neurochem Res (2015) 40:139–150
DOI 10.1007/s11064-014-1478-9

ORIGINAL PAPER

Montelukast, a Cysteinyl Leukotriene Receptor-1 Antagonist Protects Against Hippocampal Injury Induced by Transient Global Cerebral Ischemia and Reperfusion in Rats

M. A. Saad · R. M. Abdelsalam · S. A. Kenawy · A. S. Attia

Received: 8 June 2014 / Revised: 11 October 2014 / Accepted: 11 November 2014 / Published online: 18 November 2014
© Springer Science+Business Media New York 2014

Abstract Cysteinyl leukotrienes (CysLTs) are potent pro-inflammatory and immune modulating lipid mediators involved in inflammatory diseases and were boosted in human brain after acute phase of cerebral ischemia. The antagonism of CysLTs receptors may offer protection against ischemic damage. Therefore it seemed interesting to study the possible neuroprotective effect of Montelukast, a CysLTR1 antagonist in global cerebral ischemia/reperfusion (IR) injury in rats. Global cerebral ischemia–reperfusion was induced by bilateral carotid artery occlusion for 15 min followed by 60 min reperfusion period. Animals were randomly allocated into three groups (n = 30 per group): Sham operated, I/R control and rats treated with montelukast (0.5 mg/kg, po) daily for 7 days then I/R was induced 1 h after the last dose of montelukast. After reperfusion rats were killed by decapitation, brains were removed and both hippocampi separated and the following biochemical parameters were estimated; lactate dehydrogenase activity, oxidative stress markers (lipid peroxides, nitric oxide and reduced glutathione), inflammatory markers (myeloperoxidase, tumor necrosis factor-alpha, nuclear factor kappa-B, interleukin-6 and interleukin-10), apoptotic biomarkers (caspase 3 and cytochrome C), neurotransmitters (glutamate, gamma aminobutyric acid), CysLTs contents and CysLT1 receptor expression; as well as total brain infarct size and histopathological examination of the hippocampus were assessed. Montelukast protected hippocampal tissue by reducing oxidative stress, inflammatory and apoptotic markers. Furthermore, it reduced glutamate and lactate dehydrogenase activity as well as infarct size elevated by I/R. These results were consistent with the histopathological findings. Montelukast showed a neuroprotective effects through antioxidant, anti-inflammatory and antiapoptotic mechanisms.

Keywords Montelukast · Ischemia/reperfusion · Oxidative stress · Apoptosis · Inflammation

Introduction

Leukotrienes are a group of inflammatory mediators produced in leukocytes from arachidonic acid by the enzyme 5-lipoxygenase [1, 2]. They were first discovered in leukocytes and hence the name leukotrienes, but have since been found in other immune cells. As a rebuttal to this point, it might be convincingly stated that the production of leukotrienes is usually accompanied by the production of histamine and prostaglandins, which act also as inflammatory mediators.

LTC4, LTD4, LTE4 and LTF4 are often called cysteinyl leukotrienes (CysLTs) due to the presence of the amino acid cysteine in their structure. Pharmacological studies demonstrated that cysteinyl leukotrienes activate at least two receptors, cysteinyl leukotriene receptor 1 (CysLTR1) and Cysteinyl leukotriene receptor 2 (CysLTR2) which are present on mast cells, eosinophil, and endothelial cells. Upon activation they can stimulate proinflammatory activities such as endothelial cell adherence and chemokine production by mast cells. Along similar lines, Samitas et al.

M. A. Saad (✉) · R. M. Abdelsalam · S. A. Kenawy · A. S. Attia
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt
e-mail: muhammed.abdullatif@gmail.com

Present Address:
M. A. Saad
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

[3] argues that the levels of CysLTs, have been increased in asthmatic patients and their level was correlated with disease severity. In addition to that, excess CysLTs can induce anaphylactic shock [4].

Reperfusion after a period of global cerebral ischemia results in accumulation, activation, and adherence of circulating leukocytes to the endothelium of blood vessels [5, 6]. There is an unambiguous relationship between the enhanced leukocyte aggregation and the accumulation of high amounts of CysLTs which in turn will stimulate toxic oxygen radicals and hydrolytic enzymes that contribute to the full manifestation of ischemia reperfusion injury [7, 8]. CysLTs, have been implicated in the process of leukocyte accumulation, adhesion to the microvascular endothelium, emigration from the blood stream at sites of inflammation [9, 10], degranulation, and release of lysosomal enzymes [11] as well as generation of oxygen radicals [12].

Research findings by Corser-Jensen et al. [13] points towards the increased production of CysLTs in the ischemic brains, and this increase was correlated with blood–brain barrier (BBB) dysfunction and brain damage. Therefore, accumulation of CysLTs in the brain may play a key role in cerebral ischemia.

These findings lend support to the claim that attenuating CysLTs may confer a plausible therapeutic strategy for cerebral ischemia. Previous studied on 5-lipoxygenase inhibitors have been reported to offer protective effects on cerebral ischemia [14]. In addition to that, pranlukast a selective CysLT1 receptor antagonist, protected mice and rats against focal and global cerebral ischemia [15]. In the present study, the issue under scrutiny is whether Montelukast, the prototype CysLT1 receptor antagonist has the same neuroprotective effect as pranlukast. Additionally, this experimental investigation was conducted to unveil the exact mechanism of its neuroprotective effect which needs further clarification.

The main theoretical premise behind the preference of montelukast over other leukotriene receptor antagonists returns to the fact that it has a lower dose in comparison to other leukotriene receptor antagonists and it is given once daily making it easier for the patients to adhere to the regimen. It was found that, montelukast 10 mg once daily has the same efficacy as pranlukast 225 mg twice daily and zafirlukast 40 mg twice daily in adults as cited in Keam et al. [16] work. Although Churg–Strauss syndrome has been noted in pranlukast recipient [16], no clinically significant differences in adverse event profiles between pranlukast, zafirlukast or montelukast were shown in comparisons. The consensus view seems to be that montelukast has a higher penetration to the BBB compared to other leukotriene receptor antagonists, as zafirlukast [17] and pranlukast [18] which were found to minimally cross the BBB.

However, far too little attention has been paid to the prophylactic effect of CysLTs receptor antagonists, as the previous studies mostly focused on the curative effect of CysLTs receptor antagonists. Furthermore, the oral activity of CysLTs receptor antagonists is still clouded by the fact that most of the previously mentioned reports administered CysLTs receptor antagonists intraperitoneally and acutely after induction of ischemia reperfusion. Hence, it deemed of importance to study the prophylactic effect of montelukast on global cerebral ischemia reperfusion injury and to focus on oral administration since it is an orally active agent. To that end, this dissertation seeks to investigate the effects of montelukast administered orally for 7 days prior to the induction of ischemia reperfusion. Furthermore, to portray the mechanisms of neuroprotective effects elicited by montelukast, its effects on oxidative stress biomarkers, apoptotic factors, inflammatory mediators, neurotransmitters and infarct size were addressed.

Materials and Methods

Animals

Male Wistar rats weighing; 250–300 g were obtained from the National Scientific Research Centre (Giza, Egypt). Animals were housed for at least 1 week in the laboratory room prior to testing. They were kept under controlled environmental conditions; room temperature (24–27 °C), constant humidity (60 ± 10 %), with alternating 12 h light and dark cycles. Food (standard pellet diet) and water were allowed ad libitum. The Ethics Committee of Faculty of Pharmacy Cairo University approved this study. All animals' procedures were performed in accordance to the institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. Unnecessary disturbance of animals was avoided. Animals were treated gently; squeezing, pressure and tough maneuver were avoided.

Experimental Design

Animals were randomly allocated into three groups (n = 30 rats per group). As follows;

Group I: Sham operated (SO).
Group II: Ischemia–reperfusion group (I/R).
Group III: Montelukast pretreatment; Rats were given montelukast (0.5 mg/kg, po) daily for 7 days [19], and then I/R was induced 1 h after the last dose of montelukast.

Each group was subdivided into two subsets. The first subset (n = 24 rats) was used for biochemical estimations,

while the second subset (n = 6 rats) served for measurement of infarction size and histopathological examination.

In all groups rats were anaesthetized with thiopental (50 mg/kg, i.p.) and midline ventral incision was made in the neck. Bilateral carotid artery occlusion using small artery clips was done to induce global cerebral ischemia for 15 min followed by 60 min reperfusion period except for the SO group in which the arteries were exposed for 75 min without occlusion. After reperfusion rats were killed by decapitation, brains were removed and both hippocampi separated and used for biochemical estimations.

Methods

Activity of Lactate Dehydrogenase (LDH) and Protein Content Assay in Rat Hippocampus

Evaluation of the activity of Lactate dehydrogenase in rat hippocampus was through Colorimetric Kinetic Determination using kit supplied by Biosystems (Biosystems, S.A. Costa Brava, 30. 08030 Barcelona, Spain) according to the method of Lorentz et al. [20], while the protein content of tissue supernatant was determined using the method of Lowry et al. [21].

Estimation of Hippocampal Oxidative Stress Biomarkers Contents

Lipid peroxides formation was investigated in hippocampal tissue homogenate by estimation of thiobarbituric acid reactive substances (TBARs) according to the method of Mihara and Uchiyama [22]. Furthermore, inspection of GSH content in rat hippocampus was performed according to the method of Beutler et al. [23]. Additionally, nitric oxide measurement in rat hippocampus was according to the method described by Miranda et al. [24].

Determination of Pro-inflammatory and Anti-inflammatory Mediators in Rat Hippocampus

Myeloperoxidase (MPO) enzyme, being a plentiful constituent of neutrophils, serves as a marker for tissue neutrophil content. Since MPO is located within the primary granules of neutrophils, extraction of MPO depends upon procedures to disrupt the granules which render MPO soluble in aqueous solution. This could be achieved by sonication in potassium phosphate buffer (50 mM, pH 6) containing 0.5 % hexadecyltrimethylammonium bromide (HTAB) [25], where HTAB is a detergent that releases MPO from the primary granules of the neutrophil [26].

On the other hand, the content of nuclear factor–kappa B was assayed by enzyme-linked immunosorbent assay (ELISA) using kit supplied by EIAab (E1824r, EIAab Science Co., Wuhan, China). Also, assessment of the content of tumor necrosis factor alpha was by ELISA using kit supplied by R&D Systems (Quantikine Rat TNF-α ELISA, Catalog #RTA00, R&D systems, Inc., Minneapolis, MN, USA).

Furthermore, rating the contents of interleukin-6 and interleukin-10 were made using kit supplied by R&D systems (Quantikine® ELISA, Rat IL-6 Immunoassay, Catalog Number R6000B, R&D systems, Inc., Minneapolis, MN, USA) and (Quantikine® ELISA, Rat IL-10 Immunoassay, Catalog Number R1000, R&D systems, Inc., Minneapolis, MN, USA), respectively.

Enzyme-linked Immunosorbent Assay (ELISA) of Apoptotic Biomarkers in Rat Hippocampus

The content of caspase-3 enzyme and the content of cytochrome-c were figured out by ELISA using kit supplied by R&D systems (Quantikine Active Caspase-3 ELISA, Catalog #KM300, R&D systems, Inc., Minneapolis, MN, USA) for caspase 3 content, and using kit supplied by EIAab (E0594r, EIAab Science Co., Wuhan, China) for cytochrome c content.

High Performance Liquid Chromatography (HPLC)

Hippocampus was homogenized in 70 % HPLC methanol (1/10 weight/volume) and was used for the estimation of glutamate and GABA using a fully automated high-pressure liquid chromatography system (HPLC; Perkin-Elmer, MA, USA). Brain amino acids were inspected by the phenylisothiocyanate derivatization technique described by Heinrikson and Meredith [27]. Hippocampal tissues were dried under vacuum following reconstitution with 2:2:1 mixture (v) of methanol:1 M sodium acetate trihydrate:triethylamine. The derivatization procedure using a 7:1:1:1 mixture (v) of methanol:triethylamine:double-distilled deionized water:phenylisothiocyanate, was performed for 20 min at room temperature then re-subjected to vacuum until dryness. Subsequently, derivatized amino acids were reconstituted with sample diluent consisting of 5:95 mixture (v) of acetonitrile:5 mM phosphate buffer (pH = 7.2). Samples were then sonicated and filtered (0,45 μm; Millipore, USA). A Pico-Tag physiological free amino acid analysis C18 (300 mm × 9 3.9 mm i.d) column from Waters (MA, USA) and a binary gradient of Eluents 1 and 2 (Waters) were used, the column temperature was at set 46 ± 1 °C. A constant flow rate of 1 ml/min was maintained throughout the experiment. 20 μl of samples were injected and the absorbance of the derivatized amino acids was measured at 254 nm. Glutamate standard was prepared in double-distilled deionized water, while

GABA standard was prepared in polyethylene vials to prevent adhesion to glass.

Measurement of Extracellular Cysteinyl Leukotrienes and CysLT1 Receptor Expression

Assessment of the contents of CysLTs (LTC4, LTD4 and LTE4) in hippocampal homogenate supernatants were by ELISA using kit supplied by Cayman Chemical Co. (Ann Arbor, MI, USA) and calculated as pg/mg protein. On the other hand, cysteinyl leukotriene receptor 1 expression was gauged using CYSLTR1 ELISA Kit (antibodies-online GmbH, Schloss-Rahe-Str., Germany) according to the manufacturer's instructions and the results were expressed as the ratios to β-actin which was evaluated by ELISA method using kit supplied by Cell Signaling Technology, Inc. (PathScan® Total β-Actin Sandwich ELISA Kit, Danvers, MA, USA).

Infarct Size Estimation

At the end of 60 min reperfusion period, animals (n = 4) were intracardially perfused with isotonic saline and sacrificed by spinal dislocation. Brains were then sliced into 2 mm coronal sections and incubated with 1 % triphenyltetrazolium chloride (TTC) at 37 °C in 0.2 M Tris buffer (pH 7.4) for 20 min. While viable cells stain bright red when TTC is converted to red formazone pigment by NAD and lactate dehydrogenase, infracted cells lose the enzyme as well as cofactor and thus remain unstained or stain dull yellow. The brain slices were placed over glass plate and the infarcted areas were traced by a 100 squares in 1 cm^2 transparent plastic grid. In each brain slice, the average infarcted area of both sides as well as the non infarcted area was computed. Infarcted area was expressed as a percentage of total brain area [28, 29].

Histopathological Investigation

Histopathological examination was performed on the brains of 3–4 rats randomly selected from each group. Following transcardiac perfusion, brains were removed, placed in 10 % formalin/PBS and kept until they became hard enough to be sectioned. Each brain was embedded in paraffin blocks. Coronal sections of 5 μm were obtained and stained with haematoxylin and eosin (H&E) for standard histological examination according to the method of Banchroft et al. [30]. In brief, deparaffinization of sections was performed using xylene, while hydration was carried out using descending grades of alcohol and finally water. The sections were stained with Haematoxylin for 10 min. and then counterstained with Eosin for 1 min., followed by rapid rinsing with distilled water to remove excess stain then dehydration with ascending grades of alcohols. Finally, clearing with xylene and mounting in Canada balsam was performed. For quantification of the number of pycnotic neuronal cells, three random regions were examined at ×400 magnification and the number of pycnotic neurons in three areas per section of hippocampal dentate gyrus (DG) region were identified and counted on the basis of the presence of pycnotic nuclei and shrunken cytoplasm. Then the number of the pycnotic neuronal cells was calculated as an average per rat.

Statistical Analysis

Values were expressed as mean ± S.E.M. using a computer software program statistical package for the social sciences "SPSS" (Version 16.0.). One-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons post hoc. test was used for comparing the means of the different groups. The criterion for statistical significance was set at the $P < 0.05$ level. Graphical representation was conducted using GraphPad Prism (Version 5).

Results

Lactate Dehydrogenase (LDH) Activity in Hippocampal Tissue After Montelukast (0.5 mg/kg, po) Administration

Figure 1 shows the summary statistics for LDH activity, were transient global cerebral I/R was accompanied with elevated hippocampal LDH activity to about 263 % of the SO group. Montelukast decreased LDH activity to about quarter the I/R group. However, no significant reduction in montelukast group was found compared with SO group.

Effect of Montelukast (0.5 mg/kg, po) on Oxidative Stress, Inflammatory and Apoptotic Biomarkers as Well as Neurotransmitters Contents in Hippocampal Tissue

As shown in Fig. 2, I/R resulted in a significant increase in hippocampal TBARs and NOx contents to 209 and 199 % of the SO group, respectively, accompanied with a reduction in GSH content to 28 % compared to SO group. On average, montelukast were shown to have a potential antioxidant power through a reduction in TBARs (Fig. 2a) and NOx contents (Fig. 2c) to about 57 and 26 % of that in I/R group, respectively. Furthermore, montelukast prevented the reduction in hippocampal reduced GSH content by attaining more than two folds the I/R group but the level was still lower than that of SO group (Fig. 2b).

There was a significant positive correlation between global cerebral I/R and neutrophil infiltration as observed by

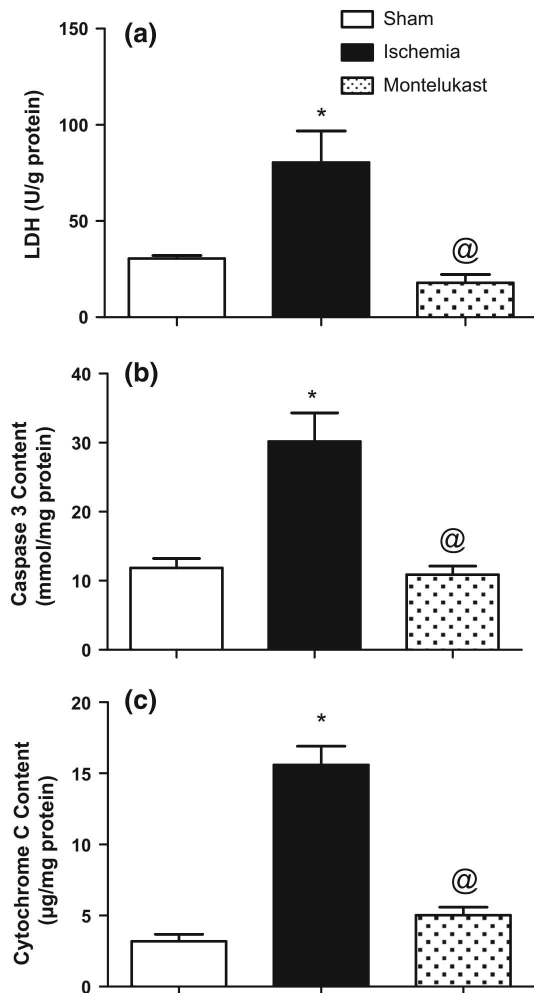

Fig. 1 Effect of Montelukast (0.5 mg/kg, po) on hippocampal **a** cytosolic lactate dehydrogenase (LDH) activity; and apoptotic markers **b** caspase-3 and **c** cytochrome-c (Cyt-c) contents in rats subjected to global cerebral ischemia reperfusion. Values were expressed as mean ± SEM of six rats. *Significantly different from Sham operated control at $P < 0.05$. @Significantly different from Ischemia reperfusion at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

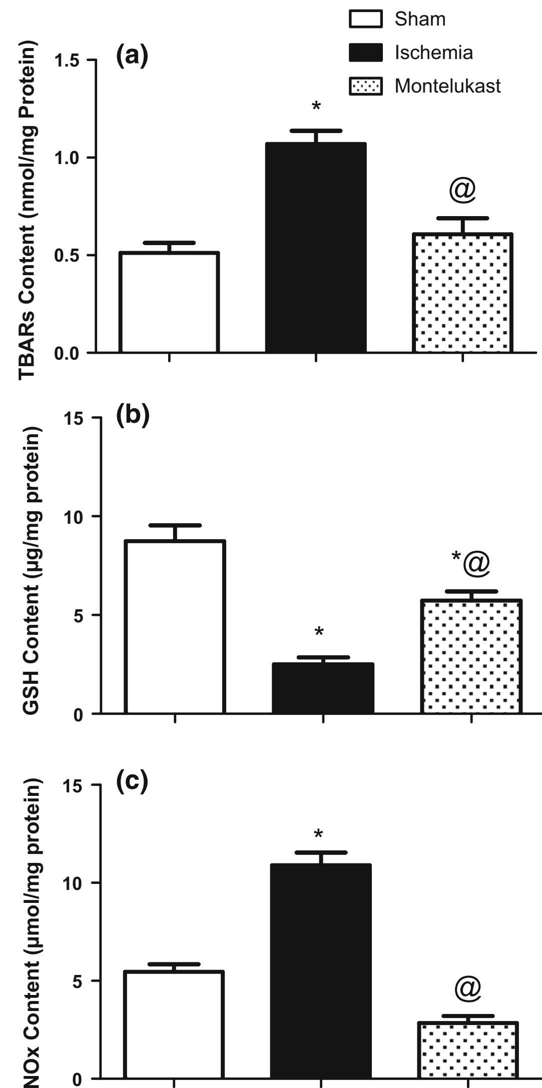

Fig. 2 Effect of Montelukast (0.5 mg/kg, po) on hippocampal oxidative stress biomarkers **a** thiobarbituric acid reactive substances (TBARs), **b** reduced glutathione (GSH) and **c** nitric oxide (NOx) contents in rats subjected to global cerebral ischemia reperfusion. Values were expressed as mean ± SEM of six rats. *Significantly different from Sham operated control at $P < 0.05$. @Significantly different from Ischemia reperfusion at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

the elevation in hippocampal MPO activity in I/R group to about 1.5 times that in the SO group. This effect was offset by montelukast in which MPO activity decreased to about half the I/R group. Such positive correlation was found also between global cerebral I/R and inflammatory markers as I/R showed a significant increase in hippocampal NF-κB, TNF-α, IL-6 contents together with reduction in IL-10 content. The mean score for NF-κB reached 331 % of that in the SO group, meanwhile TNF-α and IL-6 were increased by fivefold and 1.5-fold respectively. On the other hand IL-10 content reached 29 % of the SO group.

Montelukast prevented the increase in MPO, NF-κB, TNF-α and IL-6 contents to be 50, 43, 32 and 61 % of that in I/R group, respectively. However, the ANOVA (one way) showed that the result of montelukast on the reduced IL-10 content was not statistically significant. These results were highlighted in Table 1.

From the data in Fig. 1, it is apparent that global cerebral I/R resulted in obvious increase in hippocampal caspase-3 content to 254 % of the SO group. Additionally, hippocampal Cyt-c content was elevated to about five folds following I/R compared to the SO group. Prior administration of montelukast significantly prevented the increase in both caspase-3 and cytochrome c contents by showing a reduction to about 36 and 32 % compared to I/R group (Fig. 1).

Table 1 Effect of montelukast (0.5 mg/kg, po) on hippocampal inflammatory biomarkers: myeloperoxidase (MPO) activity, nuclear factor kappa-B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10) and interleukin-6 (IL-6) contents in rats subjected to global cerebral ischemia reperfusion

Groups	Parameter				
	MPO activity (mU/mg protein) Mean ± SE	NF-κB content (ng/mg protein) Mean ± SE	TNF-α content (Pg/mg protein) Mean ± SE	IL-10 content (Pg/mg protein) Mean ± SE	IL-6 content (Pg/mg protein) Mean ± SE
Sham operated control	26.83 ± 4.24	0.11 ± 0.01	3.02 ± 0.39	16.71 ± 2.34	27.11 ± 1.74
Ischemia reperfusion	42.53* ± 2.42	0.35* ± 0.02	14.85* ± 0.96	4.96* ± 1.22	40.41* ± 5.38
Montelukast (0.5 mg/kg, po)	20.5@ ± 3.22	0.15@ ± 0.03	4.78@ ± 0.72	6.98* ± 1.33	24.79@ ± 1.28

Values were expressed as mean ± SEM of six rats

* Significantly different from Sham operated control at $P < 0.05$

@ Significantly different from Ischemia reperfusion at $P < 0.05$

Statistical analysis was performed by ANOVA followed by Tukey's Post-hoc test

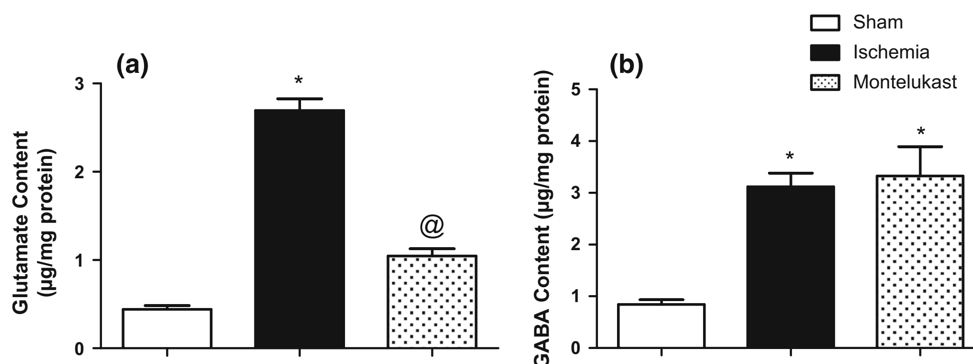

Fig. 3 Effect of Montelukast (0.5 mg/kg, po) on hippocampal neurotransmitters **a** glutamate and **b** gamma amino butyric acid (GABA) contents in rats subjected to global cerebral ischemia reperfusion. Values were expressed as mean ± SEM of six rats. *Significantly different from Sham operated control at $P < 0.05$. @Significantly different from Ischemia reperfusion at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

Global cerebral I/R showed a significant increase in both glutamate and GABA contents in the hippocampus. Glutamate content reached to about six folds that measured in the SO group while GABA content increased to 368 % of the SO group. Montelukast failed to change the GABA content (Fig. 3b), but could prevent the rise in glutamate content to reach 39 % of the I/R group (Fig. 3a).

Effect of Montelukast (0.5 mg/kg, po) on Cysteinyl Leukotrienes and CysLT1 Receptor Expression in Hippocampal tissue

Transient cerebral I/R resulted in a significant increase in cysteinyl leukotrienes contents (Fig. 4a) and cysteinyl leukotriene receptor 1 expression (Fig. 4b) to attain 194 and 130 % of the SO group, respectively. Treatment with montelukast attenuated the elevated CysLT1 receptor expression to attain less than half the I/R group, but failed to show any significant change in the contents of cysteinyl leukotrienes contents in rat hippocampus.

Cerebral Infarct Size Following Montelukast (0.5 mg/kg, po) Administration

Global cerebral I/R resulted in a significant increase in infarct size to 230 % of the SO group. Montelukast prevented the elevated infarct size to reach 57 % of that in I/R group (Fig. 5).

Effect of Montelukast (0.5 mg/kg, po) on the Histopathology of Hippocampal Areas in Rats Subjected to I/R

As shown in Fig. 6, sections of the SO rat hippocampi showed normal histological structures, while sections of the I/R rat hippocampi presented the occurrence of

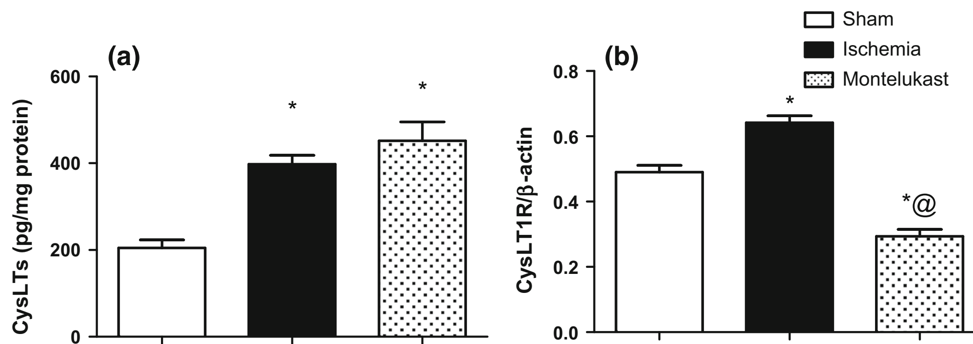

Fig. 4 Effect of Montelukast (0.5 mg/kg, po) on hippocampal **a** CysLTs contents and **b** CysLT1 receptor expression in rats subjected to global cerebral ischemia reperfusion. Values were expressed as mean ± SEM of six rats. *Significantly different from Sham operated control at $P < 0.05$. @Significantly different from Ischemia reperfusion at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

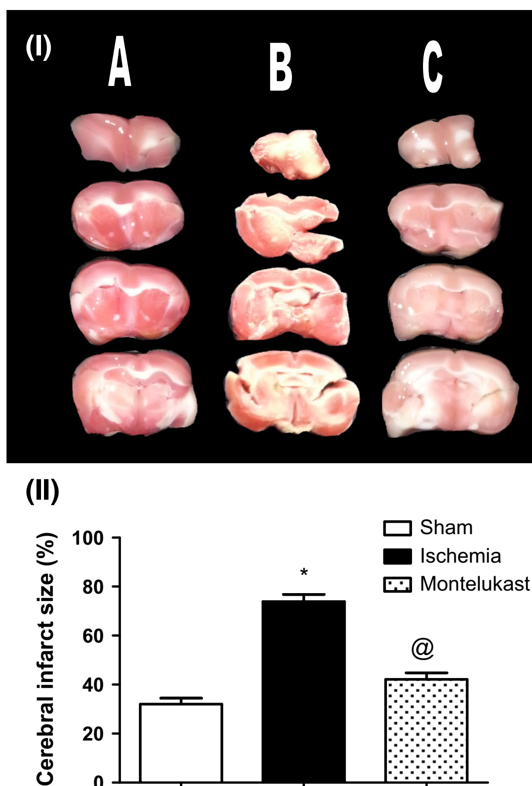

Fig. 5 Effect of Montelukast (0.5 mg/kg, po) on brain coronal sections. **I** coronal sections showing the infarct areas (in *white*) in (*A*) Sham operated control, (*B*) ischemia/reperfusion brain, (*C*) and Montelukast (0.5 mg/kg, po). **II** Summary of the quantitative analysis of infarct areas. Values were expressed as mean ± SEM of four rats. *Significantly different from Sham operated control at $P < 0.05$. @Significantly different from Ischemia reperfusion at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

necrosis, atrophy and pyknosis of pyramidal cells of the hippocampus. Rats treated with montelukast (0.5 mg/kg, po) for 7 days before the induction of I/R, demonstrated a significant improvement in the I/R induced changes where the hippocampal cellular structures were nearly preserved (Figs. 6, 7).

Discussion

Cerebral ischemia elicits an acute inflammatory response that is greatly augmented by reperfusion. Polymorphonuclear leukocytes (PMNs) are the first line defense that reaches the ischemic area [5]. They have a vital role in the tissue injury following ischemia and reperfusion as it has the capacity to produce oxygen-derived free radicals, proteases, leukotrienes and myeloperoxidase when activated by appropriate stimuli [31]. A significant part of the total cerebral injury after ischemia and reperfusion is attributable to these effects.

There is overwhelming evidence corroborating the notion that oxidative stress is a key step in the damage induced by I/R. For instance, it has been reported that exposure of rats to transient cerebral ischemia showed an elevation in lipid peroxidation, and a decline in the reduced glutathione content and superoxide dismutase (SOD) activity [32]. Further evidence supporting Vaibhav et al. [32] work, may lie in the findings of Akhtar et al. [33], who, demonstrated that median cerebral artery occlusion showed an elevation in the levels of TBARs with a reduction in the levels of glutathione and antioxidant enzymes as SOD and catalase. The results of the current work provide confirmatory evidence to the previously reported work, as exposure of rats to global cerebral ischemia reperfusion in our model showed a marked decrease in reduced GSH content and an elevation in lipid peroxidation and peroxynitrite as evidenced by the elevated contents of TBARs and NOx confirming the involvement of oxidative stress in the damage induced by I/R. NOx produced react rapidly with superoxide produced in excess during reperfusion to form peroxynitrite contributing to

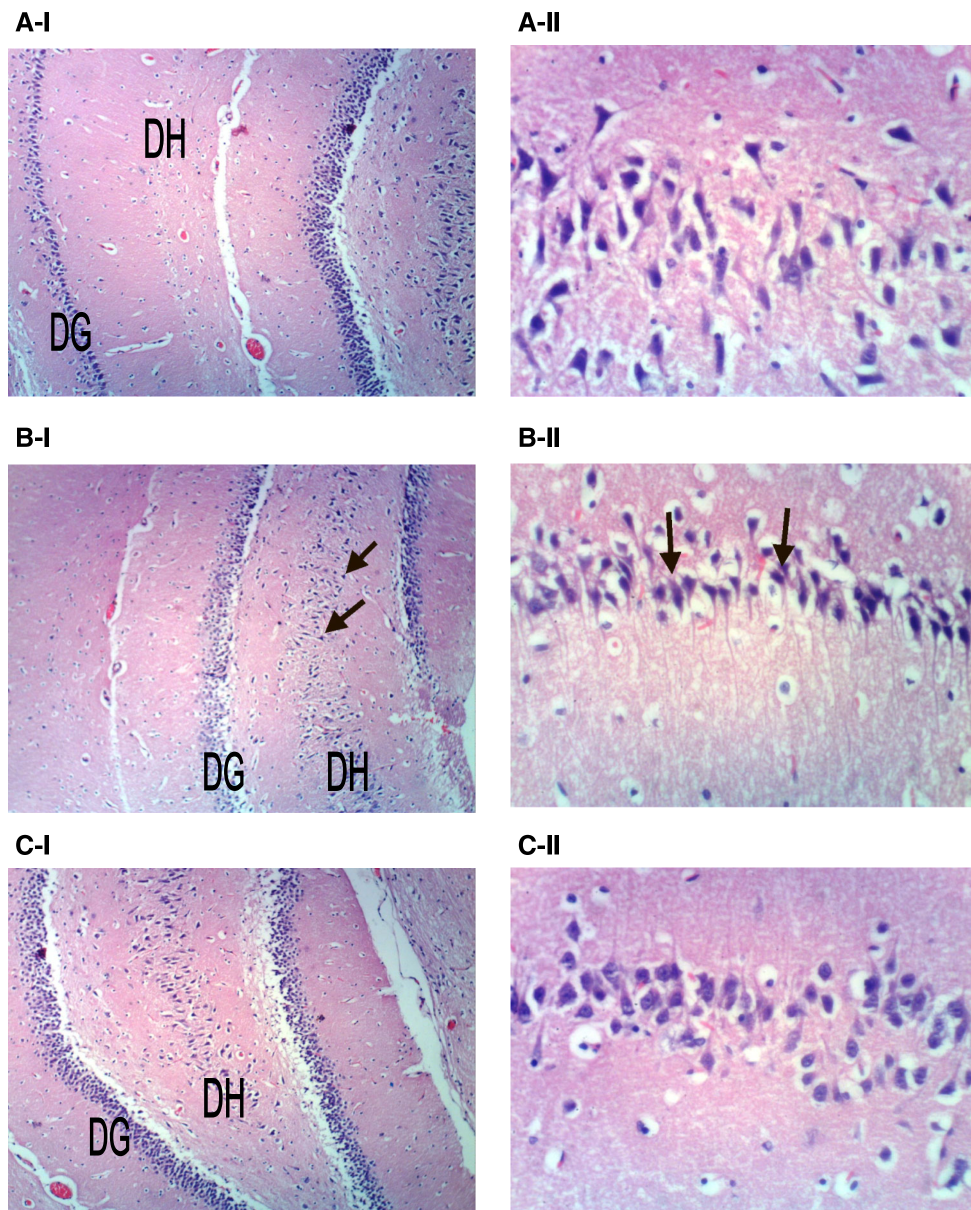

Fig. 6 Representative photomicrographs of the hilar region of the dentate gyrus sector of the hippocampal sections of **a** control animals showing normal architecture of hippocampus, **b** ischemia reperfusion (I/R) animals showing nuclear pyknosis (*arrow*), **c** rats treated with Montelukast (0.5 mg/kg, po) for 7 days before induction of I/R showing preserved cellular structures. (*DH* dentate hilus, *DG* dentate gyrus). I = (H&E ×100) and II = (H&E ×400)

cell death as seen with vaculations and pyknotic nuclei upon histopathological examination.

On logical grounds, there is compelling reason to affirm that the oxidative stress induced after exposure to ischemia reperfusion may be as a result of elevated 5-lipoxygenase activity [5-LO] and leukotrienes [LT] content in ischemic areas. It has been confirmed that arachidonic acid level was greater in brain regions subjected to I/R injury [34] and 5-LO expression as well as LT level were elevated in the ischemic brain [35], indicating an important role for LT in cerebral ischemia. The elevation in LT was accompanied by the increase in TBARs content and the decrease in tissue GSH level, and SOD activity [36]. This finding corroborates the ideas of Hagar and Abd El Tawab [37], who suggested that induction of ischemia–reperfusion resulted in elevation of serum nitrite and nitrate, TNF-α, malondialdehyde (MDA) concentration and a reduction in reduced glutathione content. Administration of zafirlukast, a cysteinyl leukotriene receptor antagonist before ischemia–reperfusion improved the functions of ischemic organ and abolished the pervious changes induced by I/R [37]. It is encouraging to compare these findings with that found by

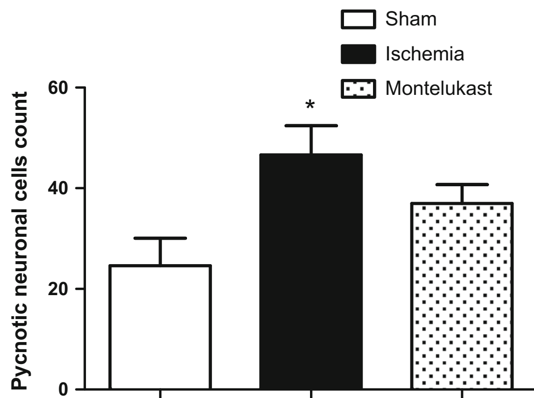

Fig. 7 Effect of montelukast (0.5 mg/kg, po) on hippocampal pycnotic neuronal cells count in rats subjected to global cerebral ischemia reperfusion. Values were expressed as mean ± SEM of six rats. *Significantly different from Sham operated control at $P < 0.05$. Statistical analysis was performed by ANOVA followed by Tukey's post-hoc test

Daglar et al. [38], who found that tissue MDA levels and glutathione consumptions were decreased significantly in the groups treated with montelukast before and during the surgical operation compared with the I/R group. The results of the present investigation were in harmony with the previously reported work as treatment of rats with montelukast for 7 days before induction of I/R succeeded to diminish the elevated TBARs and NOx contents and elevated reduced GSH content in rats' hippocampi. The data gathered in the current study strongly suggests that montelukast has a potential antioxidant action that can be used to treat many other disorders in which oxidative stress is a hallmark process.

Many studies have propounded the view that inflammatory mediators are involved in the damage induced by focal and global cerebral ischemia reperfusion [39, 40].

In the present work the levels of inflammatory mediators MPO, NF-κB, TNF-α and IL-6 were elevated, while the level of the anti-inflammatory cytokine IL-10 was markedly declined. The view that inflammatory process was induced after I/R is very much in line with the previously reported data.

Cysteinyl leukotrienes are considered one of the most important inflammatory mediators that are implicated in the pathogenesis of many inflammatory disorders such as asthma [41], rheumatoid arthritis [42], ischemia [43] and many other disorders.

TNF-α were known to stimulate the arachidonate cascade leading to the synthesis of LT in vivo [44]. Furthermore, LT enhance TNF-α induced cytokines production through enhancing NF-κB activity [45]. Together these results provide important insights into the mutual enhancement relationship between leukotrienes and TNF-α. Also, leukotriene B4 enhances the production of IL-6 [46]. On the other hand, the absence of 5-LO derived leukotrienes results in increased IL-10 production with a concomitant decrease in the production of pro-inflammatory cytokines, including TNF-α and IL-12 [47].

Blockade of leukotriene receptor by montelukast in the present work could decrease MPO, NF-κB, TNF-α and IL-6 levels but failed to induce IL-10. In harmony with our results, montelukast could normalize the TNF-α, NF-κB levels and MPO activity elevated by methotrexate in renal tissues [48]. Furthermore, blockade of leukotriene receptor [49], or inhibition of leukotriene synthesis [50] significantly decreased serum TNF-α and IL-6 in rats subjected to hemorrhagic shock as compared to untreated rats. However, the findings of the current study do not support the previous research of Yuksel et al. [51] who demonstrated that montelukast significantly increased serum IL-10 level. This discrepancy may be due to the longer period of montelukast treatment in Yuksel et al. [51] work as compared to the 1 week treatment period in our work.

Many reports studied the role of neurotransmitters in the pathogenesis of ischemia reperfusion. For instance, occlusion of the median cerebral artery showed an increase in excitatory and inhibitory amino acids in the CSF relative to the sham-operated rats [52]. Furthermore, Transient forebrain ischemia increased glutamate and GABA contents as well as NMDA receptor expression in rats' hippocampi [53]. There are similarities between the attitudes expressed by I/R in this study and those described by Wang et al. [52] and Cai et al. [53], as the present investigation showed that exposure of rats to median cerebral artery occlusion resulted in a significant rise in glutamate and GABA contents in rats' hippocampi.

It has been shown that NMDA injection upregulated the expression of CysLT1 receptor in neurons which indicates that the increased CysLT1 receptor is involved in NMDA induced excitotoxicity [54]. Also, the CysLT1 receptor was found to modulate brain cryoinjury induced by applying a liquid nitrogen-cooled metal probe to the surface of the skull for 30 s [55]. This link between CysLT1 receptor and brain damage was confirmed in our model as the expression of CysLT1 receptor was induced after the period of I/R which may be mediated through the activation of NMDA receptors by the increased glutamate level. Prophylactic use of montelukast before the induction of I/R succeeded to suppress the elevation of CysLT1 receptor which was in harmony with the results shown by Ding et al. [54] who demonstrated that NMDA-induced responses are inhibited by CysLT1 receptor antagonists.

Turning now to the experimental evidence on the effect of I/R on CysLTs contents, Ciceri et al. [56] showed that cysteinyl-leukotriene formation is associated with NMDA receptor activation, and that it represents a neurotoxic event. It has been postulated also that severe forebrain

ischemia in rats increased free arachidonic acid by approximately 8.5 times compared with the preischaemic level which resulted also in accumulation of CysLTs in brain tissue after reperfusion [57]. This induction in CysLTs content was also seen in our model of I/R. However, debate continues about the effect of CysLT receptor antagonists on the content of CysLTs. In the present work montelukast reduced the elevated glutamate content and hence the NMDA receptor activity which was expected to reduce the production of CysLTs as shown with Ge et al. [58]. Additionally, montelukast reduced the concentration of leukotrienes in the respiratory tract of children with persistent asthma [59, 60]. Contrary to expectations of the latterly mentioned studies which suggest that montelukast can suppress CysLTs contents, it failed to change CysLTs in rats' hippocampi elevated by I/R. The results of the current investigation go straightforward with the study of Nakamori et al. [61] who demonstrated that pranlukast had no effect on the mucosal CysLT levels in the stomach after I/R treatment.

Apoptotic cell death is a genetically programmed mechanism(s) that allows the cell to commit suicide. The two major well-studied apoptotic processes are the extrinsic and intrinsic pathways [62, 63]. The extrinsic pathway is mediated by tumor necrosis factor receptors. Activation of these so called death receptors leads to the recruitment and activation of initiator caspases such as caspases 8 and 10. This leads to the activation of an effector caspase, typically caspase 3. The active caspase 3 is responsible for the cleavage of a number of so-called death substrates that lead to the well-known characteristic hallmarks of an apoptotic cell [64]. The intrinsic pathway is largely centered around and/or regulated by the mitochondria [65]. The most widely studied form of intrinsic apoptosis is initiated by the release of cytochrome c from the mitochondria that results in the formation of the apoptosome. The apoptosome then activates initiator caspase, mostly caspase 9, which leads to the activation of the executioner caspase 3. For the sake of discussion, I would like to argue that intrinsic pathway leads to similar type of apoptotic response as observed for the extrinsic pathway.

The available evidence seems to confirm the role of free radicals [66], inflammatory mediators [67] and glutamate excitotoxicity [68] in the induction of apoptosis. The shown induction in oxidative stress, inflammatory process and oxidative stress by the current model of ischemia reperfusion can finally end in an increase in cell death and apoptosis as evidenced by the elevation in caspase 3 and cytochrome c contents in rats' hippocampi. Moreover, we traced the infarct size and lactate dehydrogenase (LDH) activity and found that they were elevated following I/R which corroborated with histopathological findings that confirmed the presence of necrosis, atrophy and pyknosis in the pyramidal cells of the hippocampus. The data yielded by this investigation provides convincing evidence that both intrinsic and extrinsic pathway were involved in the damage shown with I/R.

It has conclusively been shown that leukotrienes can regulate the viability and apoptosis of most cell cultures. It decreased the number of living cells and increased the number of necrotic cells [69]. Treatment with montelukast could reduce apoptosis in cardiomyocytes in vitro and in vivo [70]. In addition, according to Daglar et al. [38] blockade of leukotrienes receptors with montelukast or inhibition of leukotrienes synthesis can reduce apoptosis in liver and intestine of rats subjected to I/R. The above findings are consistent with the study by Lai et al. [71], who demonstrated that the blockade of leukotrienes receptors by repeated treatment with montelukast reduced TNF-α and caspase-3 activation in the hippocampus and cortex.

In accordance with the previously mentioned results, treatment of rats with montelukast before induction of I/R in the present investigation could normalize the elevated Cyt c, TNF-α and caspase 3 contents, LDH activities together with a significant decline in infarct size compared to untreated rats. These results were confirmed in rats treated with montelukast by amending the occurrence of pyknotic nuclei and valuations as shown in histopathological examination compared to I/R group. A closer look at these data indicates that prophylactic use of montelukast orally for 7 days was enough to suppress most of the damage induced by I/R.

In conclusion, our findings clearly indicate that blockade of leukotrienes receptors with montelukast impose a strong antioxidant, anti-inflammatory and antiapoptotic effects. Our results add a new evidence for the neuroprotective effects of leukotriene antagonists and highlight the need for further experimental and clinical studies that will define the clinical scope of therapy with montelukast as a sole agent or as an adjunctive therapy for patient suffering from stroke.

Acknowledgment This research was funded by the Faculty of Pharmacy Cairo University.

References

1. O'Byrne PM, Israel E, Drazen JM (1997) Antileukotrienes in the treatment of asthma. Ann Intern Med 127:472–480
2. Salmon JA, Higgs GA (1987) Prostaglandins and leukotrienes as inflammatory mediators. Br Med Bull 43:285–296
3. Samitas K, Chorianopoulos D, Vittorakis S, Zervas E, Economidou E, Papatheodorou G, Loukides S, Gaga M (2009) Exhaled cysteinyl-leukotrienes and 8-isoprostane in patients with asthma and their relation to clinical severity. Respir Med 103:750–756

4. Kumar S, Verma AK, Das M, Dwivedi PD (2012) Molecular mechanisms of IgE mediated food allergy. Int Immunopharmacol 13:432–439
5. Kitano K, Usui S, Ootsuji H, Takashima S, Kobayashi D, Murai H, Furusho H, Nomura A, Kaneko S, Takamura M (2014) Rho-kinase activation in leukocytes plays a pivotal role in myocardial ischemia/reperfusion injury. PLoS One 9:e92242
6. Zhao H, Perez JS, Lu K, George AJ, Ma D (2014) Role of Toll-like receptor 4 in renal graft ischemia-reperfusion injury. Am J Physiol Renal Physiol. doi:10.1152/ajprenal.00469.2013
7. Grisham MB, Hernandez LA, Granger DN (1986) Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 251:G567–G574
8. Parks DA, Bulkley GB, Granger DN, Hamilton SR, McCord JM (1982) Ischemic injury in the cat small intestine: role of superoxide radicals. Gastroenterology 82:9–15
9. Dahlen SE, Bjork J, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA, Samuelsson B (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 78:3887–3891
10. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ (1980) Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286:264–265
11. Rae SA, Smith MJ (1981) The stimulation of lysosomal enzyme secretion from human polymorphonuclear leucocytes by leukotriene B4. J Pharm Pharmacol 33:616–617
12. Serhan CN, Radin A, Smolen JE, Korchak H, Samuelsson B, Weissmann G (1982) Leukotriene B4 is a complete secretagogue in human neutrophils: a kinetic analysis. Biochem Biophys Res Commun 107:1006–1012
13. Corser-Jensen CE, Goodell DJ, Freund RK, Serbedzija P, Murphy RC, Farias SE, Dell'acqua ML, Frey LC, Serkova N, Heidenreich KA (2014) Blocking leukotriene synthesis attenuates the pathophysiology of traumatic brain injury and associated cognitive deficits. Exp Neurol. doi:10.1016/j.expneurol.2014.03.008
14. Shi SS, Yang WZ, Tu XK, Wang CH, Chen CM, Chen Y (2013) 5-Lipoxygenase inhibitor zileuton inhibits neuronal apoptosis following focal cerebral ischemia. Inflammation 36:1209–1217
15. Zhang SH, Wei EQ, Zhu CY, Chen Z, Zhang SF (2004) Protective effect of ONO-1078, a leukotriene receptor antagonist, on focal cerebral ischemia induced by endothelin-1 in rats. Yao Xue Xue Bao 39:1–4
16. Keam SJ, Lyseng-Williamson KA, Goa KL (2003) Pranlukast: a review of its use in the management of asthma. Drugs 63:991–1019
17. Anon (1996) Zafirlukast for asthma. Med Lett Drugs Ther 38:111–112
18. Kobayashi H, Ide H, Handa Y, Aradachi H, Arai Y, Kubota T (1992) Effect of leukotriene antagonist on experimental delayed cerebral vasospasm. Neurosurgery 31:550–555
19. Zhao R, Shi WZ, Zhang YM, Fang SH, Wei EQ (2011) Montelukast, a cysteinyl leukotriene receptor-1 antagonist, attenuates chronic brain injury after focal cerebral ischaemia in mice and rats. J Pharm Pharmacol 63:550–557
20. Lorentz K, Klauke R, Schmidt E (1993) Recommendation for the determination of the catalytic concentration of lactate dehydrogenase at 37 degrees C. Standardization committee of the German society for clinical chemistry, enzyme working group of the German society for clinical chemistry. Eur J Clin Chem Clin Biochem 31:897–899
21. Lowry OH, Rosebrough NJ, FARR AL (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
22. Mihara M, Uchiyama M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278
23. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
24. Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62–71
25. Bradley PP, Christensen RD, Rothstein G (1982) Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood 60:618–622
26. Schultz J, Kaminker K (1962) Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. Arch Biochem Biophys 96:465–467
27. Heinrikson RL, Meredith SC (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. Anal Biochem 136:65–74
28. Malik ZA, Singh M, Sharma PL (2011) Neuroprotective effect of Momordica charantia in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. J Ethnopharmacol 133:729–734
29. Rehni AK, Bhateja P, Singh N, Jaggi AS (2008) Implication of mast cell degranulation in ischemic preconditioning-induced prevention of cerebral injury. Fundam Clin Pharmacol 22: 179–188
30. Banchroft J, Stevens A, Turner D (1996) Theory and practice of histological techniques. Churchil Livingstone, London, pp 257–262
31. Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320:365–376
32. Vaibhav K, Shrivastava P, Tabassum R, Khan A, Javed H, Ahmed ME, Islam F, Safhi MM, Islam F (2013) Delayed administration of zingerone mitigates the behavioral and histological alteration via repression of oxidative stress and intrinsic programmed cell death in focal transient ischemic rats. Pharmacol Biochem Behav 113:53–62
33. Akhtar M, Maikiyo AM, Najmi AK, Khanam R, Mujeeb M, Aqil M (2013) Neuroprotective effects of chloroform and petroleum ether extracts of Nigella sativa seeds in stroke model of rat. J Pharm Bioallied Sci 5:119–125
34. Katsuki H, Okuda S (1995) Arachidonic acid as a neurotoxic and neurotrophic substance. Prog Neurobiol 46:607–636
35. Ohtsuki T, Matsumoto M, Hayashi Y, Yamamoto K, Kitagawa K, Ogawa S, Yamamoto S, Kamada T (1995) Reperfusion induces 5-lipoxygenase translocation and leukotriene C4 production in ischemic brain. Am J Physiol 268:H1249–H1257
36. Yang SL, Huang X, Chen HF, Xu D, Chen LJ, Kong Y, Lou YJ (2007) Increased leukotriene c4 synthesis accompanied enhanced leukotriene c4 synthase expression and activities of ischemia-reperfusion-injured liver in rats. J Surg Res 140:36–44
37. Hagar HH, Abd El Tawab R (2012) Cysteinyl leukotriene receptor antagonism alleviates renal injury induced by ischemia-reperfusion in rats. J Surg Res 178:e25–e34
38. Daglar G, Karaca T, Yuksek YN, Gozalan U, Akbiyik F, Sokmensuer C, Gurel B, Kama NA (2009) Effect of montelukast and MK-886 on hepatic ischemia-reperfusion injury in rats. J Surg Res 153:31–38
39. Luo Y, Yang YP, Liu J, Li WH, Yang J, Sui X, Yuan X, Nie ZY, Liu YQ, Chen D, Lin SH, WangYA (2014) Neuroprotective effects of madecassoside against focal cerebral ischemia reperfusion injury in rats. Brain Res
40. Park JH, Park OK, Cho JH, Chen BH, Kim IH, Ahn JH, Lee JC, Yan BC, Yoo KY, Lee CH, Hwang IK, Kwon SH, Lee YL, Won MH, Choi JH (2014) Anti-inflammatory effect of tanshinone I in

neuroprotection against cerebral ischemia-reperfusion injury in the gerbil hippocampus. Neurochem Res. doi:10.1007/s11064-014-1312-4
41. Kawano T, Matsuse H, Tsuchida T, Fukahori S, Fukushima C, Nishino T, Kohno S (2014) Cysteinyl leukotriene receptor antagonist regulates allergic airway inflammation in an organ- and cytokine-specific manner. Med Sci Monit 20:297–302
42. Yousefi B, Jadidi-Niaragh F, Azizi G, Hajighasemi F, Mirshafiey A (2014) The role of leukotrienes in immunopathogenesis of rheumatoid arthritis. Mod Rheumatol 24:225–235
43. Ni NC, Ballantyne LL, Mewburn JD, Funk CD (2014) Multiple-site activation of the cysteinyl leukotriene receptor 2 is required for exacerbation of ischemia/reperfusion injury. Arterioscler Thromb Vasc Biol 34:321–330
44. Huber M, Beutler B, Keppler D (1988) Tumor necrosis factor alpha stimulates leukotriene production in vivo. Eur J Immunol 18:2085–2088
45. Kanda N, Watanabe S (2007) Leukotriene B(4) enhances tumour necrosis factor-alpha-induced CCL27 production in human keratinocytes. Clin Exp Allergy 37:1074–1082
46. Rola-Pleszczynski M, Stankova J (1992) Leukotriene B4 enhances interleukin-6 (IL-6) production and IL-6 messenger RNA accumulation in human monocytes in vitro: transcriptional and posttranscriptional mechanisms. Blood 80:1004–1011
47. DiMeo D, Tian J, Zhang J, Narushima S, Berg DJ (2008) Increased interleukin-10 production and Th2 skewing in the absence of 5-lipoxygenase. Immunology 123:250–262
48. Abdel-Raheem IT, Khedr NF (2014) Renoprotective effects of montelukast, a cysteinyl leukotriene receptor antagonist, against methotrexate-induced kidney damage in rats. Naunyn Schmiedebergs Arch Pharmacol 387:341–353
49. Al-Amran FG, Hadi NR, Hashim AM (2013) Cysteinyl leukotriene receptor antagonist montelukast ameliorates acute lung injury following haemorrhagic shock in rats. Eur J Cardiothorac Surg 43:421–427
50. Al-Amran FG, Hadi NR, Hashim AM (2011) Leukotriene biosynthesis inhibition ameliorates acute lung injury following hemorrhagic shock in rats. J Cardiothorac Surg 6:81
51. Yuksel B, Aydemir C, Ustundag G, Eldes N, Kutsal E, Can M, Demirtas S, Tomac N (2009) The effect of treatment with montelukast on levels of serum interleukin-10, eosinophil cationic protein, blood eosinophil counts, and clinical parameters in children with asthma. Turk J Pediatr 51:460–465
52. Wang L, Huang Y, Wu J, Lv G, Zhou L, Jia J (2013) Effect of Buyang Huanwu decoction on amino acid content in cerebrospinal fluid of rats during ischemic/reperfusion injury. J Pharm Biomed Anal 86:143–150
53. Cai Q, Wang HW, Hua SY, Tan JZ, Zhou T, Li CS (2012) Neutroprotective efficacy of sodium tanshinone B on hippocampus neuron in a rat model of focal cerebral ischemia. Chin J Integr Med 18:837–845
54. Ding Q, Wei EQ, Zhang YJ, Zhang WP, Chen Z (2006) Cysteinyl leukotriene receptor 1 is involved in N-methyl-D-aspartate-mediated neuronal injury in mice. Acta Pharmacol Sin 27:1526–1536
55. Ding Q, Fang SH, Zhou Y, Zhang LH, Zhang WP, Chen Z, Wei EQ (2007) Cysteinyl leukotriene receptor 1 partially mediates brain cryoinjury in mice. Acta Pharmacol Sin 28:945–952
56. Ciceri P, Rabuffetti M, Monopoli A, Nicosia S (2001) Production of leukotrienes in a model of focal cerebral ischaemia in the rat. Br J Pharmacol 133:1323–1329
57. Mabe H, Nagai H, Suzuka T (1990) Role of brain tissue leukotriene in brain oedema following cerebral ischaemia: effect of a 5-lipoxygenase inhibitor, AA-861. Neurol Res 12:165–168
58. Ge QF, Hu X, Ma ZQ, Liu JR, Zhang WP, Chen Z, Wei EQ (2007) Baicalin attenuates oxygen-glucose deprivation-induced injury via inhibiting NMDA receptor-mediated 5-lipoxygenase activation in rat cortical neurons. Pharmacol Res 55:148–157
59. Knorr B, Franchi LM, Bisgaard H, Vermeulen JH, LeSouef P, Santanello N, Michele TM, Reiss TF, Nguyen HH, Bratton DL (2001) Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2–5 years. Pediatrics 108:E48
60. Volovitz B, Tabachnik E, Nussinovitch M, Shtaif B, Blau H, Gil-Ad I, Weizman A, Varsano I (1999) Montelukast, a leukotriene receptor antagonist, reduces the concentration of leukotrienes in the respiratory tract of children with persistent asthma. J Allergy Clin Immunol 104:1162–1167
61. Nakamori Y, Komatsu Y, Kotani T, Kojima S, Takeuchi K (2010) Pathogenic importance of cysteinyl leukotrienes in development of gastric lesions induced by ischemia/reperfusion in mice. J Pharmacol Exp Ther 333:91–98
62. Fulda S, Gorman AM, Hori O, Samali A (2010) Cellular stress responses: cell survival and cell death. Int J Cell Biol. doi:10.1155/2010/214074
63. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE (2009) Cell death. N Engl J Med 361:1570–1583
64. Whelan RS, Kaplinskiy V, Kitsis RN (2010) Cell death in the pathogenesis of heart disease: mechanisms and significance. Annu Rev Physiol 72:19–44
65. Gupta S, Kass GE, Szegezdi E, Joseph B (2009) The mitochondrial death pathway: a promising therapeutic target in diseases. J Cell Mol Med 13:1004–1033
66. Park S, Yoon J, Bae S, Park M, Kang C, Ke Q, Lee D, Kang PM (2014) Therapeutic use of HO-responsive anti-oxidant polymer nanoparticles for doxorubicin-induced cardiomyopathy. Biomaterials. doi:10.1016/j.biomaterials.2014.03.084
67. Shi SS, Yang WZ, Chen Y, Chen JP, Tu XK (2014) Propofol reduces inflammatory reaction and ischemic brain damage in cerebral ischemia in rats. Neurochem Res 39:793–799
68. Dief AE, Kamha ES, Baraka AM, Elshorbagy AK (2014) Monosodium glutamate neurotoxicity increases beta amyloid in the rat hippocampus: a potential role for cyclic AMP protein kinase. Neurotoxicology. doi:10.1016/j.neuro.2014.04.003
69. Korniichuk HM, Makohon NV, Aleksieieva IM, Lushnikova IV (2002) Effect of exogenous leukotrienes and lipoxygenase inhibitors on apoptosis and necrosis in cultured rat hepatocytes. Fiziol Zh 48:34–40
70. Becher UM, Ghanem A, Tiyerili V, Furst DO, Nickenig G, Mueller CF (2011) Inhibition of leukotriene C4 action reduces oxidative stress and apoptosis in cardiomyocytes and impedes remodeling after myocardial injury. J Mol Cell Cardiol 50:570–577
71. Lai J, Hu M, Wang H, Hu M, Long Y, Miao MX, Li JC, Wang XB, Kong LY, Hong H (2014) Montelukast targeting the cysteinyl leukotriene receptor 1 ameliorates Abeta1-42-induced memory impairment and neuroinflammatory and apoptotic responses in mice. Neuropharmacology 79:707–714