EVALUATION OF THE ANTI-ISCHAEMIC EFFECT OF SELECTIVE COX-2 INHIBITOR (ROFECOXIB) IN PERMANENT LEFT MIDDLE CEREBRAL ARTERY OCCLUSION (FOCAL CEREBRAL ISCHEMIA MODEL) IN RATS

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ABSTRACT

Cyclooxygenase-2 (COX-2) enzyme is induced in the central nervous system after various insults. It has been localized to neurons and in cells associated with the cerebral vasculature where the system is involved in the inflammatory component of the ischaemic cascade. COX-2 is part of the initial reaction that involves the arachidonic acid cascade, which produces molecules that involved in inflammatory response. The present study evaluated the pharmacological effects of a specific COX-2 inhibitor (rofecoxib), in a permanent focal cerebral ischaemia model in albino rats and its effects were compared to those of calcium channel blocker (nimodipine).

Experiments were carried out on sixty male albino rats. Focal cereberal ischemia was induced by middle cerebral artery occlusion.

Rofecoxib and nimodipine were administered 30 minutes after the occlusion of middle cerebral artery [MCA] and then daily IP for successive 6 days during which neurobehavioral evaluation was done. On the 7th day of occlusion, the infarction size, was measure and the remote hippo-

campal cell death were determind . Treatment with either rofecoxib or nimodipine caused significant equal improvement of the neurological score, significant attenuation of the infarction size and hippocampal cell death. While, the decrease of the infarction size was 50% by both drugs, the percentage of reduction of hippocampal degeneration was only 10% by both drugs. This difference in the percentage of improvement of infarction sizes and hippocampal degeneration may be due to presence of the hippocampus in a remote site from MCA blood supply.

The present study suggest that COX-2 plays an important role in the ischaemic cascade of events. Furthermore, selective COX-2 inhibitors may be useful in the treatment of ischaemic stroke to improve motor functions.

INTRODUCTION

Ischemia activates a cascade that leads to the induction and expression of genes in a variety of cell types throughout the central nervous system (CNS) [1,2]. The inflammatory mediator pathway has been implicated as a potential contributor to ischemia-induced deficits[3]. Cyclooxygenase-2 (COX-2), has become the focus of attention because it is the rate-limiting enzyme involved in arachidonic acid metabolism, thereby generating prostaglandins and thromboxanes, molecules that play important roles in supporting and sustaining the inflammatory response [4]. COX-2 can be induced in neurons and in cells associated with the cerebral vasculature after various CNS insults, including global [5,6] and focal ischemia [7].

The possibility that COX-2 may be a crucial component in ischemia induced neurodegeneration has been the subject of several recent studies that used rodent models of focal ischemia[7]. The COX-2 inhibitors SC-58125 NS-398 have been shown to prevent delayed death of hippocampal neurons[8] and reduce infarct size[9] after global ischemia.

The main purpose of the present study was to investigate the effect of COX-2 inhibitor, rofecoxib, or the Ca+2 channel blocker, nimodipine, on the behavioral deficits, the infarction

volume, the hippocampal cell death in a rat model of focal cerebral ischaemia induced by permanent unilateral middle cerebral artery occlusion. The effect of rofecoxib was compared with those of nimodipine.

MATERIALS AND METHODS

Selective COX-2 inhibitor: Rofecoxib-powder (Vioxx, Global-Napi) and calcium channel blocker: Nimodipine- tablets 30 mg (Nimotop, Bayer)

Animals and Experimental protocol :

Sixty male albino rats each weighing 250-300 grams, were used. Rats were put under similar housing condition, and were allowed to eat and drink ad Libitum. Rats were subdivided into 6 equal groups (10 rats for each); 3 groups were sham operated in which rats were subjected to the same surgical procedure as focal cerebral ischaemia groups but without diathermic occlusion of the middle cerebral artery. The other 3 groups were subjected to left middle cerebral artery (MCA) occlusion. All animals received medications intraperitoneally 30 minutes after surgery and then once daily for successive 6 days. Drugs were dissolved in saline and each dose was given in 1 mL

Animal groups :

1- Non treated SHAM operated group: Rats were given 1 mL saline
2- Rofecoxib treated SHAM operated group: Rats were treated with rofecoxib at a dose of 10 mg/kg body weight/day (10).
3- Nimodipine treated SHAM - operated group: Rats were treated with nimodipine at a dose of 5mg/kg body weight/day (11).
4- Non treated focal cerebral ischaemic group: Rats were given 1 mL saline
5- Rofecoxib treated focal cerebral ischaemic group: Rats were treated with rofecoxib at a dose of 10 mg / kg body weight /day (10).
6- Nimodipine treated focal cerebral ischaemic group: Rats were treated with nimodipine at a dose of 5 mg /kg body weight /day(11).

Surgical Procedures :

The technique of middle cerebral artery occlusion described by Tamura et al., [12] was used in this study. Each animal was anesthetized by thiopental sodium 30 mg /kg b.wt/day

intraperitoneal (13). The MCA was approached through a temporal incision, and the bone overlying the vessel was removed using a dental drill. The dura was opened, and the arachnoid membrane was gently removed. The vessel was occluded by thermal coagulation, from a point proximal to the lenticulostriate branches to the rhinal fissure. The incision was closed, and the rats were returned to their home cage after full recovery from anesthesia.

Effect of MCA occlusion on neurological score :

Neurobehavioral evaluation was started on the next day after surgery and carried out for successive 6 days, using the neurological grading system developed by Garcia et al (14). This method includes the evaluation of the grads of :

1-spontaneous activity
2-Symmetry in the movement of four limbs.
3-Forepaw out stretching
4-Climbing
5-Body propioception
6-Response to vibrissae touch

The score which given to each rat at the completion of the evaluation is the summation of all six individual test scores.

Effect of MCA occlusion on the infarction size and remote "hippocampal" histopathological changes :

On the 7th day after MCA occlusion, the rats were killed by knifing and their brains were quickly removed.

A. Evaluation of ischaemic area by 2,3,5 tripheny1 tetrazolium chloride (TTC) staining of the brain (15).

On the 7 days of MCA occlusion the rats were killed by knifing, five brains of each group were quickly removed and placed in ice-cold saline for 5 minutes and then cut into 2-mm. coronal slices. Sections were incubated in TTC-containing saline solution (Sigma chemical Co.) for 20 minutes. Then, the slices were refrigerated in 10 % formalin over night. The infracted areas were outlined in white (15). The longitudinal and transverse axis were measured in mm. between the farthest two points. The percentage of infracted size was measured by adobe photoshop-5 program

B. Histopathological examination of the left hippocampus:

After decapitation, the other five rat brains of each group were, dissected and the left hippocampi were removed and placed in formalin as preservative. Longitudinal sections (5μm thick) obtained using a microtome. Sections were stained by Glees' method according to Clark, (16). Cell counts were performed using a light microscope at a magnification of 100x and 400x and expressed as the percentage of the ischaemic cell to the total cell count per section (17).

Statistical analysis :

Statistical significance was assessed with the ANOVA test according to Armitage & Berry, (18). $P < 0.05$ was considered to be significant.

RESULTS

1- Effect of rofecoxib and nimodipine on neurological score in permanent focal cerebral ischaemia (MCA occlusion) : [table1 , chart1]

The mean neurological scores of all sham groups (either treated or non- treated) were 18 starting from the second postoperative day and maintained till the end of duration of follow up (7days).

The mean neurological scores were significantly decreased in the focal cerebral ischaemic groups as compared to sham groups starting from the second postoperative day until time of sacrifaction.

There was a significant increase in the mean neurological score starting in the 3rd postoperative day in the ischaemic groups treated by rofecoxib or nimodipine (gps 5 & 6) as compared to the non-treated ischaemic group (gp 4). Also, in the rofecoxib treated focal ischaemic group (gp5), there was significant increase in the mean neurological score in the 4th postoperative day versus the 2nd and 3rd post operative days. Meanwhile, in the nimodipine treated focal cerebral ischaemic group (gp 6), there was significant increase in the mean neurological score in the 4th postoperative day versus the 2nd postoperative day. The levels of scores noted on the 4th day were maintained till the 7th postoperative day. These levels of scores were significantly higher than those obtained in the non-treated ischaemic group, but still significantly less than those of sham groups.

2- Effect of rofecoxib and nimodipine on the infarction size and remote "hippocampal" histopathological changes in permanent focal cerebral ischaemia (MCA occlusion) :

A- Evaluation of the infracted area by 2,3,5 tripheny1 tetrazolium chloride (TTC) stain of the brain sections: [table2 , fig 1]

In the MCA occlusion group (gp 4), the infracted areas was extended from the outer limit of the lateral surface to the outer limit of the inferior surface of the parietal cortex. The infarction affected all the thickness of the cortex. It also affected the lateral part of the caudate nucleus.

The infracted size was significantly decreased in the MCA occluded groups treated with either rofecoxib or nimodipine (gps 5 or 6) versus the non treated MCA occluded group (gp 4). There was non-significant difference between the effect of rofecoxib or nimodipine on the size of infracted areas

Both drugs resulted into withdrawal of the infarction boundaries of the dorsal and inferior surfaces of the parietal cortex. Also, treatment with both drugs made the infarction areas more superficial with decrease in the depth of the cortex lesion. This led to sparing of the adjacent part of the cortex to the corpus callosum. The latter observation was noted in 3 of 5 rats treated with nimodipine versus 2 of 5 rats treated with rofecoxib.

B- Histopathological examination of the left hippocampi : [table3 , fig2]

The ischaemic groups showed significant degeneration in the ipsilateral (left) hippocampus as compared to the sham groups. [Fig2].

The degeneration was in the pyramidal cell layer which lost its normal architecture, the nucleus became clumped, chromatic and picknotic. The cytoplasm had argyrophillic condensations. The axons of degenerated neurons in white matter appeared beaded and tortuous.

Treatment with either rofecoxib or nimodipine in the focal ischaemic groups (gps 5 & 6) caused significant decrease in the percentage of the degenerated cell in comparison to the non-treated ischaemic group (gp 4). But there was non-significant difference between the effect of nimodipine or rofecoxib on the histopathological picture of the hippocampus.

Table (1): Effect of Rofecoxib and Nimodipine on Neurological Scores in Permanent Focal Cerebral Ischaemia (MCA Occlusion) in Albino Rats *(One-way ANOVA, Mean±SEM, P<0.05 indicates significance, n=10)*

	2nd day	3rd day	4th day	5th day	6th day	7th day
Sham groups **Gp1 : Non treated** **Gp 2 : Rofecoxib treated** *(10 mg/Kg IP for 7 days)* **Gp 3: Nimodipine treated** *(5 mg/Kg IP for 7 days)*	18 ± 0.0	18 ± 0.0	18 ± 0.0	18 ± 0.0	18 ± 0.0	18 ±0.0
Gp 4: Non treated focal cerebral ischaemia group	4.0±0.43 $^{\Psi}$	5.5±0.22 $^{\Psi}$	5.7±0.15* $^{\Psi}$	5.7±0.15* $^{\Psi}$	5.7±0.15* $^{\Psi}$	5.7±0.15* $^{\Psi}$
Gp 5: Rofecoxib treated focal cerebral ischaemia group *(10 mg/Kg IP for 7days)*	5.8±0.36$^{\Psi}$	9.4±0.67$^{*\Psi\$}$	11.4±0.50 $^{*\#\Psi\$}$	11.4±0.50 $^{*\#\Psi\$}$	11.4±0.50$^{*\#\Psi\$}$	11.4±0.50 $^{*\#\Psi\$}$
Gp 6: Nimodipine treated focal cerebral ischaemia group *(5 mg/Kg IP for 7 days)*	5.4±0.48 $^{\Psi}$	9.7±0.68$^{*\Psi\$}$	11.2±0.79$^{*\Psi\$}$	11.2±0.79$^{*\Psi\$}$	11.2±0.79 $^{*\Psi\$}$	11.2±0.79$^{*\Psi\$}$

$^{\Psi}$ *versus sham groups* $^{\$}$ *versus non treated focal cerebral ischaemia group*
* *versus the 2nd day* $^{\#}$ *versus the 3rd day*

Table 2: Effect of Rofecoxib and Nimodipine on the Infracted size of Brain Sections Stained by 2,3,5 Triphenyl Tetrazolium Chloride (TTC) stain After 7 Days of Permanent Focal Cerebral Ischaemia (MCA Occlusion) in Albino Rats

(one way ANOVA, Mean±SEM, P<0.05 indicates significance, n=5)

	Trans. axis of total infracted area (mm)	long. axis of total infracted area (mm)	Percentage of total infracted area
Gp 4: Non treated focal cerebral ischaemia group	19.4±1.2	44.8±1.8	19.1±1.2
Gp 5: Rofecoxib treated focal cerebral ischaemia group *(10 mg/Kg IP for successive 7 days*	11.8±1.8*	33.2±2.2*	8.5±0.8*
GP 6: Nimodipine treated focal cerebral ischaemia group *(5 mg/Kg IP for successive 7 days)*	10±0.8*	33±1.3*	8.5±0.7*

* *versus non treated focal cerebral ischaemia group*

Table(3): Effect of Rofecoxib and Nimodipine on Histopathological Examination of the Left Hippocampi (modified Glees method) After 7 Days of Permanent Focal Cerebral Ischaemia (MCA occlusion) in Albino Rats

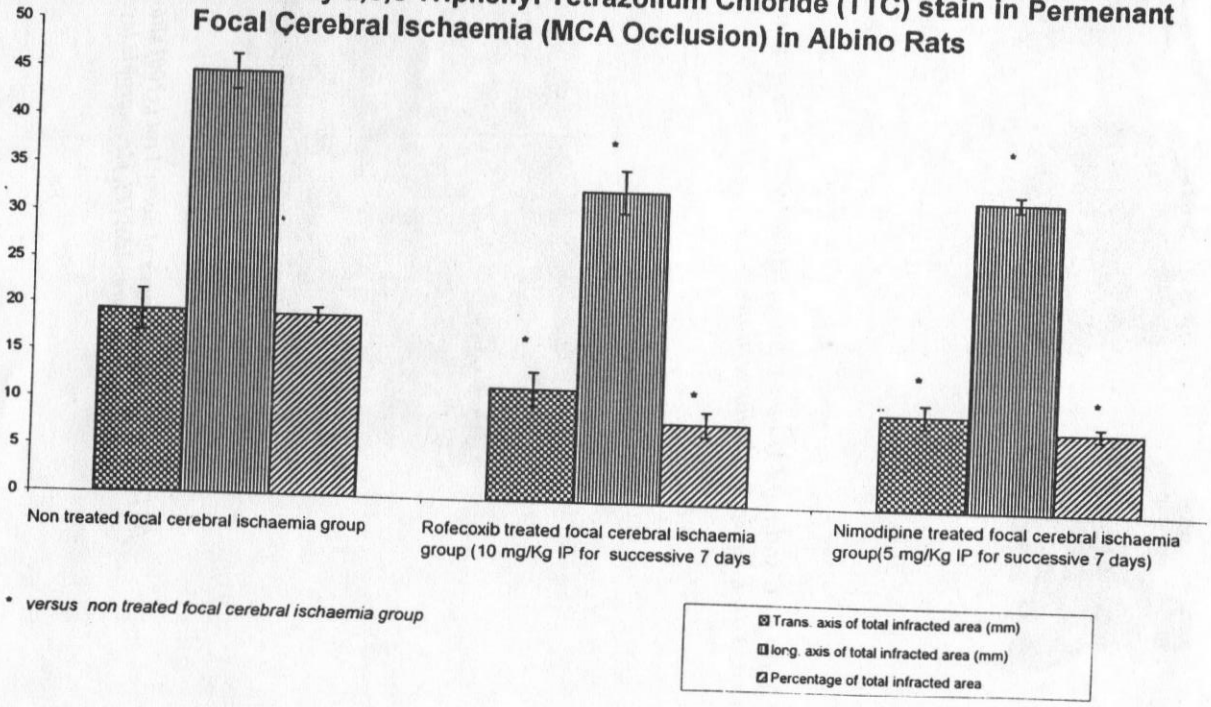
(one way ANOVA, Mean ± SEM, P < 0.05 indicate significance, n=5)

Groups	Percentage of degenerated cell per section
Non treated focal cerebral ischaemic group	90.5 ± 0.8
Rofecoxib treated focal cerebral ischaemic group *(10 mg/kg ip for 7 days)*	77.9 ± 0.5*
Nimodipine treated focal cerebral ischaemic group *(5 mg kg ip for 7 days)*	78.5 ± 1.1*

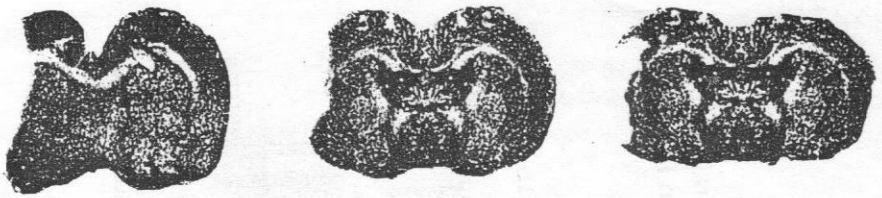
* *versus non treated focal cerebral ischaemic group.*

Chart (1): Effect of Rofecoxib and Nimodipine on Neurological Scores in Permenamt focal Cerebral Ischaemia (MCA Occlusion) in Albino Rats
20
18
16
14
12
10
8
6
4
2
0
2 nd day
3 rd day
4 th day
5 th day
6 th day
7 th day
SHAM groups (GPs1,2,3)
Non treated focal cerebral ischaemia (GP4)
Rofecoxib treated focal cerebral ischaemia group (GP5) 10 mg/kg IP for 7 days
Nimodipine treated focal cerebral ischaemia group (GP6) (5 mg/Kg IP for 7 days)
* versus the 2nd day
# versus the 3 rd day
Ψ versus sham groups
$ versus non treated focal cerebral ischaemia group

Chart (2): Effect of Rofecoxib and Nimodipine on the Infracted size of Brain Sections Stained by 2,3,5 Triphenyl Tetrazolium Chloride (TTC) stain in Permenant Focal Cerebral Ischaemia (MCA Occlusion) in Albino Rats



* *versus non treated focal cerebral ischaemia group*



A B C

Fig (1) TTC stain of (A) non-treated, (B) rofecoxib treated and (C) nimodipine treated ischaemic forebrain sections.

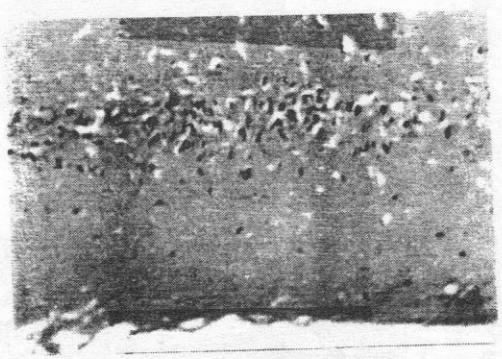


fig [2] L.S of hippocampus of normal rat [x100] showing the 3 layers; molecular [M], pyramidal [P], pleomorphic layers [PM]

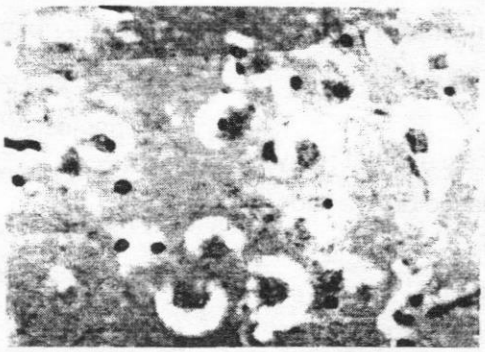


Fig [3] L.S of hippocampus of ischaemic non-treated rat [x400] showing degenerated pyramidal cell layer ; shrunken cell, loss of cellular architecture and replaçement by vaculated areas

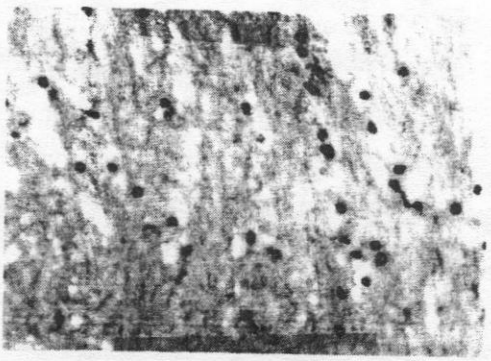


Fig [4] L.S of hippocampus of ischaemic rofecoxib treated rat [x400] showing less degeneration in the pyramidal cell layer with little vaculation

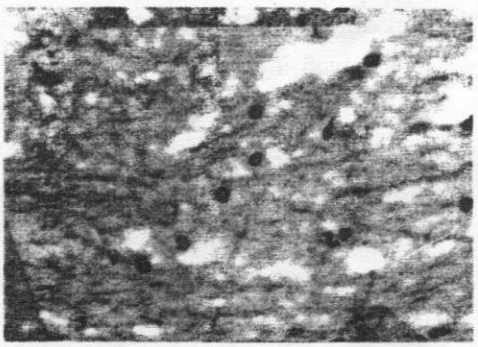


Fig [5] L.S of hippocampus of ischaemic nimodipine treated rat [x400] showing less degeneration in the pyramidal layer with little vaculation

DISCUSSION

The two major mechanisms causing brain damage in stroke are, ischaemia and hemorrhage [22].

One of the processes that may play a role in the delayed progression of the damage is post-ischaemic inflammation [3,19]. Cerebral ischaemia is followed by infiltration of neutrophils in the ischaemic brain, a process initiated by local expression of cytokines, chemokines and adhesion molecules [20]. Expression of cyclooxygenase-2 has emerged an important determinant of cytotoxicity associated with inflammation [21]. COX-2 expression may be induced by activation of glutamate receptors [23]. Expression of COX-2 has been shown to be determinant of cytotoxicity through modulation of neurotransmitter release [24].

It was noted also that the time course of COX-2 induction ran in parallel with the expression profile of inflammatory genes for cytokines, adhesion molecules [3]. Another mechanism by which COX-2 induces neuronal cell death is associated with the generation of highly reactive oxygen species (ROS) during the synthesis of prostanoids [4]. Also, COX-2 reaction product PGE2 could contribute to ischaemic cell death through induction of apoptosis [26]. Permanent focal cerebral ischemia rat model simulates the human stroke due to cerebral embolus or thrombus [27].

Rofecoxib was chosen on the basis of its high COX-2 selectivity, potency [28], and can readily cross the B.B.B [29]. Its dose (10 mg/kg body weight /day) was the dose that inhibits COX-2 enzyme activity and therefore PGs formation in rodents [30]. Nimodipine was chosen because it is the most widely studied calcium channel blocker in the treatment of acute cerebral ischaemia [31]. It is the most lipophilic member of 1,4-dihydropyridire group. So, its distribution volume in the brain of rats is very high [32]. The mechanism of action of nimodipine in acute focal cerebral ischaemia relates to its ability to produce selective cerebral vasodilatation and to block calcium entry into neurons by direct action on L-type voltage-sensitive calcium channels [33].

In our study, neurobehavioral evaluation was done according to Garcia et al.,[15]. Pantoni et al., [34] stated that the neurological score designed by Garcia et al., [14] represents a useful tool for the assessment of functional outcome in animals with ischaemic brain damage. Roof et al., [35] stated that the experimental used sensorimotor and cognitive tests are sensitive enough to detect the effects of therapeutic agents and for tracking behavioral recovery over time that occurs after MCA occlusion, making them useful for comparing the effects of different doses and/or drugs.

The mean neurological scores of all sham groups, either treated or non treated, were maintained at 18 till time of sacrifaction. While in the focal cerebral ischaemia group the neurological score was significantly decreased on the 2nd postoperative day The score was then increased on the 3rd postoperative day to be maximum on the 4th postoperative day and maintained till time of scarification. In agreement with this result Garcia et al., [14] found that the mean neurological scores were decreased after permanent MCA occlusion in comparison to the control rats. Another more complex behavioral testing was done by Roof et al., [35] who used Tamura et al., [12] model of proximal right MCA occlusion. They found that the sensorimotor function was impaired significantly by about 60% on the 7th day of MCA occlusion compared with shamoperated control rats, which is in accordance to our result. They showed also non-significant improvement at the end of 30 days of testing in comparison to the 7th day of occlusion. They related the intensity of the behavioral alteration to the extensive ischaemic changes in the caudoputamenal tissues, which is situated furthest from the collateral arterial connections on the brain surface. The caudate nucleus, the putamen and the globus pallidus are the components of the basal ganglia [36]. The motor function is primarily carried out by the putamen, whereas cognition and emotion are primarily carried out by the caudate nucleus [37].

In our study, treatment with the selective COX-2 inhibitor, rofecoxib after occlusion of the MCA caused improvement of the mean neurologi-

cal score since the 2nd postoperative day, which was significantly increased by the 3rd day. This increase was maximum by the 4th postoperative day, a level that was maintained till the 7th day. These results were supported by Cernak et al., (38), as they found that administration of the selective COX-2 inhibitor nimesulide after brain injury resulted in a significant improvement in cognitive deficits and motor dysfunction compared with vehicle treated control (38).

Furthermore, Candelario et al (7) found that treatment with the selective COX-2 inhibitor nimesulide at doses of 3,6,12 mg/kg given. before the onset of permanent MCA occlusion reduced the neurological deficits and the motor impairment 24 hours after occlusion .

The study of Gupta et al. showed that, administration of meloxicam at a dose of 2.5/kg 4 hours after transient MCA occlusion in rats produced a non-significant improvement in the motor performance (39). However, combination between meloxicam and melatonin at a dose of 20 mg/kg as an antioxidant led to significant improvement in the motor performance. This synergestic neuroprotective effect of melatonin plus meloxicam was related to the prevention of the initial damage of free radical by the antioxidant,melatonin and the protection of the neurons from further delayed progression of neuronal death by the COX-2 inhibitor, meloxicam (47).

In our study treatment with nimodipine resulted in improvement of the mean neurological score since the 2nd postoperative day, which was significantly increased by the 3rd. day. This increase was maximum by the 4th postoperative day, a level that was maintained till the 7th day. These levels of score were significantly higher than those obtained in the non-treated ischaemic group, but still significantly less than those of sham group. There are some discrepancies about the successful effect of nimodipine in treatment of stroke both experimentally and clinically. In experimental models, some authors reported that nimodipine induced significant improvement of neurological outcome after induction of ischaemia (37,40,41,42). Other authors noted non-significant improvement of neuro-

logical outcome following MCA occlusion and nimodipine treatment [43, 44]. Scriabine & Kerckhoff, [32] found that nimodipine reduced neurological deficits after permanent MCA occlusion in rats. They showed significant improvement in the behaviour outcome on the 2nd day of focal ischaemia and gradually increased till the 7th day.

Several clinical trials with nimodipine had been done in human stroke. Gelmers et al., [45] reported a significantly improved neurological outcome in patients treated with nimodipine (begun within 24 hours of the onset of symptoms of an acute ischaemic stroke. On the other hand, Infeld et al.,[31] and Horn et al., [46] found no effect of oral nimodipine on the functional outcome after stroke. Infeld et al.,[31] noted that oral nimodipine administered within 12 hours (30 mg every 6 hours) for 2 weeks enhanced acute reperfusion but with adverse neurological and functional outcome in patients with MCA infarction. So the beneficial effect of nimodipine may be counteracted by the reperfusion injurious products as toxic free radicals and numerous enzymatic processes which exacerbates tissue damage [47]. Also, very early use of nimodipine 30 mg within 6 hours after the onset of stroke and for 10 days showed poor functional outcome compared with placebo [46].

In our study, MCA occlusion resulted in an infracted areas which was extended from the outer border of the dorsal surface to the outer border of the inferior surface of the parietal cortex. The infarction affected all the thickness of the cortex. It also affected the lateral part of the caudate nucleus. These results were in agree with Tamura et al.,[12], Garcia et al [48] and Roof et al., [35]

In our study, I.P administration of rofecoxib immediately after the occlusion of MCA and daily for successive 7 days caused significant decrease in the size of the infracted areas by 50% nearly in comparison to the non treated focal cerebral ischaemia group. This result was supported by Candelario-Jalil et al., [7] who reported that administration of the selective COX-2 inhibitor, nimesulide. before permanent MCA reduced the total infarct

volume. In addition, Govani et al., [49] found that in vivo rat model of cerebral ischaemia induced by Rose Bengal injection and light activation, the selective COX-2 inhibitors SC58236 adminstered just before the occlusion decreased the infarction volume.

In contrast, Hara et al., [50] reported that the total infarct volume was unaffected by the selective COX-2 inhibitor administered just before and 2 hours after transient focal cerebral ischaemia. Gupta et al., [39] demonstrated that the selective COX-2 inhibitor, meloxicam administered 4 hours after transient MCA occlusion showed a non-significant decrease in the infarct volume

This contradiction between these bibliographic data could be explained by the presence of both toxic and protective prostaglandin (EP) receptors [51]. Genetic deletion of EP2 receptors in vivo models of transient MCA occlusion led to increased infarct volume compared with wild mice [52]. Activation of the EP2 receptor can prevent injury in acute excitotoxicity and ischaemia. Activation of EP2 receptor resulted in activation of cAMP signaling which is neuroprotective [53]. McCullough et al.,[52] thought that the toxicity of COX-2 enzymatic activity is mediated through the PG receptor subtypes that are not positively coupled to cAMP e.g. EP1, EP3 receptors. EP3 receptors are coupled negatively to cAMP whereas EP1 receptor coupled to increased phosphoinositol turnover with elevation of intracellular calcium [51,54].

In our study, treatment with nimodipine IP for successive 7 days after permanent MCA occlusion showed 50% significant decrease in the size of infracted area delineated by TTC stain as compared with non-treated MCA occluded group. Other previous studies also had demonstrated that nimodipine significantly decreased the infraction size in focal cerebral ischaemia [40, 41, 42]. Moreover, prolonged intravenous administration of nimodipine starting before ischaemia reduced infarct size by 20 to 60% [50]

In the present study, the ischaemic groups showed significant degener-

ation in the ipsilateral (left) hippocampus as compared to the sham groups. States et al., [56] reported that hippocampal neurons die with DNA fragmentation in 50% of animals following permanent MCA occlusions. They related the mechanism of this hippocampal injury to transynaptic activation of N-methyl-D-aspartate (NMDA) receptors that mediate induction of early genes as, heat shock protein (HSP70) that are responsible for cell death in the hippocampus. Also Candelario-Jalil et al., [17] reported that there was progressive and significant decrease in neuronal density in the hippocampus starting in the 2nd day following transient global ischaemia and reperfusion and up to the 7th day of recirculation compared with sham groups.

In our study, I.P administration of rofecoxib for successive 7 days after permanent MCA occlusion caused significant decrease of ischaemic cells in the hippocampi by 10% in comparison to the non-treated ischaemic group. Previous study of Nakayama et al., [8] noted that expression of COX2-mRNA and protein was increased after ischaemia in hippocampal neurons before their death. Furthermore, hippocampal neurons survival was increased in rats treated with selective COX-2 [8] The neuroprotective effects observed with COX-2 selective inhibitors are partly mediated through reduction of COX-2 induced damage in the hippocampus following excitotoxic brain injury [57]. Also, Deng and Feng [11] reported that treatment with rofecoxib kainate infusion reduced kainate-induced cell death in the rat hippocampus and specially protected pyramidal cell from death.

In our research, we found that I.P. administration of nimodipine after permanent MCA occlusion caused significant decrease in the percentage of degenerated cells in the left hippocampi sections by 10%. This protective effect was in accordance with Nuglisch et al., [58] who noted that IP administration of nimodipine 60 minutes prior to 10 minutes bilateral carotid clamping led to significant reduction of neuronal damage in hippocampai. Nuglisch et al., [58] related these findings to the direct action of nimodipine on the neurons and not to the post-ischaemic cerebral va-

sodilation. The beneficial cytoprotective effect of nimodipine was related to normalization of calcium homeostasis and BBB permeability after ischaemia .

In conclusion both rofecoxib and nimodipine caused equal improvement of the neurological score, infarction sizes and hippocampal degenerations. While, the decrease of the infarction size was 50% by both drugs, the percentage of reduction of hippocampal degeneration was only 10% by both drugs, because the hippocampus is a remote site from the MCA blood supply and its degeneration may be due to induction of genes that are responsible for cell death.

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تقييم تأثير مثبط إنزيم السيكلواوكسچينيز (٢) (روفيكوكسيب) فى حالة قصور بؤرى دائم للدورة الدموية المخية فى الجرذان البيضاء

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لاتزال السكتة الدماغية من الأمراض التى تؤدى إلى العجز وأحياناً الموت حيث أنه لايوجد دواء فعال لعلاج هذه المشكلة. وقد ثبت زيادة إنزيم السيكلواوكسجينيز (٢) فى مناطق مختلفة من المخ والتى تؤدى لإحداث الضرر إما عن طريق زيادة الناقلات الكيميائية للإشارات العصبية أو زيادة المؤكسدات التى تؤدى لموت الخلايا بالمخ.

وجاءت فكرة هذا البحث لتقييم تأثير تثبيط إنزيم السيكلواوكسچينيز (٢) بدواء الروفيكوكسيب فى حالة قصور الدورة المخية فى الجرذان البيضاء.

وقد أجرى هذا البحث على ٦٠ فأراً بيضاء حيث قسمت الى ٦ مجموعات كل منها ١٠ فئران : ٣ مجموعات ضابطة و٣ مجموعات يتم فيها ربط دائم للشريان المخى الأوسط الأيسر. تتلقى هذه الجرذان الدواء عن طريق الحقن فى التجويف البريتونى بعد اذابة التركيز المطلوب فى ١سم٣ محلول ملح يومياً لمدة ٧ أيام متتالية وتنقسم هذه الجرذان الى المجموعات التالية :

١- مجموعة ضابطة غير معالجة : أعطيت ١سم٣ محلول يومياً لمدة ٧ أيام.

٢- مجموعة ضابطة معالجة بمثبط انزيم السيكلواوكسچينيز(٢) :

تم علاجها بالروفيكوكسيب ١٠ مجم/كجم يومياً لمدة ٧ أيام.

٣- مجموعة ضابطة معالجة بغلق قنوات الكالسيوم:

تم علاجها بالنيموديبين ٥ مجم/ كجم يومياً لمدة ٧ أيام.

٤- مجموعة مصابة بقصور الدورة الدموية البؤرى الدائم غير معالجة :

اعطيت ١سم٣ محلول ملح يومياً لمدة ٧ أيام.

٥- مجموعة مصابة بقصور الدورة الدموية البؤرى الدائم معالجة بمثبط انزيم السكلواوكسچينيز(٢): تم علاجها بالروفيكوكسيب ١٠ مجم / كجم يومياً لمدة ٧ أيام.

٦- مجموعة مصابة بقصور الدورة الدموية البؤرى الدائم معالجة بغالق قنوات الكالسيوم: تم علاجها بالنيموديبين ٥ مجم / كجم يوميا لمدة ٧ أيام.

وقد تم تقييم تأثير الأدوية المستخدمة على الخلل الناتج فى الوظائف نتيجة لقصور الدورة الدموية للمخ فى هذا النموذج التجريبى فى الفئران بدراسة :

- السلوك الحركى للفئران من اليوم التالى لربط الشريان المخى الأوسط (٧ أيام متتالية).
- فى اليوم السابع ، تم أخذ المخ من كل مجموعة وتقسيمهم الى مجموعتين تحتوى كل منهما على ٥ عينات : أحداهما لتحديد حجم التنكرز باستخدام صبغة ٢ ، ٣ ، ٥ ترای فينيل تترازوليم كلورايد، والأخرى لتحديد الخلايا المصابة فى منطقة الهيبوكامباس .

وكانت النتائج هى نجاح كل من الروفيكوكسيب والنيموديبين فى تقليل الإصابة من ناحية السلوك الحركى أو حجم التنكرز أو الخلايا المصابة فى الهيبوكامباس .

ولوحظ فى هذا الجزء من البحث تأثر الهيبوكامباس رغم بعدة عن التوزيع الدموى للشريان المخى الأوسط. وكذلك فاعلية كل من الروفيكوكسيب والنيموديبين فى تقليل إصابته ولكن ليس بالتناسب فى فاعليتهما على درجة التنكرز أو السلوك الحركى مما يشير الى وجود ميكانيكة خاصة فى تأثر الهيبوكامباس فى حالة القصور الدموى المخى. كما يشير أيضاً الى الحاجة لعلاج دوائى يتناسب مع هذه الميكانيكية المفترضة.