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

By
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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-
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
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 (7 days).

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 sacrifice.

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.

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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)

<table>
<thead>
<tr>
<th></th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
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<tbody>
<tr>
<td>Sham groups</td>
<td></td>
<td></td>
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<tr>
<td>Gp 1: Non treated</td>
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<tr>
<td>Gp 2: Rofecoxib treated (10 mg/Kg IP for 7 days)</td>
<td>18 ± 0.0</td>
<td>18 ± 0.0</td>
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<td>18 ± 0.0</td>
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<td>18 ± 0.0</td>
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<tr>
<td>Gp 3: Nimodipine treated (5 mg/Kg IP for 7 days)</td>
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<tr>
<td>Gp 4: Non treated focal cerebral ischaemia group</td>
<td>4.0±0.43*</td>
<td>5.5±0.22*</td>
<td>5.7±0.15*</td>
<td>5.7±0.15*</td>
<td>5.7±0.15*</td>
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</tr>
<tr>
<td>Gp 5: Rofecoxib treated focal cerebral ischaemia group (10 mg/Kg IP for 7 days)</td>
<td>5.8±0.36*</td>
<td>9.4±0.67*</td>
<td>11.4±0.50*</td>
<td>11.4±0.50*</td>
<td>11.4±0.50*</td>
<td>11.4±0.50*</td>
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<tr>
<td>Gp 6: Nimodipine treated focal cerebral ischaemia group (5 mg/Kg IP for 7 days)</td>
<td>5.4±0.48</td>
<td>9.7±0.68</td>
<td>11.2±0.79</td>
<td>11.2±0.79</td>
<td>11.2±0.79</td>
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</tbody>
</table>

* versus sham groups  
* versus non treated focal cerebral ischaemia group  
* versus the 2nd day  
* versus the 3rd day
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)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of degenerated cell per section</th>
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</thead>
<tbody>
<tr>
<td>Non treated focal cerebral ischaemic group</td>
<td>90.5 ± 0.8</td>
</tr>
<tr>
<td>Rofecoxib treated focal cerebral ischaemic group (10 mg kg lp for 7 days)</td>
<td>77.9 ± 0.5*</td>
</tr>
<tr>
<td>Nimodipine treated focal cerebral ischaemic group (5 mg kg lp for 7 days)</td>
<td>78.5 ± 1.1*</td>
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</tbody>
</table>

* versus non treated focal cerebral ischaemic group.
Chart (2): Effect of Rofecoxib and Nimodipine on the Infarcted size of Brain Sections Stained by 2,3,5 Triphenyl Tetrazolium Chloride (TTC) stain in Permanent Focal Cerebral Ischaemia (MCA Occlusion) in Albino Rats

* versus non treated focal cerebral ischaemia group

- Trans. axes of total infarcted area (mm)
- Long. axes of total infarcted area (mm)
- Percentage of total infarcted area
Fig [3] L.S of hippocampus of ischaemic non-treated rat [x400] showing degenerated pyramidal cell layer; shrunken cell, loss of cellular architecture and replacement 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.
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 infarcted 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 infarcted 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.
iation 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-


flammation by COX-2.


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- مجموعة مصابة بقصور الدورة الدموية البؤري الدائم غير معالجة:
  أعطيت اسهم 3 محلول ملح يومياً لمدة 7 أيام.
- مجموعة مصابة بقصور الدورة الدموية البؤري الدائم معالجة بمشتبه ازيم السكلاوكسيجينزيمات: تم علاجها بالروفوكوكسيبي 10 مجم / كجم يومياً لمدة 7 أيام.
- مجموعة مصابة بقصور الدورة الدموية البؤري الدائم معالجة بتفاعل قنوات الكالسيوم: تم علاجها بالنيموسيسين 5 مجم / كجم يومياً لمدة 7 أيام.

وقد تم تقييم تأثير الأدوية المستخدمة على الخلل الناتج في الوظائف الناتجة لقصور الدورة الدموية للمخ في هذا النموذج التجيري في الفئران بدراسة:
- السلوك الحركي للفئران من اليوم التالي لربط الشريان المخ الأوسط (7 أيام متتالية).
- في اليوم السابع، تم أخذ المخ من كل مجموعة وتقسيمهم إلى مجموعتين تحتوي كل منهما على 5 عينات، أخذوا تحديد حجم التتكز باستخدام صبغة 2، 3، 4، 6 تراي فينيل تترازوليم
- كلوريد، والخ ليتم تحقيق الخلايا المضادة في منطقة الهيبوكاماس.

وكانت النتائج هي نجاح كل من الروفوكوكسيبي والنيموسيسين في تقليل الإصابة من ناحية السلوك البؤري أو حجم التتكز أو الخلايا المضادة في الهيبوكاماس.

ولوحت في هذا الجزء من البحث تأثير الهيبوكاماس رغم بعدة عن التوزيع الدموي للخلي الأعظم. وكذلك فاعلية كل من الروفوكوكسيبي والنيموسيسين في تقليل إصابته ولكن ليس بالتناسب في فاعليةهما على درجة التتكز أو السلوك البؤري مما يشير إلى وجود ميكانيكا خاصة في تأثير الهيبوكاماس في حالة القصور الدموي المخ. كما يشير أيضاً إلى الحاجة لعلاج دوالي يتناسب مع هذه الميكانيكا المفترضة.