Differential Effect of Three Cyclooxygenase Inhibitors on Human Cerebral Blood Flow Velocity and Carbon Dioxide Reactivity

Hugh S. Markus, MRCP; Patrick Vallance, MRCP; Martin M. Brown, MRCP

Background and Purpose Prostaglandins are believed to play an important role in maintenance of cerebral blood flow and possibly in the vasodilatory response to carbon dioxide. Therefore, the nonsteroidal anti-inflammatory drugs and aspirin, which inhibit cyclooxygenase, might be expected to reduce cerebral blood flow and the response to hypercapnia. This may be estimated by measuring cerebral vascular reserve using carbon dioxide. It can be assessed by measuring cerebral blood flow velocity and vasoconstrictor responses to hypercapnia (induced by hyperventilation) from 37.5% to 20.7% (P<.003). There was a nonsignificant reduction in the vasodilatory response to 8% carbon dioxide (mean: predrug, 87.7%; postdrug, 61.0%), with marked intersubject variability. In contrast, basal middle cerebral artery velocity and vasoconstrictor and vasodilatory responses to changes in carbon dioxide were unchanged after aspirin or sulindac administration.

Conclusions The lack of effect of aspirin on basal cerebral blood flow velocity and on vasodilatory reserve is reassuring; aspirin will not reduce cerebral blood flow or the response to a reduced perfusion pressure in patients with critically impaired cerebral hemodynamics. However, indomethacin should be avoided in such patients. (Stroke. 1994;25:1760-1764.)

Key Words: aspirin • ultrasonics • cerebral circulation • indomethacin • prostaglandins

Current opinion favors embolism as the major mechanism underlying stroke in patients with carotid stenosis. However, hemodynamic factors may also play a role; this is suggested by the steadily increasing stroke rate with increasing degrees of carotid stenosis, even in patients with carotid stenosis of 70% and greater diameter reduction. The hemodynamic effect of a carotid stenosis depends not only on the degree of stenosis but also on the collateral supply via both the circle of Willis and extracranial-intracranial communications. It can be assessed by measuring cerebrovascular reserve. This may be estimated by measuring the change in blood flow, or flow velocity, in response to the administration of a vasodilator such as carbon dioxide or acetazolamide. In patients with internal carotid artery stenosis or occlusion, the vasodilatory response to CO2 may be impaired; this is thought to reflect the fact that the cerebral vessels are already maximally vasodilated. Recently it has been demonstrated that an impaired cerebral vasodilatory response to CO2 is associated with a significantly increased risk of stroke in patients with carotid occlusion. This measurement of cerebrovascular reserve is used in many laboratories to assess long-term stroke risk and also to determine the need for intervention such as carotid endarterectomy in patients with asymptomatic carotid stenosis. One might expect that drugs that reduce either resting cerebral blood flow (CBF) or the vasodilatory response would increase the risk of stroke in patients with markedly reduced cerebrovascular reserve. Such drugs also would interfere with the measurement of cerebrovascular reserve. The widely used inhibitors of prostaglandin synthesis, such as aspirin and other nonsteroidal anti-inflammatory drugs, could cause such problems.

There is evidence that prostacyclin is important in both the regulation of resting CBF and the vasodilatory response to hypercapnia. It has a vasodilatory action in vivo,5,6 and inhibition of prostaglandin synthesis by the cyclooxygenase inhibitor indomethacin reduces basal CBF by 20% to 40%, both in a variety of animal models7-11 and in the few studies performed in humans.12-14 In addition, in some animal models indomethacin reduces the cerebral vasodilatory response to hypercapnia,12,14 although studies in humans have revealed conflicting results.12-15 Many patients are treated with indomethacin or other nonsteroidal anti-inflammatory drugs that inhibit cyclooxygenase and prostaglandins. Furthermore, most patients with symptomatic vascular disease are now treated with aspirin, which is also a potent cyclooxygenase inhibitor. A drug-induced decrease in CBF and reduced vasodilatory response could critically reduce...
CBF in patients with already reduced cerebrovascular perfusion reserve. These drugs could also interfere with the measurement of cerebrovascular reserve, making predictions based on this test unreliable if the patient is taking aspirin.

To investigate this further we compared the effects of indomethacin and two other cyclooxygenase inhibitors, aspirin and sulindac, on CBF velocity and on the vasodilatory response to hypercapnia. We used the noninvasive technique of transcranial Doppler ultrasound to measure changes in blood flow velocity.

Methods

Subjects

In each drug group 7 healthy normal volunteers were studied. All were nonsmokers with no significant past medical history. They had taken no aspirin or nonsteroidal inflammatory drugs in the previous 2 weeks. There was an equal ratio of men to women in each group (6:1). Mean (SD) age in the three groups was not significantly different: indomethacin, 32.6 (6.3) years; aspirin, 35.1 (7.7) years; and sulindac, 34.3 (8.1) years.

Measurement

CBF velocity was measured using a transcranial pulsed Doppler ultrasound machine fitted with a 2-MHz probe (TC2000, EME Ltd). The right middle cerebral artery was insonated at a depth of 45 to 52 mm. The probe was held in a fixed position in a specifically designed holder held in place by an elastic strap. All subjects were lying in a semirecumbent position on a couch with the upper half of the body at 30 degrees while measurements were taken. Mean middle cerebral artery blood velocity (V_max) was recorded continuously onto an IBM-compatible microcomputer for off-line analysis. Subjects wore a face mask; both inspiratory and expiratory ports were fitted with one-way valves. End-tidal CO_2 was monitored continuously (Normacap 200, Datex).

At each measurement time point, baseline V_max during normocapnia was measured, followed by V_max during inspiration of 8% CO_2 and then during hyperventilation. For these measurements subjects rested until V_max and end-tidal CO_2 were stable, and then mean V_max over 30 seconds was recorded.

Subjects then breathed 8% CO_2 in air; this was continued until end-tidal CO_2 and V_max were stable, when mean V_max over 30 seconds was recorded. The period of hypercapnia lasted 3 to 6 minutes. Then after breathing room air for 2 minutes the subject was instructed to vigorously hyperventilate. This was continued until end-tidal CO_2 and V_max were stable, when mean V_max over 30 seconds was recorded. The period of hyperventilation lasted 2 to 3 minutes.

All studies began at 9 to 9:30 AM. Subjects had fasted from the previous midnight and drank only water. The initial baseline measurements were repeated after a rest period of 10 minutes. An oral prostaglandin synthesis inhibitor (100 mg indomethacin, 1200 mg soluble aspirin, or 300 mg sulindac) was then given, and further measurements were taken at 90 and 150 minutes.

Blood pressure and pulse during normocapnia were recorded at the start and at 90 and 150 minutes. Pulsatility index of the middle CBF Doppler waveform was recorded during normocapnia at baseline and at 90 and 150 minutes. This is calculated as (maximum velocity during systole−maximum velocity during diastole)/mean velocity. The mean value from 10 seconds of recording was calculated.

Data Analysis

The vasodilatory response to hypercapnia was calculated from the formula (V_max during 8% CO_2−V_max during normocapnia)/V_max during normocapnia×100. As assessed using transcranial Doppler, a 8% CO_2 results in a maximal vasodilatory response, and therefore the increase in flow velocity was not divided by the rise in end-tidal CO_2.

The vasoconstrictor response to hypercapnia (hyperventilation) was calculated from the formula (V_max during hyperventilation−V_max during normocapnia)/V_max during normocapnia×100. The hyperventilation protocol usually results in maximal vasoconstriction; therefore, the change in flow velocity was not divided by the change in end-tidal CO_2 in the primary analysis. However, in case hyperventilation had not been sufficiently vigorous to result in maximum vasoconstriction in some subjects, a separate analysis was performed in which the fall in V_max during hyperventilation was divided by the fall in end-tidal CO_2 occurring during hyperventilation.

Changes in measured parameters over time were compared among the three groups using the measurement of the area under the curve 17 for three time points: 0 minutes (mean of two pretreatment readings [pre-1 and pre-2]), 90 minutes, and 150 minutes. Values at times 90 and 150 minutes were expressed as a percentage of the value at time 0 for each subject. A one-way ANOVA was then performed on the summed data for individual subjects followed by Scheffé's multiple-comparisons test to identify differences between the different groups. In addition, for individual groups mean predrug values were compared with values at 90 minutes using paired t tests. Significance was declared at the P<.05 level.

Results

Results for the three drugs are summarized in Table 1 (indomethacin), Table 2 (aspirin), and Table 3 (sulindac). V_max during normocapnia fell significantly by a mean of 30.4% after the administration of indomethacin, and this reduction was maintained at 150 minutes. This reduction was seen in all subjects (Figure). In contrast, after both aspirin and sulindac there was no change in V_max during normocapnia (ANOVA, P<.001; indomethacin versus aspirin, P<.005; indomethacin versus sulindac, P<.005). Pulsatility index increased after indomethacin from a mean (SD) of 0.80 (0.18) to 0.94 (0.21) at 90 minutes (t test, P<.05). In contrast, pulsatility index did not change after aspirin or sulindac; however, differences between the three groups did not reach significance (ANOVA, P>.05). There was no change in pulse, blood pressure, or end-tidal CO_2 during normocapnia after administration of any of the three drugs (Tables 1 through 3).

After indomethacin there was a highly variable reduction in the vasodilatory response to hypercapnia; mean fall was 30.4%, but there were great interindividual differences (Figure), with some subjects showing an almost complete abolition of vasodilatory response, and others showing little change. There was no difference in the levels of end-tidal CO_2 reached during inspiration of 8% CO_2 between the studies before and after indomethacin. There was no change in the vasodilatory response to hypercapnia after either aspirin or sulindac. Differences between the three groups were not significant (ANOVA, P=.4).

After indomethacin administration all subjects showed a reduced vasoconstrictor response to hyperventilation by a mean of 44.7%. The levels of end-tidal CO_2 reached during hyperventilation were unchanged after indomethacin. The results were similar when the fall in V_max during hyperventilation was divided by the fall in end-tidal CO_2 during hyperventilation: before indomethacin, 26.1% (SD, 11.5); 90 minutes after indomethacin, 13.0% (SD, 9.19); change, 50.4%. In contrast, there was no change in the response to hyperventilation.
after aspirin or sulindac (ANOVA, $P<.0005$; indomethacin versus aspirin, $P<.001$; indomethacin versus sulindac, $P<.005$).

**Reproducibility**

Repeating the predrug measurements twice allowed estimation of the reproducibility of the measurements of $V_{max}$ and cerebrovascular reserve. Mean (SD) percentage difference was estimated from absolute value of $(\text{measurement } 1 - \text{measurement } 2)/\text{measurement } 2 \times 100$. Mean (SD) difference was for $V_{max}$ during normocapnia, 4.13 (3.98)%; for vasodilatory response to 8% CO$_2$, 12.10 (14.53)%; and for vasoconstrictor response to hyperventilation, 8.60 (11.39)%.

**Discussion**

Our results demonstrate that indomethacin results in a rapid fall in CBF velocity. The 20% to 40% reduction in basal CBF velocity after indomethacin is similar to those attained in our study, and in humans. This coupled with an increase in pulsatility index indicates that it exerts a vasoconstrictor action. We also found that indomethacin significantly reduces the vasoconstrictor response to hyperventilation induced by hyperventilation. In contrast to the uniformity of the reduction in resting $V_{max}$ and in the vasoconstrictor response, which was seen in all subjects (Figure), indomethacin resulted in a variable reduction in the vasodilatory response to CO$_2$. In some individuals this response was markedly decreased, whereas in others there was little change. This marked interindividual variability in the inhibition of the vasodilatory response may be the reason why previous studies in humans have revealed conflicting results. In these studies the mean data from a group of subjects were analyzed, and this method of presentation may obscure the interindividual variability. In one study no control group received hypercapnia; a significant increase in CBF was seen with a very modest increase in CO$_2$ concentration from 5.0 to 5.5 kPa, but this may represent a nonspecific response to the stress of the mask and the CO$_2$. Furthermore, without a control group it is unclear whether the response to hypercapnia was reduced compared with normal subjects, as the trend in our study suggested, or was unaltered as found by Pickles et al. However, other studies revealing conflicting results induced greater changes in end-tidal CO$_2$ concentrations, similar to those attained in our study, and used ade-

### Table 1. Values of Cerebral Blood Flow Velocity, Vasodilatory and Vasoconstrictor Responses, Pulsatility Index, and Arterial Blood Pressure Before and 90 and 150 Minutes After 100 mg Indomethacin

<table>
<thead>
<tr>
<th>Time</th>
<th>$V_{max}$, cm/s</th>
<th>Vasodilatory Response, %</th>
<th>Vasoconstrictor Response, %</th>
<th>PI</th>
<th>ETCO$_2$, Normocapnia, kPa</th>
<th>Systolic BP, mm Hg</th>
<th>Diastolic BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-1</td>
<td>48.9</td>
<td>87.7</td>
<td>37.5</td>
<td>0.80</td>
<td>4.56</td>
<td>113.0</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td>(11.0)</td>
<td>(42.2)</td>
<td>(14.0)</td>
<td>(0.18)</td>
<td>(0.91)</td>
<td>(10.8)</td>
<td>(7.2)</td>
</tr>
<tr>
<td>Pre-2</td>
<td>48.0</td>
<td>85.8</td>
<td>37.4</td>
<td>0.79</td>
<td>4.27</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>(11.1)</td>
<td>(40.1)</td>
<td>(11.8)</td>
<td>(0.17)</td>
<td>(0.85)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>90 Min</td>
<td>34.0</td>
<td>61.0</td>
<td>20.7</td>
<td>0.94</td>
<td>4.34</td>
<td>109.9</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td>(12.8)</td>
<td>(35.8)</td>
<td>(17.0)</td>
<td>(0.21)</td>
<td>(0.54)</td>
<td>(9.3)</td>
<td>(6.5)</td>
</tr>
<tr>
<td>150 Min</td>
<td>34.8</td>
<td>81.9</td>
<td>20.0</td>
<td>0.94</td>
<td>4.36</td>
<td>111.6</td>
<td>63.7</td>
</tr>
<tr>
<td></td>
<td>(11.8)</td>
<td>(39.7)</td>
<td>(14.9)</td>
<td>(0.21)</td>
<td>(0.80)</td>
<td>(9.2)</td>
<td>(5.3)</td>
</tr>
</tbody>
</table>

$P$ values:

pre-1 vs 90 min .002 NS .003 .028 NS NS NS

$V_{max}$ indicates middle cerebral artery blood velocity; PI, pulsatility index; ETCO$_2$, end-tidal CO$_2$; BP, blood pressure; Pre, pretreatment; and NS, not significant.

Standard deviations are shown in parentheses. Probability values from $t$ test comparisons between the first baseline measurement and the measurement at 90 minutes are shown.

### Table 2. Values of Cerebral Blood Flow Velocity, Vasodilatory and Vasoconstrictor Responses, Pulsatility Index, and Arterial Blood Pressure Before and 90 and 150 Minutes After 1200 mg Aspirin

<table>
<thead>
<tr>
<th>Time</th>
<th>$V_{max}$, cm/s</th>
<th>Vasodilatory Response, %</th>
<th>Vasoconstrictor Response, %</th>
<th>PI</th>
<th>ETCO$_2$, Normocapnia, kPa</th>
<th>Systolic BP, mm Hg</th>
<th>Diastolic BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-1</td>
<td>45.1</td>
<td>103.5</td>
<td>41.1</td>
<td>0.63</td>
<td>4.46</td>
<td>109.4</td>
<td>76.1</td>
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<tr>
<td></td>
<td>(10.4)</td>
<td>(54.7)</td>
<td>(12.1)</td>
<td>(0.08)</td>
<td>(0.67)</td>
<td>(4.7)</td>
<td>(5.7)</td>
</tr>
<tr>
<td>Pre-2</td>
<td>45.9</td>
<td>101.8</td>
<td>40.4</td>
<td>0.61</td>
<td>4.45</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>(12.0)</td>
<td>(57.5)</td>
<td>(12.9)</td>
<td>(0.07)</td>
<td>(0.68)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>90 Min</td>
<td>42.9</td>
<td>100.3</td>
<td>40.1</td>
<td>0.66</td>
<td>4.45</td>
<td>112.6</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>(7.5)</td>
<td>(45.1)</td>
<td>(9.8)</td>
<td>(0.10)</td>
<td>(0.58)</td>
<td>(3.6)</td>
<td>(3.6)</td>
</tr>
<tr>
<td>150 Min</td>
<td>43.7</td>
<td>107.7</td>
<td>38.0</td>
<td>0.63</td>
<td>4.59</td>
<td>110.0</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>(9.1)</td>
<td>(50.7)</td>
<td>(15.8)</td>
<td>(0.09)</td>
<td>(0.34)</td>
<td>(2.5)</td>
<td>(6.2)</td>
</tr>
</tbody>
</table>

**Abbreviations as in Table 1.**

Standard deviations are shown in parentheses. There were no significant changes in any parameter after aspirin.
TABLE 3. Values of Cerebral Blood Flow Velocity, Vasodilatory and Vasoconstrictor Responses, Pulsatility Index, and Arterial Blood Pressure Before and 90 and 150 Minutes After 300 mg Sulindac

<table>
<thead>
<tr>
<th>Time</th>
<th>$V_{max}$ cm/s</th>
<th>Vasodilatory Response, %</th>
<th>Vasoconstrictor Response, %</th>
<th>PI</th>
<th>$ETCO_2$ Normocapnia, kPa</th>
<th>Systolic BP, mm Hg</th>
<th>Diastolic BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-1</td>
<td>49.3</td>
<td>81.4</td>
<td>44.1</td>
<td>0.69</td>
<td>5.31</td>
<td>109.4</td>
<td>78.1</td>
</tr>
<tr>
<td></td>
<td>(7.5)</td>
<td>(51.1)</td>
<td>(8.0)</td>
<td>(0.07)</td>
<td>(0.39)</td>
<td>(4.7)</td>
<td>(5.7)</td>
</tr>
<tr>
<td>Pre-2</td>
<td>49.6</td>
<td>77.8</td>
<td>45.6</td>
<td>0.65</td>
<td>4.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.4)</td>
<td>(46.4)</td>
<td>(8.7)</td>
<td>(0.08)</td>
<td>(0.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 Min</td>
<td>50.0</td>
<td>77.9</td>
<td>40.9</td>
<td>0.69</td>
<td>4.80</td>
<td>112.6</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>(11.5)</td>
<td>(53.0)</td>
<td>(12.3)</td>
<td>(0.11)</td>
<td>(0.33)</td>
<td>(3.6)</td>
<td>(3.6)</td>
</tr>
<tr>
<td>150 Min</td>
<td>47.0</td>
<td>84.5</td>
<td>40.6</td>
<td>0.66</td>
<td>4.87</td>
<td>110.0</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>(6.9)</td>
<td>(58.1)</td>
<td>(13.8)</td>
<td>(0.11)</td>
<td>(0.41)</td>
<td>(2.5)</td>
<td>(6.2)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. Standard deviations are shown in parentheses. There were no significant changes in any parameter after sulindac.

Graphs showing effect of 100 mg indomethacin on middle cerebral artery (MCA) blood flow velocity ($V_{max}$) (top), on vasodilatory response to 8% carbon dioxide (middle), and on vasoconstrictor response to hypocapnia induced by hyperventilation (bottom). Points show individual values in each subject at the first pretreatment measurement and at 90 minutes after indomethacin.

Sulindac is the nonsteroidal agent most similar structurally to indomethacin. The lack of effect of aspirin and sulindac on CBF suggests that indomethacin may reduce CBF by a method other than inhibition of prostaglandin synthesis, as proposed by Eriksson et al.18 Indomethacin is known to have inhibitory actions on a number of enzyme and cellular systems in addition to its inhibition of prostaglandin synthesis.19 In a study in anesthetized pigs it had a rapid-acting (within 60 seconds) systemic vasoconstrictor effect that preceded any change in prostaglandin release and was thought to be independent of cyclooxygenase inhibition. Few animal studies have looked at the effect of prostaglandin inhibitors other
than aspirin, and the results have been conflicting.\textsuperscript{5,20} Only one human study, using the nitrous oxide washout technique, has looked at the effect of prostaglandin inhibitors other than indomethacin in normal adults; naproxen and aspirin had no effect on CBF or reactivity to CO\textsubscript{2}, whereas indomethacin did reduce both parameters in this study.\textsuperscript{18} Our results support these findings.

It is possible that effects on \( V_{\text{max}} \) were not seen with aspirin and sulindac because the doses given did not adequately inhibit prostaglandin synthesis. However, both sulindac and aspirin, at lower doses than those given in our study, result in marked inhibition of platelet aggregation in humans in vivo.\textsuperscript{21} It is more difficult to assess the inhibition of vascular prostacyclin in vivo; however, 300 mg of aspirin (a dose much smaller than we used) results in over 80\% inhibition of prostacyclin production by human vein.\textsuperscript{22} It has been suggested that aspirin has no effect because it does not adequately cross the blood-brain barrier,\textsuperscript{18} and this remains a possible explanation. However, studies in pigs have demonstrated that aspirin and other nonsteroidal agents inhibited the cerebrovascular production of prostaglandins to as great a degree as indomethacin, yet only indomethacin reduced CBF.\textsuperscript{20}

In summary, our results have direct clinical implications in that indomethacin should be avoided in patients with critical cerebral hemodynamics or in CO\textsubscript{2} cerebrovascular reserve testing. In contrast, aspirin and sulindac in the doses we used appear safe and will not interfere with cerebrovascular reserve testing. Our results raise the possibility that the predominant effect of indomethacin on the cerebral circulation is through mechanisms other than prostaglandin inhibition. This should be borne in mind when studying the cerebral circulation, and other cyclooxygenase should be used in addition to indomethacin in pharmacological studies of prostaglandin inhibition.

Acknowledgments

Hugh Markus is supported by the British Heart Foundation. We thank Dr Martin Bland for statistical help.

References

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Stroke. 1994;25:1760-1764
doi: 10.1161/01.STR.25.9.1760
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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