Beneficial Effect of OKY-046, a Selective Thromboxane A\textsubscript{2} Synthetase Inhibitor, on Experimental Cerebral Vasospasm

Hidetada KOMATSU, Yasuo TAKEHANA, Shuichiro HAMANO, Arao UJIIE and Seiji HIRAKU*

Central Research Laboratories, Kissei Pharmaceutical Co., Ltd., Matsumoto 399, Japan
*Research Institute, Ono Pharmaceutical Co., Ltd., Shimamoto, Mishima, Osaka 618, Japan

Accepted April 10, 1986

Abstract—Effects of OKY-046, a selective inhibitor of thromboxane (TX)A\textsubscript{2} synthetase and a platelet aggregation inhibitor, on in vitro and in vivo models of cerebral vasospasm were studied. The contraction of the isolated rabbit basilar artery by an exposure to 1.0 ml of whole rabbit blood plus 0.05 or 0.1 units/ml of thrombin was diminished by the treatment with 10^{-4} M of OKY-046 and/or 10^{-6} M of cinanserin. When the whole blood of rabbits treated intravenously with 1 mg/kg/min of OKY-046 was used, the contraction of the basilar artery was decreased to about half of the control contraction. Angiographically recognized cerebral vasospasm in vivo, by a transorbital injection of 5.0 to 7.0 ml of autologous arterial blood into the cisterna magna of dogs, was suppressed by 0.05 and 0.5 µg of OKY-046. Moreover, the decrease in the regional cerebral blood flow in autologous blood infused-dogs was inhibited by 0.5 µg of OKY-046. The increase in TXB\textsubscript{2} in the cerebrospinal fluid of dogs was significantly inhibited, and the level of 6-keto-PGF\textsubscript{1α} was slightly increased by the treatment of OKY-046. The ratio of 6-keto-PGF\textsubscript{1α}/TXB\textsubscript{2} was increased from 1.5 to 5.2 in OKY-046-treated dogs. No effect on the basal tone and response to vasoactive agonists such as norepinephrine, KCl and PGE\textsubscript{1} was observed in the isolated spiral thoracic aorta of guinea pigs or rabbits. Taken together with our previous findings, we conclude that the inhibition of cerebral vasospasm in the in vitro and in vivo models by the treatment of OKY-046 might be due to an inhibition of platelet aggregation, an inhibition of TXA\textsubscript{2} generation and an increase in the ratio of PGI\textsubscript{2}/TXA\textsubscript{2}.

It is well known that cerebral vasospasm followed by aneurysmal rupture and subarachnoid hemorrhage (SAH) is a significant cause of mortality and morbidity in humans. However, the basic pathogenesis of vasospasm still remains obscure. Recently, important roles of platelets and vascular endothelium in cerebrovascular diseases have been recognized, and several studies have suggested that arachidonic acid metabolites may play an important role in the pathophysiology of those disorders (1–8). It has been also reported that thromboxane (TX)A\textsubscript{2}, released mainly from platelets, induces platelet aggregation and has a strong vasoconstrictive effect on cerebral arteries (1, 5, 9). More recently, (E)-3-[p-(1 \textsubscript{H}-imidazol - 1 - ylmethyl)phenyl] - 2 - propenoic acid (OKY-046), as shown in Fig. 1, was

\[
\begin{align*}
\text{Structure of OKY-046} \\
\text{IC}_{50} & 4-11 \times 10^{-9} M \\
\text{PGI}_{2}\text{-synthetase} & > 10^{-3} M \\
\text{PGF}_{1α}\text{-synthetase} & > 10^{-3} M
\end{align*}
\]

Fig. 1. Structure and inhibitory effect of OKY-046 on arachidonic metabolizing enzymes. The IC\textsubscript{50} shows the concentration which gave 50% inhibition (from reference No. 10 and Hiraku et al., unpublished observation).
reported to be a potent and selective inhibitor of TXA₂ synthetase, and it was found to inhibit platelet aggregation and TXA₂ generation in platelets (10). In this study, we investigated the effect of OKY-046 on cerebral vasospasm models in vitro by an exposure to whole blood from rabbits and observed the effect on in vivo models by an intracranial injection of autologous blood in dogs.

**Materials and Methods**

**In vitro cerebral vasospasm model**

Rabbits weighing 3.0 to 4.0 kg were sacrificed by a rapid exsanguination via the common carotid artery. After removal of the brain, the basilar artery was dissected free and cut spirally under an operating microscope. The vessel was mounted at a load of 0.5 g in a 50-ml bath filled with the Tyrode’s solution and aerated with 95% O₂ and 5% CO₂ at 37°C. The vessel was tested for a viability by replacing the Tyrode’s solution with the high potassium (140 mM) solution.

The spiral cut basilar artery in a 50-ml bath was exposed to whole blood, which was drawn from the auricular artery of non-treated conscious rabbits with one-tenth volume of 3.8% of sodium citrate, in the presence of 0.05 or 0.1 units/ml of thrombin (Sigma, St. Louis, U.S.A.). The contraction was isometrically recorded using a force-displacement transducer (Model SB-IT, Nihon Kohden, Tokyo) and observed for 60 min. When whole blood plus thrombin was repeatedly added to the 50-ml bath, no satisfactory reproducibility of the response could be obtained. Therefore, we did not perform the evaluation of test drugs in the same preparation. To examine the effect of drugs on contraction of the basilar artery, each drug was applied to the 50-ml bath 5 min before an addition of whole blood plus thrombin. For ex vivo whole blood application, whole blood was obtained from conscious rabbits before drug application, at 10 min after an intravenous injection of 10 mg/kg of drug, or at 30 or 120 min during a continuous infusion of 100 µg/kg/min of OKY-046, 1 mg/kg/min of OKY-046, or 1 mg/kg/min of ticlopidine. When ex vivo whole blood treated with a continuous infusion of 1 mg/kg/min of OKY-046 was used, the level of TXB₂ in the 50-ml bath was measured by the radioimmunoassay (RIA) method (11).

**In vivo cerebral vasospasm model**

1) **Production of cerebral vasospasm:** Adult mongrel dogs weighing 6 to 26 kg were anesthetized with 30 mg/kg of sodium pentobarbital intravenously. The femoral artery and cephalic vein were catheterized for monitoring of arterial blood pressure and for an injection of anesthetic, respectively. Experimental cerebral vasospasm was produced by a transorbital injection of 5.0 to 7.0 ml of fresh arterial blood into the chiasmatic cisterna magna after the removal of an equivalent amount of clear cerebrospinal fluid (CSF) of dogs. In dogs given a sham operation, CSF was introduced from the basal cisterna magna and again restored transorbitally.

2) **Measurement of caliber of the basilar artery:** The right vertebral artery of dogs was catheterized for an injection of 2.5 to 5.0 ml of an Angiographin (Schering AG, Berlin, West Germany) through an Auto-Injector (AP-100B, Fukuoka Radiological, Fukuoka, Japan). Angiograms were obtained before and after the autologous blood injection. The caliber of the basilar artery was measured at a point about 1.0 cm distant from the circle of Willis.

3) **Measurement of regional cerebral blood flow (rCBF):** The rCBF in normal and autologous blood infused-dogs was determined by a hydrogen clearance technique. One platinum electrode (M.T. Giken, Chofu, Japan), 0.3 mm in diameter, was inserted through a drill hole into the gyrus sygmoideus in the frontal lobe, 5 mm in length, and then the drill hole was filled with a dental cement. The H₂ clearance curve was recorded with a pH₂ monitor apparatus (PHG300, M.T. Giken).

4) **Measurement of TXB₂ and 6-keto-prostaglandin (PG)F₁α:** Two ml of CSF in dogs was obtained through a needle inserted in the cisterna magna from the foramen magnum before and 3 hr after an intracisternal injection of autologous blood. The concentrations of TXB₂ and 6-keto-PGF₁α in CSF were measured by RIA (11).

5) **Drug administration:** Test drugs were
transorbitally administered at the same time as an injection of autologous blood into the cisterna magna.

**Isolated thoracic aorta**

The thoracic aorta of rabbits and guinea pigs was isolated and cut spirally. The preparation was mounted at a load of 1 g in the Krebs’ solution and aerated with 95% O₂ and 5% CO₂ at 37°C. Test drugs were applied to the aorta 5 min before the addition of vasoactive agonists (norepinephrine, KCl, and PGE₁). The response was isometrically measured using a force-displacement transducer (Model SB-1T, Nihon Kohden).

**Test drugs**

The following drugs were used: OKY-046 (used as sodium, Kissei and Ono, Japan), indomethacin (Sigma, St. Louis, U.S.A.), aspirin (Sanko, Tokyo, Japan), ticlopidine hydrochloride (synthesized in Kissei), verapamil hydrochloride (Knoll, Ludwigshafen, West Germany), cinanserin (synthesized in Kissei), norepinephrine (Sankyo, Tokyo, Japan) and PGE₁ (Sigma). These drugs were dissolved in physiological saline, 0.2 M tris buffer (pH 8.0) or 99.5% ethanol and then diluted to the desired concentration by physiological saline.

**Results**

**In vitro cerebral vasospasm model**

1) **In vitro drug application:** Figures 2, 3, 4 and 5 indicate the effects of the drugs on the in vitro models of cerebral vasospasm. The contraction is expressed in percentage of response induced by depolarization in the high potassium (140 mM) solution. The administration of 1.0 ml of whole blood plus 0.05 or 0.1 units/ml of thrombin (at final concentration) to the rabbit basilar artery in a 50-ml bath resulted in a rapid contraction, reaching a maximum response of 77% in 5 min. This contraction was persistent, with a gradual decrease to approximately 60% at 60 min. A slight contraction of the vessel by thrombin alone continued over a 60-min observation period. When whole blood alone was added, the contraction was gradually produced, reaching a maximum of 43% at 30 min and sustained over a 60-min period. The contraction by whole blood plus thrombin was significantly inhibited by 10⁻⁶ and 10⁻⁵ M of verapamil, a Ca antagonist, dose-dependently (Figs. 2 and 4). When 10⁻⁴ M of OKY-046, 10⁻⁶ M of cinanserin or 10⁻⁵ M of indomethacin was added to the bath, the contraction induced by whole blood plus thrombin was inhibited for over
60-min. When $10^{-4}$ M of OKY-046 together with $10^{-6}$ M of cinanserin was used, the contraction was further suppressed. The degree

Fig. 3. Tracings showing inhibitory effects of OKY-046 and/or cinanserin and indomethacin on the contraction of the basilar artery induced by an exposure to whole blood (containing one-tenth volume of 3.8% sodium citrate) plus 0.05 or 0.1 units/ml of thrombin. Potassium contraction was induced by replacing the Tyrode's solution with the high potassium (140 mM) solution. Test drugs were applied in a 50-ml bath 5 min before the addition of whole blood plus thrombin. Each point indicates the mean of 4 to 15 experiments. Vertical bars show the S.E. of the mean. (A): control, (B): $10^{-4}$ M of OKY-046, (C): $10^{-6}$ M of cinanserin, (D): $10^{-4}$ M of OKY-046 plus $10^{-6}$ M of cinanserin, (E): $10^{-5}$ M of indomethacin.

Fig. 4. The time course of contractile response of the rabbit basilar artery by an exposure of whole blood and/or thrombin in a 50-ml bath. Test drugs were applied 5 min before the addition of whole blood plus thrombin. Each point indicates the mean of 4 to 15 experiments. Vertical bars show the S.E. of the mean. (0): 1.0 ml of whole blood (containing one-tenth volume of 3.8% sodium citrate) plus 0.05 or 0.1 units/ml of thrombin (final concentration), (■): thrombin alone, (▲): 1.0 ml of whole blood (one-tenth volume of 3.8% sodium citrate) alone, (●): whole blood plus thrombin in the presence of $10^{-6}$ M of verapamil in a 50-ml bath. (☑): whole blood plus thrombin in the presence of $10^{-5}$ M of verapamil in a 50-ml bath. *, ** and ***: significantly different from the contraction induced by whole blood plus thrombin at P<0.05, P<0.01 and P<0.001, respectively.

Fig. 5. Inhibitory effects of OKY-046 and/or cinanserin and indomethacin on the contraction of the basilar artery of rabbits induced by an exposure to 1.0 ml of whole blood (containing one-tenth volume of 3.8% sodium citrate) plus 0.05 or 0.1 units/ml of thrombin. Test drugs were applied 5 min before the addition of whole blood plus thrombin. Each point indicates the mean of 5 to 15 experiments. Vertical bars show the S.E. of the mean. (0): control, (●): $10^{-4}$ M of OKY-046, (■): $10^{-6}$ M of cinanserin, (☐): $10^{-4}$ M of OKY-046 plus $10^{-6}$ M of cinanserin, (▲): $10^{-6}$ M of indomethacin. *, ** and ***: significantly different from the control at P<0.05, P<0.01 and P<0.001, respectively.
Table 1. *Ex vivo* effects of OKY-046 and ticlopidine on cerebral vasospasm by exposure to whole blood plus thrombin in rabbits

<table>
<thead>
<tr>
<th>Drugs</th>
<th>n</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controla</td>
<td>5</td>
<td>59.9±14.6</td>
<td>61.1±8.47</td>
<td>57.7±8.83</td>
<td>56.1±5.35</td>
<td>53.8±5.97</td>
<td>52.7±5.73</td>
<td>50.7±7.23</td>
</tr>
<tr>
<td>OKY-046 10 mg/kg, i.v.</td>
<td>4</td>
<td>34.9±12.65</td>
<td>50.2±15.22</td>
<td>37.8±9.62</td>
<td>40.9±9.95</td>
<td>38.1±12.4</td>
<td>41.9±9.43</td>
<td>41.7±10.02</td>
</tr>
<tr>
<td>OKY-046 100 μg/kg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controla</td>
<td>5</td>
<td>46.7±8.96</td>
<td>77.1±8.45</td>
<td>61.6±8.98</td>
<td>63.3±8.66</td>
<td>65.1±9.03</td>
<td>65.2±8.71</td>
<td>68.4±9.71</td>
</tr>
<tr>
<td>30 min</td>
<td>4</td>
<td>73.5±13.34</td>
<td>79.2±14.59</td>
<td>79.3±17.73</td>
<td>68.3±9.71</td>
<td>58.2±4.28</td>
<td>58.1±5.13</td>
<td>61.7±8.51</td>
</tr>
<tr>
<td>120 min</td>
<td>4</td>
<td>48.4±7.82</td>
<td>76.9±10.40</td>
<td>71.7±7.39</td>
<td>73.7±5.61</td>
<td>73.8±2.30</td>
<td>74.9±0.61</td>
<td>73.8±2.30</td>
</tr>
<tr>
<td>OKY-046 1 mg/kg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controla</td>
<td>9</td>
<td>60.8±10.33</td>
<td>65.3±5.01</td>
<td>54.1±4.88</td>
<td>54.9±4.57</td>
<td>56.8±6.52</td>
<td>50.8±5.57</td>
<td>50.8±5.94</td>
</tr>
<tr>
<td>30 min</td>
<td>8</td>
<td>27.4±10.74*</td>
<td>35.8±6.79**</td>
<td>33.4±8.22*</td>
<td>34.0±6.87*</td>
<td>36.1±7.04*</td>
<td>36.6±7.52**</td>
<td>38.4±7.37</td>
</tr>
<tr>
<td>120 min</td>
<td>7</td>
<td>31.0±5.32*</td>
<td>57.1±8.20</td>
<td>35.9±7.10*</td>
<td>33.5±7.22*</td>
<td>33.5±6.56*</td>
<td>33.5±6.68</td>
<td>32.3±6.71</td>
</tr>
<tr>
<td>Ticlopidine 1 mg/kg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controla</td>
<td>4</td>
<td>69.0±3.88</td>
<td>52.6±4.95</td>
<td>52.7±9.02</td>
<td>50.3±10.01</td>
<td>49.6±7.03</td>
<td>56.8±7.18</td>
<td>56.8±7.18</td>
</tr>
<tr>
<td>30 min</td>
<td>4</td>
<td>65.2±21.84</td>
<td>57.4±15.76</td>
<td>52.3±17.02</td>
<td>51.7±14.9</td>
<td>52.3±15.42</td>
<td>57.9±14.95</td>
<td>49.9±14.00</td>
</tr>
<tr>
<td>120 min</td>
<td>4</td>
<td>66.8±19.03</td>
<td>53.4±14.14</td>
<td>46.5±15.50</td>
<td>70.8±21.28</td>
<td>60.1±18.65</td>
<td>63.4±18.54</td>
<td>63.4±25.21</td>
</tr>
</tbody>
</table>

Drugs were administered from the auricular vein. *Ex vivo* whole blood was obtained from conscious rabbits 10 min after an intravenous injection of 10 mg/kg of drug and at 30 and 120 min during a continuous infusion of 100 μg and 1 mg/kg/min of OKY-046 or 1 mg/kg/min of ticlopidine. Each value is indicated as a percentage of the maximum response by high potassium (140 mM) solution and is the mean±S.E. n: number of animals, * and **: significantly different from each control at P<0.05 and P<0.01, respectively. a: whole blood before *ex vivo* drug application was used.
of inhibition by a combination of $10^{-4}$ M of OKY-046 and $10^{-6}$ M of cinanserin was greater than that by $10^{-5}$ M of indomethacin (Figs. 3 and 5).

2) Ex vivo drug application: The results obtained using ex vivo whole blood treated with OKY-046 or ticlopidine are shown in Table 1. The contraction induced by whole blood from the rabbit with an intravenous injection of 10 mg/kg or an infusion of 100 µg/kg/min of OKY-046 and 1 mg/kg/min of ticlopidine did not significantly reduce the contraction before drug application over a 60-min observation period. However, when whole blood of rabbits infused for 30 and 120 min with 1 mg/kg/min of OKY-046 was used, a significant or moderate decrease in the contraction was observed. Table 2 indicates the concentration of TXB2 at 5 min after the addition of whole blood treated with OKY-046. The concentration of TXB2 was significantly lower than the level before drug application.

**In vivo cerebral vasospasm model**

1) Caliber of the basilar artery: Figure 6 indicates the effect of OKY-046 and other drugs on the caliber of the basilar artery in autologous blood infused-dogs. Severe and sustained cerebral vasospasm was angiographically observed by an injection of autologous arterial blood into the cisterna magna of dogs. The caliber is expressed as a percentage of the value before the injection of autologous arterial blood. The caliber of

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Concentration of TXB2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.61±0.073 (4)</td>
</tr>
<tr>
<td>30</td>
<td>0.20±0.020** (4)</td>
</tr>
<tr>
<td>120</td>
<td>0.23±0.051** (4)</td>
</tr>
</tbody>
</table>

One ml of whole blood drawn from a rabbit infused with 1 mg/kg/min of OKY-046 for 30 and 120 min was applied to a 50-ml bath in the presence of the basilar artery of rabbits together with 0.05 or 0.1 units/ml of thrombin (final concentration). Each value indicates the mean±S.E. of 4 experiments. **: significantly different from the control (0 min) at P<0.01.

Fig. 6. Effects of OKY-046, indomethacin and ticlopidine on the cerebral vasospasm induced by a transorbital injection of autologous arterial blood into the cisterna magna of dogs. Test drugs were administered into the cisterna magna together with autologous arterial blood. Each point indicates the mean of 2 to 11 experiments. Vertical bars show the S.E. of the mean. (○): control, (▲): 0.005 µg of OKY-046, (▲): 0.05 µg of OKY-046, (●): 0.5 µg of OKY-046, (□): 5 µg of indomethacin. ** and ***: significantly different from the control at P<0.05, P<0.01 and P<0.001, respectively.
the basilar artery was 81% at 45 min, 88% at 90 min, 70% at 6 hr and 82% at 24 hr after the injection of autologous arterial blood. A transorbital injection of 0.005 μg of OKY-046 into the cisterna magna with autologous arterial blood did not significantly reduce the contraction. However, when 0.05 and 0.5 μg of OKY-046 were used, the vasospasm was significantly inhibited for 6 hr; in particular, the inhibition was marked up to 3 hr. However, at 24 hr, the effect of OKY-046 was not clear. During the same period, no change in systemic arterial blood pressure by an injection of the drug was observed (data not shown). Although an injection of 0.5 μg of indomethacin or 5 μg of ticlopidine into the cisterna magna did not affect the vasospasm (data not shown), 5 μg of indomethacin inhibited the vasospasm during 24 hr; The level of inhibition was significant at 0.75 hr and from 3 to 24 hr. The basilar artery of dogs was not contracted by the sham operation, rather a relaxation was observed at 1.5 and 3 hr (data not shown).

2) rCBF: Figure 7 indicates the effect of OKY-046 on the rCBF in autologous blood infused-dogs. The rCBF is expressed as a percentage of the value before an injection of autologous arterial blood. The time course in changes of the rCBF was similar to that of the caliber of vessel in dogs. The transorbital injection of OKY-046 into the cisterna magna markedly blocked the decrease in the rCBF at a dose of 0.5 μg but not at 0.05 μg for 6 hr. Indomethacin at 5.0 μg improved the rCBF. However, the rCBF in non-treated dogs was not influenced by an intravenous injection of 3 and 30 mg/kg of OKY-046 (Table 3).

3) Levels of TXB2 and 6-keto-PGF1α in CSF: As shown in Fig. 8, the levels of TXB2 and 6-keto-PGF1α in CSF increased 4.4 and 3.3 times more than those before an injection of autologous arterial blood, respectively. An injection of 0.5 μg of OKY-046 into the cisterna magna markedly prevented an increase in TXB2 and slightly increased the level of 6-keto-PGF1α. Thus, the ratio of 6-keto-PGF1α/TXB2 was changed from 1.7 to 1.2 in control dogs and from 1.5 to 5.2 in OKY-046-treated dogs.

Isolated thoracic aorta

OKY-046 at 10^-6 to 10^-3 M had no apparent effect on the basal tone (data not shown) or on the dose-response curves of various agonists (norepinephrine, KCl and PGE1) in the isolated spiral thoracic aorta of rabbits and/or guinea pigs (Fig. 9).

![Fig. 7. Effects of OKY-046 and indomethacin on regional cerebral blood flow (rCBF) in autologous blood infused-dogs. Test drugs were administered into the cisterna magna together with autologous arterial blood. Each point indicates the mean of 4 to 6 experiments. Vertical bars show the S.E. of the mean. (○): control, (▲): 0.05 μg of OKY-046, (●): 0.5 μg of OKY-046, (□): 5 μg of indomethacin. * and **: significantly different from the control at P<0.05 and P<0.01, respectively.]

| Table 3. Effect of OKY-046 on regional cerebral blood flow (rCBF) in non-treated dogs |
|-----------------|-----------------|-----------------|-----------------|
| Drug          | Dose (mg/kg, i.v.) | % change 30 min | % change 60 min |
| OKY-046       | 3                | 98.8± 9.8       | 101.5±12.5      |
|               | 30               | 103.0±12.2      | 95.8± 6.5       |

The rCBF is expressed as a percentage of the value before an injection of autologous arterial blood into the cisterna magna. Each value indicates the mean±S.E. of 4 experiments.
Discussion

It has been recognized that cerebral vasospasm after SAH is caused by multiple factors. Participation of various substances such as serotonin, catecholamines, prostaglandins, oxyhemoglobin, thrombin and other vasoactive substances has been postulated (12–16). Since the cerebral artery causing prolonged vasospasm is usually surrounded by blood clotting, it has been suggested that platelets may have a role in the production of cerebral artery constriction (6–8). Early studies suggested that platelets might liberate various spasmogens, especially serotonin (12). Recently, participation of vasoactive prostaglandins (specifically TXA$_2$) has been observed in several investigations (1, 7, 9). Thus, we studied the effects of OKY-046, which inhibits TXA$_2$ generation and platelet aggregation (10), on experimental cerebral vasospasm in vitro and in vivo.

As the contraction of the isolated basilar artery of rabbits caused by whole blood and thrombin was markedly suppressed by verapamil, the contraction seems to be produced by extracellular Ca, but not by mechanical factors such as an adhesion of platelets. The relaxation is shown as a percentage of the contraction by 10$^{-6}$ M of norepinephrine. Each point indicates the mean of 3 to 4 experiments. Vertical bars show the S.E. of the mean.

![Fig. 8](image)

**Fig. 8.** Effect of OKY-046 on the levels of TXB$_2$ (A) and 6-keto-PGF$_{1\alpha}$ (B) in cerebrospinal fluid (CSF) before (0) and 3 hour (3 hr) after an injection of autologous arterial blood into the cisterna magna of dogs. Test drugs were administered into the cisterna magna together with autologous arterial blood. Each value indicates the mean of 3 to 4 experiments. Vertical bars show the S.E. of the mean.

Control: 0.5 μg of OKY-046, *: significantly different from control at P < 0.05.

![Fig. 9](image)

**Fig. 9.** Effect of OKY-046 on dose-response curve for norepinephrine (A), KCl (B) and PGE$_1$ (C) in the isolated thoracic aorta of guinea pigs. The contraction is shown as a percentage of the maximum contraction by each agonist. The relaxation is shown as a percentage of the contraction by 10$^{-6}$ M of norepinephrine. Each point indicates the mean of 6 experiments. (○): control, (■): 10$^{-6}$ M of OKY-046, (▲): 10$^{-5}$ M of OKY-046, (▲): 10$^{-4}$ M of OKY-046, (□): 10$^{-3}$ M of OKY-046, (×): 10$^{-7}$ M of phentolamine.
the basilar artery to the brim of the 50-ml organ bath or to the stick holder of the preparation. However, when thrombin alone was added to the 50-ml bath, a significant vasoconstriction was not produced. The contraction of the basilar artery by whole blood plus thrombin was moderately inhibited by the addition of OKY-046 and/or cinanserin and indomethacin alone into the 50-ml bath. Therefore, it is likely that the contraction may be induced by TXA₂, other vasoactive prostaglandins and serotonin, but not thrombin. When ex vivo whole blood infused with 1 mg/kg/min of OKY-046 in rabbits was used, the cerebral vasospasm was inhibited and the level of TXB₂ in the 50-ml bath markedly diminished. As the 1.0 ml of whole blood was diluted one-fiftieth in the 50-ml bath, the concentration of OKY-046 surrounding the basilar artery was considered to be comparable to that in an infusion of 20 μg/kg/min of OKY-046 in vivo. Thus we think that 1 mg/kg/min of OKY-046 is not too a high dose and that the suppression of cerebral vasospasm by OKY-046 is probably due to the inhibition of TXA₂ generation.

In the experiments on in vivo cerebral vasospasm in dogs, to examine the direct effects of drugs, each drug was administered into the cisterna magna. OKY-046 at 0.05 and 0.5 μg significantly inhibited the contraction of the basilar artery during 6 hr after an injection of autologous arterial blood, but it did not at 24 hr. On the other hand, indomethacin influenced the late vasoconstriction and the early vasoconstriction. Therefore, we assume that a mechanism of the early vasoconstriction is implicated in the generation of TXA₂ and that a late vasoconstriction is produced by other vasoactive prostaglandins or that the effect of OKY-046 may be abolished because of the disappearance of the drug from the cisterna magna. The ineffectiveness of OKY-046 on the late vasoconstriction in autologous infused-dogs must be further investigated.

The effectiveness of OKY-046 and other TXA₂ synthetase inhibitors on cerebral vasospasm in vivo has been reported (17-21). On the other hand, Fukumori et al. (22) reported the caliber of spastic vessel was not improved when OKY-046 was intravenously applied for 1 or 2 hr at 50 μg/kg/min in autologous blood infused-dogs, in spite of the increase in the rCBF and inhibition of platelet aggregation by the drug. The effectiveness of OKY-046 on the caliber of the basilar artery in our experiments is incompatible with the results of Fukumori et al. (22). We consider that it may be due to differences in administration route and dose of the drug or in the volume of autologous arterial blood which is injected into the cisterna magna for inducing cerebral vasospasm.

OKY-046 effected an inhibition of the cerebral vasospasm at a lower concentration than that which inhibited the decrease in rCBF of autologous blood infused-dogs, but indomethacin improved both cerebral vasospasm and the decrease in rCBF at the same concentration. The membrane permeability of both drugs may be different. Therefore, the following explanation for the differential effect of OKY-046 seems plausible: the basilar artery in dogs is directly exposed to OKY-046 by an intracisternal injection of the drug, but insufficient OKY-046 might arrive at the site (the gyrus sigmoideus in the frontal lobe) where the rCBF was measured. On the other hand, we consider that indomethacin might sufficiently permeate into the site where the rCBF was measured.

Prostaglandin metabolism has been reported to be modified in experimental cerebral vasospasm and in patients suffering from SAH and stroke (3, 4, 23). More recently, the balance of TXA₂ and prostacyclin (PGI₂) has been implicated in the control of the basal tone of cerebral vessels and circulation (1, 21, 24, 25). However, the opposite relations such as the increase in TXA₂ and decrease in PGI₂ are not always observed in animals with cerebral vasospasm and in SAH patients (26). Our results that both the levels of TXB₂ and 6-keto-PGF₁α in CSF increased in dogs did not agree with previous studies that PGI₂ generation in vessels decreased during the cerebral vasospasm (3, 4). On the
other hand, the ratio of 6-keto-PGF$_{1\alpha}$/TXB$_2$ in OKY-046-treated dogs clearly increased, because OKY-046 at 0.5 µg markedly suppressed the increase in TXB$_2$ level and slightly increased 6-keto-PGF$_{1\alpha}$. A specific inhibition of TXA$_2$ generation by OKY-046 causes PG-endoperoxide accumulation, subsequently it is used for PGI$_2$ generation (the so-called steal phenomenon) (27). Conversely, OKY-046 did not directly affect the vessel response (Fig. 9). We conclude that the efficacy of OKY-046 against the cerebral vasospasm in the in vitro and in vivo models is due to an inhibition of platelet aggregation (10), an inhibition of TXA$_2$ generation, and an increase in the ratio of PGI$_2$/TXA$_2$.

References


11 Inagawa, T., Ohki, S., Sawada, M. and Hirata, F.: Studies on extraction, separation and estimation of prostaglandin by radioimmunoassay. Yaku-gaku Zasshi 92, 1187–1194 (1972) (Abs. in English)


22 Fukumori, T., Tani, E., Maeda, Y. and Sukenaga,
A.: Effect of selective inhibitor of thromboxane 
A2 synthetase on experimental cerebral 

23 Egg, D., Herold, M., Rumpl, E. and Gunther, R.: 
Prostaglandin F2\alpha levels in human cerebrospinal 
fluid in normal and pathological conditions. J. 
Neurol. 222, 239-248 (1980)

24 Ellis, E.F., Wei, E.P. and Kontos, H.A.: Vaso-
dilation of cat cerebral arterioles by pro-
staglandin D2, E2, G2 and I2. Am. J. Physiol. 
237, H381-385 (1979)

25 Hagen, A.A., White, R.P. and Robertson, J.T.: 
Synthesis of prostaglandins and thromboxane 
(1979)

26 Ohmoto, T.: Cerebral vasospasm and prosta-
glandins. Neurol. Surg. (Tokyo) 12, 1453-1464 

27 Ujiie, A., Hiraku, S. and Naito, J.: Phar-
macological actions of OKY-046, a specific 
inhibitor of thromboxane synthetase, with 
special reference to inhibition of thromboxane A2 
production and acceleration of prostacyclin 
(Abs. in English)