EFFECT OF A PROSTACYCLIN DERIVATIVE (OP-41483) AND A HYPEROSMOTIC AGENT (GLYCEROL) ON BRAIN EDEMA AND METABOLISM IN CEREBRAL ISCHEMIA

YASUO KATAYAMA, M.D., FUMIHIKO KASHIWAGI, M.D.,
HAJIME MEMEZAWA, M.D., AND AKIRO TERASHI, M.D.

A PGI₂ derivative, OP-41483, and a hyperosmotic agent, glycerol, were tested for possible beneficial effects on brain edema, metabolism and pathological changes in cerebral ischemia. Combination treatment with these agents was also tested.

Cerebral ischemia was produced in spontaneously hypertensive rats, using bilateral common carotid artery ligation (BLCL).

OP-41483 was administered four times, hourly (500 ng/kg × 4, i.p.). Ten percent glycerol was administered intravenously (6.6 ml/kg). And, for the combination treatment, OP-41483 was administered three times, hourly (500 ng/kg × 3, i.p.), and 10% glycerol was administered intravenously (6.6 ml/kg) in the same manner as the glycerol treated group. In ischemic controls, saline was administered intravenously (6.6 ml/kg).

After 3 h of ischemia, brain water content and metabolites were determined and pathological observation was conducted using electron microscopy.

OP-41483 treated animals maintained higher levels of ATP concentration and reduced accumulation of lactate, but showed no difference in brain water content compared to saline treated controls. Glycerol treated animals showed significance in terms of reduction of brain water content and accumulation of lactate. Glycerol abated the depletion of ATP concentration. OP-41483 + glycerol treated animals showed the most significant effect on the reduction of brain water content and accumulation of lactate. The combination treatment also maintained higher levels of ATP concentration. Additionally, swelling of astrocytic foot processes and mitochondria with destroyed crista were not observed pathologically in the combination treated animals.

These results show that OP-41483, glycerol and combination treatment are beneficial in the treatment of cerebral ischemia. They also indicate that the combination treatment significantly enhances the protective effects compared to individual treatment.
(*Jpn Circ J* 1992; **56**: 1239–1247)

RECENTLY it has been reported that increases in prostonoids, such as throm-

Key words:

OP-41483
Glycerol
Cerebral Ischemia
Brain edema
Brain Metabolism

boxane A₂ (TXA₂), prostaglandin E₂, F_{2α} and leukotriene B₄, C₄ play important roles in aggravating cerebral ischemia^{1–4}. Conversely, prostacyclin (PGI₂) has potent vasodilatory and antiaggregatory actions against TXA₂⁵. In response to these findings, various derivatives of PGI₂ have been

(Received December 5, 1991; accepted April 23, 1992)

The Second Department of Internal Medicine, Nippon Medical School, Tokyo, Japan

Mailing address: Katayama Yasuo M.D., The Second Department of Internal Medicine, Nippon Medical School, 3-5-5 Iidabashi Chiyoda-ku, Tokyo, Japan

developed. OP-41483, a derivative of PGI₂, has one third of the antiaggregatory and vasodilatory action of PGI₂, but has an increased length of activity.^{6,7}

Glycerol, a hyperosmotic agent, is used routinely to treat brain edema in cerebral ischemia.^{8,9} The beneficial effects of this agent have been reported in several publications.¹⁰⁻¹²

OP-41483 and glycerol were tested independently and in combination to determine possible beneficial effects on brain edema and metabolism during cerebral ischemia. Additionally, pathological observations were conducted using electron microscopy.

MATERIALS AND METHODS

Sixteen week old, 270 gram, male, spontaneously hypertensive rats (SHR) with systolic blood pressures of about 200 mmHg were used in this experiment. Animals were fasted 12–14 h prior to experimentation, and then anesthetized with 2% fluothane. Rectal temperature was maintained at 37°C using a heating pad. A catheter was inserted into the left femoral artery for blood gas analysis and blood pressure recording. Another catheter was inserted into the left femoral vein to infuse designated animals with saline or glycerol. The common carotid arteries were exposed through a neck incision and occluded with commercial cotton thread to induce ischemia.

1. Administration of OP-41483 and Glycerol

Animals were given OP-41483 (n=6), glycerol (n=7), OP-41483+glycerol (n=7) or saline (n=7) for ischemic control. OP-41483 (S (E) 6, 9-Deoxa-6, 9-methylene-15-cyclopentyl-16, 17, 18, 19, 20-pentanor-PGI₂· α -cyclodextrin clathrate, Dainippon Seiyaku/Ono Yakuhin), dissolved in saline, was administered intraperitoneally, four times, hourly (500 ng/ml/kg $\times$ 4), beginning 1 h prior to occlusion. Ten percent glycerol dissolved in saline with 5% fructose (Chugai Seiyaku) was administered intravenously (6.6 ml/kg) immediately after occlusion through the left femoral vein using an infusion pump for 1 h. In the combination treatment, OP-41483 dissolved in saline was administered intraperitoneally, three times, hourly (500 ng/ml/kg $\times$ 3), beginning im-

mediately after occlusion. Ten percent glycerol solution was administered intravenously (6.6 ml/kg) immediately after occlusion for 1 h in the same manner as the glycerol treated group. Ischemic controls were administered saline intravenously (6.6 ml/kg) immediately after occlusion for 1 h in the same manner as the glycerol treated group. Sham operated controls (n=7) underwent neck incision only.

2. Measurement of Brain Water Content and Metabolites

After 3 h of ischemia the animals were sacrificed by microwave irradiation (Toshiba Microwave Applicator, 5 kw output for 1.5 sec) to fix brain metabolites. The forebrain was used for measurement of brain water content and metabolic analysis of ATP, lactate and pyruvate. Water content was measured using the freeze dried method.¹³ ATP was analyzed with the luciferin-luciferase reaction using fire fly extracts (Sigma Chemical).¹³ Lactate and pyruvate were measured spectrophotometrically using enzymatic methods.¹⁴

Sham animals were sacrificed and analyzed using the same methods.

Statistical comparisons for physiological parameters were made using analysis of variance (ANOVA) and Scheffe's F-test. ANOVA and Fisher's test were used for statistical analysis of brain water content and metabolic analysis.

3. Electron Microscopic Observation

Transmission electron microscopic (TEM) observation was conducted in saline, OP-41483, glycerol and OP-41483+glycerol treated groups. Cerebral ischemia was induced by clipping both common carotid arteries using aneurysm clips under 2% fluothane anesthesia. After 3 h occlusion, the clips were removed and the animals were perfused through the abdominal aorta, under 180 cmH₂O, with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Using the perfusion fixed tissue, 1 mm cubic blocks were excised from the middle cerebral artery (MCA) area of the brain and post fixed. After dehydration the specimens were passed through propylene oxide and embedded in EPON 816 (Ohken). Blocks were sectioned with a diamond knife on a MT-500

TABLE I PHYSIOLOGICAL VARIABLES 1 H BEFORE AND 3 H AFTER ISCHEMIA

Variables	Sham operated Controls	Saline	OP-41483	Glycerol	OP-41483 + Glycerol
<i>MSBP (mmHg)</i>					
Before	191±10.7	197±15.6	203±24.1	200±10.8	201±5.6
After	213±4.9	206±16.6	208±24.0	214±19.0	228±6.5
<i>pH</i>					
Before	7.36±0.03	7.39±0.03	7.36±0.06	7.41±0.03	7.40±0.06
After	7.42±0.02	7.39±0.02	7.41±0.14	7.41±0.02	7.36±0.06
<i>PaCO₂ (mmHg)</i>					
Before	41.1±7.7	39.5±5.1	41.4±8.2	41.8±1.6	36.9±5.4
After	36.0±0.8	22.4±6.2*	18.8±7.7*	24.3±6.2*	24.7±6.9
<i>PaO₂ (mmHg)</i>					
Before	122.1±11.9	95.3±13.9	108.1±10.3	81.7±6.0	99.1±12.8
After	90.6±6.9	129.9±18.7*	127.5±11.3	123.3±10.9*	128.0±16.2*

Values are mean±standard deviation. MSBP: mean systolic blood pressure.

Statistical difference between before and after in each variable showed: $p^* < 0.05$, by Scheffe's *F*-test.

There were no statistical differences in the variables among the treated groups.

ultramicrotome (Sorvall). The sections were stained with uranyl acetate and lead citrate, and examined in H-800 TEM (Hitachi). Each group contained three animals.

RESULTS

1. Physiological Parameters

Physiological parameters in each group are shown in Table I. There were no differences in mean systolic blood pressure (MSBP) before and after occlusion in each group, and there were no significant differences among the groups. Blood gas analysis showed no difference in pH before and after ischemia, between or among the groups. P_aCO_2 after occlusion decreased significantly compared to measurements before occlusion in the saline, OP-41483 and glycerol treated groups. However, there were no differences in the P_aCO_2 levels among the treated groups. P_aO_2 analysis showed the levels after occlusion increased significantly compared to those before occlusion in saline, glycerol and the combination treated groups. However, there were no differences among the treated groups. Therefore, hyperventilation was noticed after occlusion in the treated groups that were subjected to ischemia.

2. Effect of OP-41483 and Glycerol on Brain Water Content and Metabolites

Brain water contents in sham operated

and saline treated groups were $77.63 \pm 0.13\%$ and $79.45 \pm 0.57\%$ (Mean $\pm$ S.D.), respectively. The brain water contents in OP-41483, glycerol and OP-41483+glycerol treated groups were 79.02 ± 0.67 , 78.46 ± 0.38 and $77.95 \pm 0.25\%$, respectively (Fig. 1). The saline treated group showed a significant increase in brain water content compared to sham operated controls. The OP-41483 and glycerol treated groups also had significant increases in brain water contents compared to the sham operated control group. However, most importantly, there was no significant difference between the OP-41483+glycerol treated group and the sham operated controls. Compared to the saline treated group; glycerol and OP-41483+glycerol treated groups showed significant decreases in brain water contents. The OP-41483 treated group was not significantly different when compared with the saline treated group. The brain water content in the OP-41483+glycerol treated animals was significantly lower than the glycerol treated animals.

The concentration of ATP in the saline treated controls ($0.21 \pm 0.14 \mu\text{mol/g}$) was drastically lower than the sham operated controls (2.64 ± 0.11). The three treated groups also showed significant decreases in ATP concentration compared to sham operated controls. However, ATP was maintained at higher levels than saline treated

Fig.1. Effect of OP-41483 and glycerol on brain water content in ischemia. Note: glycerol and the combination treatment with OP-41483 and glycerol reduced increased water content.

animals. The glycerol (0.82 ± 0.33) and the OP-41483+glycerol (0.85 ± 0.24) treated groups showed statistical differences in ATP concentration when compared to saline treated controls. There was no significant difference in ATP concentration between the glycerol and the OP-41483+glycerol treated groups (Table II).

The saline treated controls ($24.2 \pm 7.77 \mu\text{mol/g}$) showed a significant increase in lactate concentration compared to sham operated controls (1.13 ± 0.27). The OP-41483 (16.4 ± 7.79) and glycerol (9.18 ± 5.52) treated groups also showed lactate accumulation. Yet, the levels of lactate in both groups were significantly lower than the

levels of the saline treated control group. The administration of OP-41483+glycerol (5.57 ± 2.06) remarkably suppressed the accumulation of lactate. There was no significant difference in lactate concentration between the combination treated group and the sham operated controls. The level of lactate was lowest in the combination treated group out of the three treated groups. But, there was no significant difference between the glycerol and the combination treated group (Table II).

There was an increase in pyruvate among all treated groups, when compared to the saline treated controls ($0.24 \pm 0.06 \mu\text{mol/g}$) and to the sham operated controls ($0.29 \pm$

TABLE II EFFECT OF OP-41483 AND GLYCEROL ON BRAIN METABOLITES IN ISCHEMIA

Groups	Metabolites			lactate/ pyruvate
	ATP	lactate	pyruvate	
Sham operated controls	2.64±0.11	1.13±0.27	0.29±0.05	3.80±0.68
Saline	0.21±0.14*	24.2±7.77*	0.24±0.06	113±54.5*
OP-41483	0.47±0.20*	16.4±7.79*†	0.34±0.11	38.5±25.5†
Glycerol	0.82±0.33*†	9.18±5.52*†	0.40±0.13*†	29.6±34.3†
OP-41483 + Glycerol	0.85±0.24*†	5.57±2.06†	0.37±0.13†	16.2±7.49†

Concentration of ATP, lactate and pyruvate was expressed as $\mu\text{mol/g}$ brain tissue.

Statistical differences from sham operated controls: * $p < 0.05$, and from saline treated group: † $p < 0.05$. NS: no significance

Fig.2. Transmission electron micrographs of the cortex of the MCA area, 3 h after ischemia in a saline treated control. Note: Constricted lumen (Lu) of the arteriole with cellular debris and marked swelling of astrocytic foot processes (Ac) (a: left). Swelling of mitochondria (Mc) with destroyed crista structure were clearly observed (b: right top). Projected nuclei of endothelium to the lumen also could be seen (c: right bottom).

0.05). Only the glycerol treated group (0.40 ± 0.13) had statistical significance compared to sham operated controls. When compared to saline treated controls, glycerol and the combination treated groups (0.37 ± 0.13) showed significantly higher levels of pyruvate (Table II).

Saline treated controls (113 ± 54.5) showed significant increases in the lactate/pyruvate ratio compared to sham operated controls

(3.80 ± 0.68). All three treatment groups significantly reduced the ratio compared to saline treated controls. There were no significant differences among the three treated groups nor between the treated groups and the sham operated controls (Table II).

3. Electron Microscopic Observation

In saline treated controls the arteriole lumen was constricted, and contained cellular

Fig.3. Transmission electron micrographs of the cortex of the MCA area, 3 h after ischemia in a OP-41483 treated animal. Note: marked swelling of the astrocytic foot process (Ac) and endothelium could be seen (left). Swelling of mitochondria with destroyed crista (Mc) was remarkably shown at high magnification (right).

Fig.4. Transmission electron micrographs of the cortex of the MCA area, 3 h after ischemia in a glycerol treated animal. Note: lumen of the arteriole (Lu) was well expanded and swelling of astrocytic foot process could not be seen (left). However, swelling of mitochondria (↑, Mc) was observed (right).

debris such as platelets and red blood cells. Extremely swollen astrocytic foot processes around the arteriola were observed (Fig. 2, left). Destruction of crista in mitochondria could be seen (Fig. 2, right top) and the projected nucleus of the endothelium towards the lumen was observed (Fig. 2, right bottom). In OP-41483 treated animals astrocytic foot processes were still very swollen and

mitochondria with destroyed crista were observed (Fig. 3). However, the destruction of crista seemed somewhat reduced when compared to the saline treated controls (Fig. 3, right). Conversely, in the glycerol treated animals arteriole lumen were expanded well and swelling of the astrocytic foot processes could not be seen (Fig. 4, left). The swelling of mitochondria with destroyed crista,

Fig. 5. Transmission electron micrographs of the cortex of the MCA area, 3 h after ischemia in an animal treated with OP-41483 and glycerol. Note: lumen of the arteriole (Lu) was well expanded and swelling of astrocytic foot process and mitochondria could not be seen (left). Structure of crista of mitochondria (↑) was preserved (right).

however, was still observed (Fig. 4, right). There were some areas within the glycerol treated group that exhibited less swelling of the mitochondria. In animals with the combination treatment, the lumen of the arteriole was well expanded; and, swelling of astrocytic foot processes and mitochondria could not be seen (Fig. 5, left). The structure of the mitochondria was preserved almost normally in the combination treatment group (Fig. 5, right).

DISCUSSION

In this study spontaneously hypertensive rats (SHR) were employed to induce cerebral ischemia. It has been reported that after 3 h occlusion of the bilateral common carotid arteries there is a reduction in cerebral blood flow, an increase in brain edema and metabolic disruption^{15,16}

We tested a PGI₂ derivative, OP-41483, a hyperosmotic agent, glycerol, and the combined treatment of these agents for possible beneficial effects on brain edema, metabolism and pathological changes in ischemia.

The dose of OP-41483 used for this experiment was based on previous studies¹⁶ and other reports^{6,7}. The dose of glycerol was chosen based on the volume used to treat patients with brain edema clinically. In the

combination treated group, OP-41483 was administered 3 times, hourly, beginning immediately after occlusion. The study of OP-41483, singularly, was administered 4 times, hourly, beginning 1 h prior to ischemia. The difference in the administration method was to more closely mimic clinical treatment in the combined treatment, while testing the optimal effectiveness of the OP-41483 on metabolites, with pretreatment.

In a preliminary experiment, effect of the agents, saline, OP-41483, glycerol and OP-41483 + glycerol, on hematocrit levels were examined. There were no differences in the values before and after ischemia between or among the groups.

OP-41483 had no effect on brain water content but it showed remarkable amelioration of metabolic derangement. Pathological observation using TEM showed OP-41483 to have no effect on swelling of astrocytic foot processes, and less destruction of crista when compared to saline treated animals. These pathological findings coincided with results in brain water content and metabolites.

Glycerol decreased brain edema and metabolic derangement. Pathological observation showed swelling of astrocytic foot processes, which indicates brain edema was diminished. However, swelling of mitochondria and destroyed crista structures were not improved

fully. Such pathological findings parallel the brain water content and metabolite levels measured.

The combination treatment promoted the most significant effect on lowering brain edema and metabolic derangement out of the three treatment groups. Only the combination treated group exhibited no differences in brain water content and lactate concentration when compared to the sham operated group. TEM observation showed swelling of astrocytic foot processes and mitochondria were diminished and the structure of crista were preserved. Therefore, TEM observation coincided with brain water content and metabolic levels measured.

The PGI₂ derivative, OP-41483, has one-third of the potent antiaggregatory action and vasodilatory action of PGI₂. The agent can ameliorate metabolic derangement of ischemic brain damage through these actions. There are several reports that platelets play important roles in aggravating cerebral ischemia.¹⁶⁻²³ Aggregated platelets release vasoactive substances such as serotonin, prostaglandin endoperoxides and TXA₂. Also, disproportionate prostaglandin synthesis has been reported during the acute phase of cerebral ischemia.¹⁻³ These vasoactive substances disturb microcirculation and induce brain edema. Therefore, their suppression may reduce ischemic impairment.

It is well known that glycerol is one of the most useful hyperosmotic agents to reduce brain edema.⁸⁻¹² The primary mechanism of the agent is to change the osmolarity between blood and brain tissue. It is also known that glycerol has free radical scavenging¹¹ and hemorheological actions.^{24,25} Free radical formation is one of the factors that causes cerebral edema through damage to the cell membrane during the acute phase of ischemia.²⁶ In this study we confirmed the significant effect of glycerol on brain edema and metabolites during ischemia. The effects were brought about through the dehydrating action, by changing osmolarity, and partly through other mechanisms mentioned above. Also, glycerol administration may increase cerebral blood flow by its own pharmacological action and by reducing cerebral edema.¹²

The combination treatment, OP-41483

+ glycerol, significantly enhanced the protective effects during an ischemic insult compared to the individual treatment of either agent. Therefore, the combination treatment improves cerebral blood flow and microcirculation through the complementary action of both agents more than either single agent acting alone.

In summary, these experiments demonstrate that a PGI₂ derivative, OP-41483, a hyperosmotic agent, glycerol, and a combination of the two are beneficial for the treatment of cerebral ischemia. The combination treatment was most beneficial for improvement of brain edema, metabolism and pathological findings in cerebral ischemia.

Acknowledgement

We thank Miss Ann McKee for revising the paper and Mr. Hiromi Muramatsu for his skilful technical help.

REFERENCES

- GAUDET RJ, ALAM I, LEVINE L: Accumulation of cyclooxygenase products of arachidonic acid metabolism in gerbil brain during reperfusion after bilateral common carotid occlusion. *J Neurochem* 1980; **35**: 653-658
- SHOHAMI E, ROSENTHAL J, LAVY S: The effect of incomplete cerebral ischemia on prostaglandin levels in rat brain. *Stroke* 1982; **13**: 494-499
- MINAMISAWA H, TERASHI A, KATAYAMA Y, KANDA Y, SHIMIZU J, SHIRATORI T, INAMURA K, KASEKI H, YOSHINO Y: Brain eicosanoid levels in spontaneously hypertensive rats after ischemia with reperfusion: Leukotriene C₄ as a possible cause of brain edema. *Stroke* 1987; **19**: 372-377
- KATAYAMA Y, SHIMIZU J, SUZUKI S, MEMEZAWA H, KASHIWAGI F, KAMIYA T, TERASHI A: Role of arachidonic acid metabolism on ischemic brain edema and metabolism. In Long D et al eds. *Advances in Neurology*. New York, Raven Press, 1990; **52**: p.105
- MONCADA S, VANE JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂ and prostacyclin. *Pharmacol Rev* 1979; **30**: 293-331
- ADAKIN PG, KOTTEGODA SR, LAU LC, TAI MY, KARIM SMM: Inhibition of platelet aggregation and reversal of vasopressin-induced ECG changes by a carboprostacyclin analogue, ONO 41483, in primates. *Prostaglandins. Leukotrienes Med* 1982; **9**: 307-320
- ADAKIN PG, LAU LC, TAI MY, KARIM SMM: Inhibition of platelet aggregation with intravenous and oral administration of a carboprostacyclin analogue, 15-cyclopentyl- ω -pentanor-5-(E)-D-carbacyclin (ONO 41483) in man. *Prosta-*

- glandins Leukotrienes Med* 1983; **10**: 53–64
8. MEYER JS, SHIMAZU K, OHUCHI T, OKAMOTO S, KOTO A, FUKUUCHI Y, ERICSSON AD: Cerebral metabolic effects of glycerol infusion in diabetics with stroke. *J Neurol Sci* 1974; **21**: 1–22
 9. MEYER JS, ITO Y, OKAMOTO S, WELCH KMA, MATHEW NT, OTT EO, SAKAI S, MIYAKAWA Y, CHABI E, ERICSSON AD: Circulatory and metabolic effects of glycerol infusion in patient with recent cerebral infarction. *Circulation* 1975; **51**: 701–702
 10. DOCZI T, SZERDAHELYI P, FERENC J, BODASI M: Effects of intraventricularly injection isoosmolar glycerol on brain water and electrolytes in rats. *Neurosurgery* 1987; **21**: 182–185
 11. KASHIWAGI F, KATAYAMA Y, SUZUKI S, SHIMIZU J, NAGAZUMI A, TERASHI A: The effect of glycerol on experimental cerebral ischemia -part 1. Studies on lipid peroxides, prostaglandins, brain edema and brain metabolites. *Brain Nerve* 1988; **40**: 179–185
 12. KASHIWAGI F, KATAYAMA Y, SHIMIZU J, KAMIYA T, TERASHI A: Effect of a new hyperosmotic agent, NIK-242 inj., on brain water content, metabolites and cerebral blood flow in cerebral ischemia in spontaneously hypertensive rats. *Jpn Circ J* 1991; **55**: 1246–1251
 13. KATAYAMA Y: Studies on pathogenesis of cerebral edema due to experimental cerebral hemorrhage; An approach through energy metabolism. *Nippon Ika Daigaku Zasshi* 1982; **49**: 58–68
 14. HOLL F: Methods of Enzymatic Analysis. New York, Verlag Chemie Weinham and Academic Press, 1974, p.1457
 15. KATAYAMA Y, SUGIMOTO S, INAMURA K, SOEDA T, SHIMIZU J, SUZUKI S, NAGAZUMI A, TERASHI A: Susceptibility to ischemic insult in hypertensive rats: Correlation between degree of ischemia and hypertension. *Jpn Circ J* 1986; **50**: 258–264
 16. KATAYAMA Y, TERASHI A, SHIMIZU J, SUZUKI S, KASHIWAGI F, KAMIYA T, ASHIDA S: Role of platelets as a factor aggravating cerebral ischemia. *Jpn Circ J* 1990; **54**: 1511–1516
 17. KATAYAMA Y, SHIMIZU J, MEMEZAWA H, MINAMISAWA H, SUZUKI S, SUGIMOTO S, TERASHI A: Protective effect of thromboxane A₂ synthetase inhibitor (Trapidil) on acutely-induced cerebral ischemia. *Brain Nerve* 1986; **38**: 925–931
 18. HARKER LA, RITCHIE JL: The role of platelets in acute vascular events. *Circulation* 1980; **62**(Suppl 5): 13–18
 19. FUJIMOTO T, SUZUKI H, TANOUE K, FUKUSHIMA Y, YAMAZAKI H: Cerebrovascular injuries induced by activation of platelets in vivo. *Stroke* 1985; **16**: 245–250
 20. GORDON JL, MILNER AL: Blood platelets as multifunctional cells. In Platelets in Biology and Pathology. ed by GORDON JL, North Holland Pub. Co. Amsterdam, 3, 1976, p.3
 21. SMITH JB, WILLIS AL: Formation and release of prostaglandins of platelets in release of thrombin. *Br J Pharmacol* 1970; **40**: 545–546
 22. MAJNO G, GILMORE V, LEVINTHAL M: On the mechanism of vascular leakage caused by histamine-type mediators. *Circ Res* 1967; **21**: 833–847
 23. ELLIS EF, NIES AS, OATES JA: Cerebral arterial smooth muscle contraction by thromboxane A₂. *Stroke* 1977; **8**: 480–483
 24. HARTMANN A, DETTMERS H, SCHOTT H, BEYENBURG S: Cerebral blood flow and rheologic alterations by hyperosmolar therapy in patients with brain oedema. *Acta Neurochir Suppl* 1990; **51**: 168–169
 25. OHTA K, GOTOH F, TOMITA M, TANAHASHI N, KOBARI M, TERAYAMA Y, MIHARA B, NARA M: Hypertonic glycerol solution improves erythrocyte hyperaggregability in occlusive cerebrovascular disease. *Clin Hemorheol* 1990; **10**: 515–523
 26. IKEDA Y, IKEDA K, LONG D: Comparative study of different iron-chelating agents in cold-induced brain edema. *Neurosurgery* 1989; **24**: 820–824