The Effects of a Thromboxane A_2 Receptor Antagonist on Neurologic Recovery after 15 min Complete Global Brain Ischemia in Dogs

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The effects of a thromboxane A_2 receptor antagonist (anti-TXA_2; ONO-3708) on the neurologic recovery after 15 min complete cerebral ischemia were investigated in dogs. Complete cerebral ischemia was achieved by occlusion of the trunk of the aorta, the superior and the inferior caval vein. Seventeen dogs were divided into 2 groups; 1) control group (no drug, n = 9), 2) Anti-TXA_2 group (anti-TXA_2 200 mcg·kg^{-1} in iv bolus soon after recirculation followed by continuous infusion at a rate of 10 mcg·kg^{-1}·min^{-1} for 3 days, n = 8). EEG, auditory brainstem response (ABR), middle latency response (MLR), and somatosensory evoked potential (SEP) recordings were obtained before ischemia, 120 min after re-circulation and on the 7th day after ischemia. The neurologic recovery score (NRS) were determined on the 3rd and the 7th day. Impaired EEG score was significantly higher in the anti-TXA_2 group on 7th day after ischemia ($P < 0.05$). Anti-TXA_2 increased the reappearance rates of the ABR-Vth ($P < 0.05$) and the SEP-N_3 waves ($P < 0.01$) at 120 min after ischemia. The survival rate tended to be higher in the anti-TXA_2 group. However, NRS did not significantly differ in the groups. (Key words: TXA_2 receptor antagonist, complete cerebral ischemia, evoked potential, neurologic recovery score)

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It is well recognized that complete cerebral ischemia increases Ca^{2+} influx into the brain cells. The increased Ca^{2+} triggers the activation of arachidonic acid cascade, production of oxidative free radicals, activation of proteases and the release of excitatory neurotransmitters[1–3]. These biological active substances are considered to be related to the development of brain damage after ischemia[1–3]. Thromboxane A_2 (TXA_2) is one of such substances extracted from arachidonic acid metabolism and characterized to be a strong platelet aggregator and vasoconstrictor[4,5]. The increase of TXA_2 concentration in plasma and brain tissue after brain ischemia[6] may contribute to the acceleration of the postischemic neuronal damage.

The purpose of the present study was to investigate the effects of a thromboxane A_2 receptor antagonist (anti-TXA_2; ONO-3708, Ono Pharmaceutical Co., Osaka, Japan) on the neurological recovery after 15 min complete brain ischemia in dogs.

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Table 1. The measuring condition of the evoked potentials

	ABR	MLR	SEP
Derivation (+)-(-)	Cz-A1	Cz-A1	Fz-C3' Fz-CV2
Stimulation	clic sound 90 dB 10 Hz	clic sound 90 dB 5 Hz	current 4 mA 5 Hz
Stimulation site	lt ear	lt ear	rt median nurve
Band (Hz)	100–3000	5–1000	20–1000
Sweep (ms)	10	50	100
Average count	1000	500	500

Materials and Methods

These experiments were approved by the Animal Care Committee of Tohoku University. Seventeen adult mongrel dogs weighing 8–13 kg were studied. Anesthesia was induced with iv sodium thiopental (25 $mg \cdot kg^{-1}$) and maintained with 0.3% halothane in O_2 and pancuronium. Their lungs were ventilated with a R-60 respirator (Aika) via a cuffed endotracheal tube to maintain Pa_{CO_2} between 35 and 40 mmHg under monitoring of expired CO_2 (78356A, Hewlet-Packard). A forelimb vein was cannulated for fluid and drug administration, and Lactate-Ringer solution was infused at a rate of 6–8 $ml \cdot kg^{-1} \cdot min^{-1}$. A femoral artery was cannulated for arterial blood sampling and continuous monitoring of systemic arterial pressure (BP). Lead II of the ECG was continuously monitored, and heart rate (HR) was computed from the mean values of RR intervals. Body temperature was monitored by an oropharyngeal thermistor probe and maintained between 37.0 and 38.0°C before ischemia and between 35.5 and 37.5°C after ischemia by a warming mat and a heat lump.

The leftside thoracotomy was performed aseptically at the 4th intercostal space and tapes were placed around the inferior and the superior caval vein, and the trunk of the aorta. Complete global brain ischemia for 15 min was achieved by occluding the inferior caval vein, the trunk of the aorta, and the superior caval vein by tightning of the tapes. During occlusion of the vessels, the heart was cooled with iced saline placed in the pericardial cavity. There was no animal of cardiac arrest during the 15 min occlusion. After drawing out iced saline and loosening the tapes, re-establishment of systemic circulation was achieved by iv injection of 1 mg of etilefrine and 1 $mg \cdot kg^{-1}$ of sodium bicarbonate.

The chest was closed 150 min after re-circulation. Muscle paralysis induced with pancuronium was reversed by neostigmine with atropine. When blood gas showed a normal respiratory state under enough spontaneous respiration, the endotracheal tube was extubated. An electrolyte solution with glucose was infused until the dogs could drink and eat normally, and an antibiotic was injected im daily for the first 4 days.

After re-establishment of circulation, the dogs received a randomized order either no drug (control group, n = 9) and anti-TXA_2 200 $mcg \cdot kg^{-1}$ in iv bolus followed by the continuous infusion at a rate of 10 $mcg \cdot kg^{-1} \cdot min^{-1}$ for the next 3 days (anti-TXA_2 group, n = 8).

Blood samples for hematocrit (Ht), blood gases, glucose (BS) and lactate (LA) were obtained immediately before ischemia and 120 min after re-circulation. EEG, auditory brainstem response (ABR), middle latency response (MLR) and somatosensory evoked potential (SEP) were measured with Neuropack II (Nihon Koden) immediately before ischemia, 120 min after re-circulation and on the 7th day after ischemia. The measuring conditions of the evoked potentials (EPs) were listed in table 1. EEG was scored from 1 to 5 using a modification of Scollo-Lavizzari's classification[7] (table 2). On the

Table 2. EEG score

1: Dominant, normal alpha-activity with theta-delta-activity
2: Dominant theta-delta-activity with detectable normal alpha activity
3: Theta-delta-activity without alpha-activity
4: Low voltage delta-activity or monophonic, non-reactive alpha-beta-activity possibly with short isoelectric intervals
5: very flat to isoelectric EEG

Table 3. Values of physiologic, blood gases, blood glucose (BS) and lactate (LA) before and 120 min after ischemia (mean ± SD)

	Control		Anti-TXA_2	
	before	120 min after	before	120 min after
mBP (mmHg)	125 ± 22	115 ± 15	118 ± 15	122 ± 18
HR (bpm)	186 ± 21	162 ± 14*	184 ± 33	169 ± 9
BT (C)	37.5 ± 0.3	36.5 ± 1.1*	37.6 ± 0.3	37.0 ± 0.4
HT (%)	40.5 ± 4.1	46.2 ± 4.2**	35.8 ± 4.1	44.2 ± 3.3**
Pa_{O_2} (mmHg)	492 ± 26	458 ± 63	481 ± 59	417 ± 84
Pa_{CO_2} (mmHg)	37.4 ± 2.2	35.3 ± 1.1*	38.0 ± 2.1	39.3 ± 4.5
BE ($mEq \cdot l^{-1}$)	−1.7 ± 1.3	−1.0 ± 2.5	+0.2 ± 2.0	−2.4 ± 2.2*
BS ($mg \cdot dl^{-1}$)	123 ± 23	147 ± 42	124 ± 22	178 ± 55*
LA ($mM \cdot l^{-1}$)	1.1 ± 0.7	1.6 ± 0.8*	1.1 ± 0.4	1.3 ± 0.6

*$P < 0.05$, **$P < 0.01$, versus before.

3rd and the 7th day after ischemia, the neurologic recovery score (NRS)[8] was calculated according to the method of a modification of Todd's neurologic deficit score[9].

Hemodynamic, blood gases, BS and LA values were analysed by a Student' t test. NRS and EEG scores were tested by a Wilcoxon' U test, and the reappearance rates of the evoked potential waves and the survival rate were analysed by an X^2 test. The differences were considered significant when $P < 0.05$ and to have a tendency to be significant when $0.05 < P < 0.1$.

Results

Weight and age of the control and the anti-TXA_2 groups were 10.9 ± 1.6 kg, 1.9 ± 0.6 yr, and 10.8 ± 1.0 kg, 2.2 ± 0.3 yr (mean ± SD), respectively. There were no significant differences between the two groups. Ht and LA were increased, and HR, BT and Pa_{CO_2} were decreased significantly in the control group at 120 min after ischemia, compared to those of the preischemic values. In the anti-TXA_2 group, increases in Ht and BS and a decrease in BE at 120 min after ischemia were significant. There were, however, no significant differences between the two groups at any periods (table 3).

The mean value for EEG score on the 7th day was significantly lower in the anti-TXA_2 group ($P < 0.05$), while that at 120 min after ischemia did not significantly differ between the groups (fig. 1). The reappearance rate of the ABR-Vth wave and the SEP-N_3 wave at 120 min after re-circulation were significantly higher in the anit-TXA_2 group ($P < 0.05$, $P < 0.01$). These differences became to be insignificant on the 7th day. The MLR-Pa wave did not reappear at 120 min after ischemia in both groups. On the 7th day, the reappearance rate of this wave tended to be higher in the anit-TXA_2 group (table 4).

The survival rate on the 7th day was 6/9 (67%) in the control group and 8/8

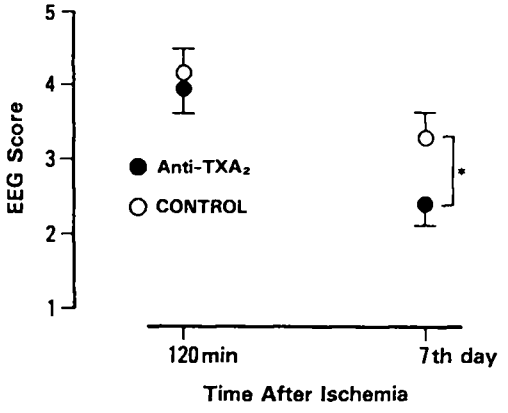

Fig. 1. EEG scores of the control group (○) and the anti-TXA_2 group (●) at 120 min and on the 7th day after ischemia. Each point represents the mean ± SE. $^*P < 0.05$, between the two groups.

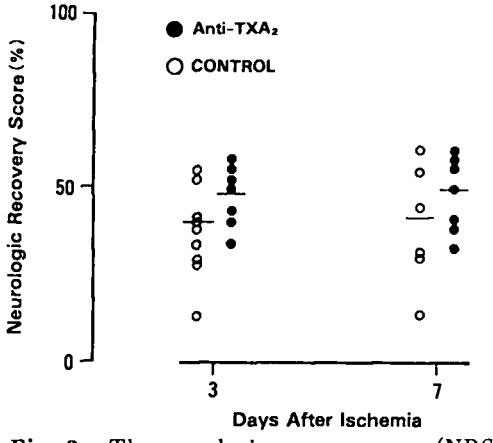

Fig. 2. The neurologic recovery scores (NRS) of surviving dogs in the control group (○) and the anti-TXA_2 group (●) on the 3rd and the 7th day after ischemia. Each point represents the NRS of each dogs and each bar represents the mean. No difference among the two groups at each period.

(100%) in the anti-TXA_2 group ($P < 0.08$). Averaged NRS values on the 3rd and 7th day did not significantly differ between the groups (fig. 2).

Discussion

Although TXA_2 has been reported to increase in plasma and the brain tissue after brain ischemia[6], little information is available concerning the effects of TXA_2 on the process of postischemic brain damage. Since TXA_2 has a strong potency of vasoconstriction and platelet aggregation[10–12], TXA_2 is considered to relate closely to the induction of the postischemic delayed hypoperfusion or the no-reflow phenomenon which may be one of the contributing phenomena to accelerate postischemic brain damages. In fact, the TXA_2 synthetase inhibitor has a protective effect on brain ischemia when administered both before and after ischemia[13]. Anti-TXA_2 has been reported to inhibit intracoronary thrombosis induced by partial occlusion of the coronary artery at a dose of 100 mcg·kg^{-1} in iv bolus and to prevent cerebral vasospasm induced by intracisternal injection of fresh blood at a dose of 10 mcg·kg^{-1}·min^{-1} iv infusion in dogs[12]. We selected the doses of anti-TXA_2 in this study with reference to these reports.

The present results demonstrated a significant improvement of EEG score in the anti-TXA_2 group on the 7th day after ischemia. The recovery of the ABR-Vth and the SEP-N_3 waves at 120 min and the MLR-Pa wave on the 7th day after ischemia were accelerated in the anti-TXA_2 group. These

Table 4. The reappearance rates of EPs at 120 min and on the 7th day after ischemia

	120 min after ischemia		7th day after ischemia	
	Control (%)	Anti-TXA_2 (%)	Control (%)	Anti-TXA_2 (%)
ABR-Vth	3/9 (33)	7/8 (88)**	5/6 (83)	8/8 (100)
MLR-Pa	0/9 (0)	0/8 (0)	1/6 (17)	5/8 (63)*
SEP-N_2	7/9 (78)	8/8 (100)	3/4 (75)	7/8 (88)
SEP-N_3	3/9 (33)	8/8 (100)***	3/4 (75)	7/8 (88)

$^*P < 0.09$, $^{**}P < 0.05$, $^{***}P < 0.01$, control vs anti-TXA_2.

results suggests the beneficial effect of anti-TXA_2 for the electrophysiological recovery from postischemic brain damages. The origins of EPs in this study are considered to be as follows[15–18]: ABR-Vth = inferior colliculus, MLR-Pa = acoustic radiation or acoustic area of cortex, SEP-N_2 = thalamus, SEP-N_3 = thalamo-cortical tract. It is well recognized that the appearance rates of EPs after ischemia depend upon their origins in the central nervous system (CNS) where EPs come from. The lower the appearance rate of EP, the upper the origin of it[14], suggesting the more weakness of this area to ischemic insult. We assumed the reason of the differences in the effects of anti-TXA_2 on EPs between the 120 min and 7th day as follows; Insignificant differences in the ABR-Vth and the SEP-N_3 waves on the 7th day are the results of the improvement in the control group. The origins of these waves are relatively lower, thus the spontaneous recovery from damages may contribute to the better reappearance rates on the 7th day. While, the MLR-Pa wave did not spontaneously recovered from damages on the 7th day because of its' higher origin. Thus the difference may become to be significant.

The changes in EEG and EPs are considered to reflect the neurologic outcome[14]. In fact, the survival rate was improved in the anti-TXA_2 group. However, the neurological outcome expressed by NRS was not improved by anti-TXA_2. There has been some reports showing the discrepancy between improvement of postischemic cerebral blood flow and neurologic recovery[2,19,20].

From this study we conclude that the postischemic administration of anti-TXA_2 accelerated the recovery of electrophysiological functions (EEG and EP) of the brain and the survival rate from 15 min complete cerebral ischemia in dogs. Anti-TXA_2, however, did not show a beneficial effect on the recovery of NRS.

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References

1. Siesjo BK, Wieloch T: Cerebral metabolism in ischemia: Neurochemical bases for therapy. Br J Anaesth 57:47–62, 1985
2. Safer P: Cerebral resuscitation after cardiac arrest: a review. Circulation 74 (Suppl IV):138–153, 1986
3. Baethmann A, Jansen M: Possible role of calcium entry blockers in brain protection. Eur Neurol 25 (Suppl 1):102–114, 1986
4. Moncada S, Vane JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. Pharmacological reviews 30:293–331, 1979
5. Wolfe LS: Eicosanoids: prostaglandins, thromboxanes, leucotrienes, and other derivatives of carbon-20 unsaturated fatty acids. J Neurochem 38:1–14, 1982
6. Katayama Y, Suzuki S, Shimizu J, Imamura K, Sugimoto S, Terashi A: Effect of bilateral common carotid artery ligation on prostaglandin levels (TXA_2, PGI_2) in spontaneously nypertensive rats (SHRSP, SHRSR) and normotensive rats (WKY). Brain 38:571–578, 1986
7. Scollo-Lavizzari G, Bassetti C: Prognostic value of EEG in post-anoxic coma after cardiac arrest. Eur Neurol 26:161–170, 1987
8. Iwatsuki N, Ono K, Takahashi M, Tazima T: The effects of nicardipine given after 10-minutes complete global cerebral ischemia on neurologic recovery in dogs. J Anesth 4:337–342, 1990
9. Todd MM, Chadwick HS, Shapiro HM: The neurologic effects of thiopental therapy following experimental cardiac arrest in cat. Anesthesiology 57:76–86, 1982
10. Toda N, Nakajima M, Okamura T, Miyazaki M: Interactions of thromboxane A_2 analogs and prostaglandins in isolated dog arteries. J Cardiovasc Pharmacol 8:818–825, 1986
11. Toda N, Inoue S, Okamura T, Okunishi H: Mechanism underlying relaxations caused by prostaglandins and thromboxane A_2 analog in isolated dog arteries. J Cardiovasc Pharmacol 11:354–362, 1988
12. Kondo K, Seo R, Kitagawa T, Omawari

N, Kira H, Ogawa T, Kawasaki A: Preventive effect of ONO-3708 on thrombosis and vasospasms in vitro and in vivo. Advances in Prostaglandin, Thromboxane, and Leukotriene Research 17:423–426, 1987

13. Katayama Y, Shimizu J, Memezawa H, Minamisawa H, Suzuki S, Sugimoto S, Terashi A: Protective effect of thromboxane A_2 synthetase inhibitor (trapidil) on acutely-induced cerebral ischemia. Brain 38:925–931, 1986
14. Ono K: The changes in EEG, evoked potentials, and neurological recovery after global brain ischemia in dogs. Jap J Anesth 39:572–580, 1990
15. Lev A, Sohmer H: Sources of averaged neuronal responses recorded in animal and human subjects during coholear audiometry (Electrocoholeography). Arch Ohr Nas Nehlk 201:79–90, 1972
16. Jewett DL, Romano ML, Williston JS: Human auditory evoked potentials: possible brain stem components detected on the scalp. Science 167:1517–1518, 1970
17. Picton TW, Hillyard SA, Krausz HI, Galambos R: Human auditory evoked potentials I. Evaluation of components. Electroencheph Neurophysiol 36:179–190, 1974
18. Machida M: Somatosensory evoked potentials: Mechanism of farfield potentials in man. Adv Neurol Sci 32:15–30, 1988
19. Iwatsuki N: The effects of calcium antagonists on brain resuscitation. J Jap Soc Reanimatol 5:34–40, 1988
20. Snyder BD, Tabbaa MA: Assessment and treatment of neurological dysfunction after cardiac arrest. Stroke 19:269–273, 1988