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Effect of KBT-3022, a new cyclooxygenase inhibitor, on experimental brain edema in vitro and in vivo

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Abstract

The effect of KBT-3022 (ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]pyrrol-1-ylacetate), a new cyclooxygenase inhibitor, on experimental brain edema was studied. In vitro, KBT-3022 (100 μM) and its metabolite desethyl KBT-3022 (10 and 100 μM), but neither acetylsalicylic acid nor indomethacin, inhibited arachidonic acid-induced swelling of guinea pig cortical slices. KBT-3022 (3–100 μM) and desethyl KBT-3022 (3–30 μM), but neither acetylsalicylic acid nor indomethacin, inhibited lipid peroxidation in guinea pig brain homogenate. In vivo, oral administration of KBT-3022 (1, 3 and 10 mg/kg) and indomethacin (10 and 30 mg/kg), but not acetylsalicylic acid, prevented brain edema induced by bilateral carotid occlusion and recirculation in gerbils. Indomethacin then prevented postischemic hyperthermia, but not KBT-3022. KBT-3022 (10 mg/kg) and indomethacin (30 mg/kg) inhibited lactate accumulation in gerbil brain after ischemia and recirculation. These results suggest that KBT-3022 prevents development of both cytotoxic edema in vitro and vasogenic edema in vivo.

Keywords: Cyclooxygenase inhibitor; KBT-3022; Arachidonic acid; Ischemic brain edema

1. Introduction

Brain edema is an important clinical complication of cerebral ischemia, and frequently leads to fatal transtentorial herniation in patients with cerebral infarction (Bounds et al., 1981). Brain edema is considered to be divided into two types. One is cytotoxic edema, characterized by disruption of cellular membranes, and the other is vasogenic edema, which is associated with breakdown of the blood-brain barrier. Ischemic brain edema is initially of the cytotoxic type, and soon progresses to the vasogenic type (Katzman et al., 1977; Siesjö, 1992). However, it is difficult to make a clear distinction between the two types in vivo. Chan and Fishman (1978) established an in vitro bioassay system for the measurement of cytotoxic edema. They demonstrated arachidonic acid-induced tissue swelling, an increased Na^+ content and a decreased K^+ content of rat brain cortical slices. It has been reported that arachidonic acid modifies membrane integrity (Klausner et al., 1980; Chan and Fishman, 1982), induces membrane perturbations with increased lipid peroxidation and free radical formation (Chan and Fishman, 1980; Chan et al., 1982), and reduces (Na^+,K^+)-ATPase activity (Chan and Fishman, 1982; Chan et al., 1983). Many studies have suggested that lipid peroxidation and nonenzymatic free radical formation may lead to membrane damage and induce cytotoxic edema (Demopoulos et al., 1980; Siesjö, 1981; Asano et al., 1989).

It has been widely accepted that arachidonic acid is related to the pathophysiology of not only cytotoxic edema but also vasogenic edema. When the brain is rendered ischemic, amounts of free fatty acids, especially arachidonic acid, and its peroxidation products are liberated from cellular membranes and accumulate in the brain (Yoshida et al., 1980; Gaudet et al., 1980; Moskowitz et al., 1984). Recent studies suggest that arachidonic acid metabolites derived from both the cyclooxygenase and lipoxygenase pathways contribute to the development of vasogenic edema through regulation of cerebral blood flow and vascular permeability (Iannotti et al., 1981; Bhakoo et al., 1984; Minamisawa et al., 1988). Moreover, the conversion of arachidonic acid to prostaglandins produces cytotoxic free radicals. These studies have shown that lipid peroxidation, free radical formation and arachidonic acid

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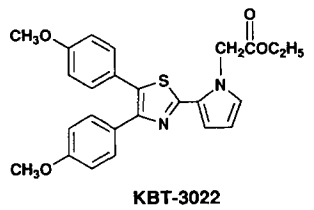
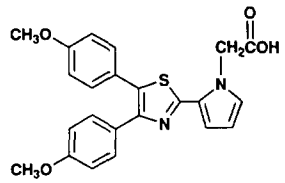


KBT-3022



Desethyl KBT-3022

Fig. 1. Chemical structures of KBT-3022 and desethyl KBT-3022.

metabolism or a combination of these factors may aggravate secondary tissue damage and lead to vasogenic edema after ischemia and recirculation.

A newly synthesized compound, KBT-3022, ethyl 2-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]pyrrol-1-ylacetate (Fig. 1), is a potent cyclooxygenase inhibitor (Yamashita et al., 1990). We have previously reported that oral administration of KBT-3022 inhibits several platelet functions (Yokota et al., 1988) and experimental thrombus formation (Yokota et al., 1995), and improves hemorheological properties (Yamamoto et al., 1995). In rats and humans, KBT-3022 is readily metabolized to desethyl KBT-3022 (Fig. 1) (Nakada et al., 1990), and in vitro the metabolite desethyl KBT-3022 is also an equipotent inhibitor of cyclooxygenase (Yamashita et al., 1990). We have investigated the effects of KBT-3022 on cytotoxic edema in vitro and vasogenic edema after ischemia and recirculation in vivo, and compared them with those of acetylsalicylic acid and indomethacin.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) weighing 320–760 g and male Mongolian gerbils (Seiwa Experimental Animals, Fukuoka, Japan) weighing 60–80 g were used.

2.2. Materials

KBT-3022 and desethyl KBT-3022 were synthesized at the New Drug Research Laboratories of Kanebo (Osaka, Japan). Acetylsalicylic acid was purchased from Wako Pure Chemical Industries (Osaka, Japan), indomethacin from Sigma Chemical Co. (St. Louis, MO, USA). The test drugs were dissolved in dimethyl sulfoxide for the in vitro experiments. For oral administration, the test drugs were dissolved or suspended in 0.5% polyoxyethylene sorbitan monooleate solution, and administered at a dose of 10 ml/kg body weight. Control animals received an equivalent volume of vehicle. Arachidonic acid (Na salt) was purchased from Sigma Chemical Co.

2.3. Arachidonic acid-induced cortical swelling

Guinea pigs were decapitated, and the brains were rapidly removed and placed on ice. Cortical slices from each hemisphere, 120–240 mg wet weight, were cut according to the method of Chan and Fishman (1978). The 'initial wet weight' of each slice was recorded. Each slice was then incubated in 3 ml Krebs-Ringer buffer (15 mM Hepes, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4, 0.7 mM $MgSO_4$, 10 mM glucose, pH 7.4) containing 0.5 mM arachidonic acid-Na and the drug solution at 37°C for 60 min. Slices in the normal group were incubated in Krebs-Ringer buffer without arachidonic acid. After incubation, each slice was lightly blotted to remove excess water and the 'final wet weight' was determined. The cortical slices were then dried at 90°C for 16 h and the 'dry weight' measured. Tissue water content and tissue swelling were calculated as follows: tissue water content (%) = (1 − dry weight/wet weight) × 100; tissue swelling (%) = (final water content/initial water content − 1) × 100.

The dried slices were incubated with 1 ml of 0.1 N HNO_3 for 16 h. After centrifugation at 3000 rpm for 10 min, the Na^+ and K^+ contents of the supernatant were measured by flame photometry (Hitachi, 205D).

2.4. Lipid peroxidation in brain homogenate

Production of lipid peroxides in the brain homogenate was measured using the method of Stocks et al. (1974). The guinea pig brains were homogenized in 4 times their weight of ice-cold 50 mM phosphate buffered saline (pH 7.4). The homogenate was centrifuged at 2800 rpm for 10 min at 4°C, and the supernatant stored at −70°C until antioxidant assay. A sample of the stock brain homogenate was thawed and diluted 10 times with 50 mM phosphate buffered saline (pH 7.4). 2 ml of aliquot was added to 20 μl of drug solution, and incubated at 37°C for 30 min. After addition of 35% $HClO_4$, the samples were centrifuged at 2800 rpm for 10 min. 1 ml of the supernatant was heated at 100°C for 15 min with 0.5 ml of 0.5% thiobarbituric acid dissolved in 50% acetic acid. Absorbance of the supernatant was measured at 532 nm. Lipid peroxidation of the supernatant was measured by the thiobarbituric acid method and expressed as malonyldialdehyde production. The inhibitory effects of drugs on lipid peroxidation were calculated as follows: inhibition % = (1 − malonyldialdehyde production (drug)/malonyldialdehyde production (control)) × 100.

2.5. Ischemic brain edema

Gerbils fasted overnight were anesthetized with 2% halothane in 70% N_2O and 30% O_2, and the rectal temperature was monitored by thermistor (Nihon Koden, MGA-III). Brain ischemia was induced by bilateral occlusion of the common carotid arteries with aneurysm clips. Exactly 15 min after carotid occlusion, the aneurysm clips were removed and cerebral circulation restored for 4 h. The animals were then decapitated, and the forebrain rapidly removed. Wet weight of the forebrain was measured within 90 s of decapitation. After drying the brain at 90°C for 16 h, the dry weight was measured. Water content of each brain was calculated as follows: water content (%) = (1 − dry weight/wet weight) × 100.

The dried brains were extracted with 2 ml of 0.1 N HNO_3 for 1 week. After centrifugation at 3000 rpm for 10 min, the Na^+ and K^+ contents of the supernatant were measured by flame photometry as before. KBT-3022, acetylsalicylic acid and vehicle were administered orally 3 h before, and indomethacin 1 h before the occlusion.

2.6. Cerebral metabolism after ischemia and recirculation

Using gerbils, the operation was done according to the above method. After 15 min of ischemia and 2 h of recirculation, the animals were killed by microwave irradiation (Toshiba, TMW-4012A, 3.5 kW microwave, 3.2 s), and the cortex, hippocampus and striatum were dissected and frozen in liquid nitrogen. The tissue samples were stored at −70°C until sample preparation. KBT-3022 and vehicle were administered orally 3 h before, and indomethacin 1 h before occlusion.

The procedures of sample preparation and fluorometry measurements of ATP, lactate, pyruvate and glucose with NADP and appropriate enzymes were done according to the method of Lowry et al. (1964). The samples were stored at −70°C until analyzed.

2.7. Statistics

Statistical significance was evaluated using one-way analysis of variance followed by Dunnett's test, but data for after-recirculation rectal temperature were compared between control and drug-treated animals using an analysis of variance for repeated measures followed by Dunnett's test. Differences of P value of less than 0.05 were considered statistically significant.

3. Results

3.1. Arachidonic acid-induced cortical swelling

Table 1 shows the effects of KBT-3022, desethyl KBT-3022, acetylsalicylic acid and indomethacin on arachidonic acid-induced tissue swelling, and Na^+ and K^+ contents in guinea pig brain cortical slices. The tissue swelling of cortical slices in Krebs-Ringer buffer without arachidonic acid (normal) was 2.42% after incubation. By addition of 0.5 mM arachidonic acid (control), tissue swelling increased 1.7-fold, the Na^+ content in the tissue increased 1.5-fold, and the K^+ content decreased 0.7-fold. Both KBT-3022 and desethyl KBT-3022 at 10–100 μM dose-dependently inhibited arachidonic acid-induced tissue swelling, and alteration of Na^+ and K^+ contents, while acetylsalicylic acid and indomethacin had no effect at 1000 μM and 100 μM, respectively.

3.2. Lipid peroxidation in brain homogenate

The inhibitory effects of KBT-3022, desethyl KBT-3022, acetylsalicylic acid and indomethacin on lipid per-

Table 1
Effects of KBT-3022, desethyl KBT-3022, acetylsalicylic acid and indomethacin on arachidonic acid-induced tissue swelling, and Na^+ and K^+ contents in guinea pig brain cortical slices

Drug	Concentration (μM)	n	Swelling (%)	Na^+ (mEq/kg dry weight)	K^+ (mEq/kg dry weight)
Normal		8	2.42 ± 0.19 [b]	728 ± 19 [b]	142 ± 4 [b]
Control		7	4.07 ± 0.08	1056 ± 67	105 ± 4
KBT-3022	1	5	3.92 ± 0.18	924 ± 73	111 ± 6
	10	5	3.45 ± 0.10	831 ± 36 [a]	128 ± 3
	100	5	3.07 ± 0.33 [b]	813 ± 57 [b]	131 ± 8 [a]
Desethyl KBT-3022	0.1	5	4.06 ± 0.22	1004 ± 46	109 ± 6
	1	5	3.75 ± 0.12	894 ± 30	116 ± 6
	10	5	3.26 ± 0.15 [a]	800 ± 37 [b]	144 ± 7 [b]
	100	5	2.94 ± 0.17 [b]	762 ± 11 [b]	142 ± 9 [b]
ASA	1000	5	3.98 ± 0.24	1000 ± 66	101 ± 6
Indomethacin	1	5	4.15 ± 0.21	982 ± 37	105 ± 12
	10	5	3.69 ± 0.23	908 ± 74	119 ± 4
	100	5	3.72 ± 0.21	907 ± 67	126 ± 7

Normal group was incubated in Krebs-Ringer buffer without arachidonic acid. Other groups were incubated in Krebs-Ringer buffer with 0.5 mM arhachidonic acid. Each value represents the mean ± S.E.M. [a] $P < 0.05$, [b] $P < 0.01$, significant difference from control (Dunnett's test). ASA: acetylsalicylic acid.

Table 2
Effects of KBT-3022, desethyl KBT-3022, acetylsalicylic acid and indomethacin on lipid peroxidation in guinea pig brain homogenate

Drug	Concentration (μM)	Inhibition (%)	IC_{50} (μM) (95% C.L.)
KBT-3022	3	15.9 ± 2.1	
	10	23.1 ± 2.0	
	30	33.8 ± 0.8	
	100	40.6 ± 1.7	
Desethyl KBT-3022	1	2.0 ± 1.5	
	3	11.5 ± 0.6	15.9
	10	21.2 ± 4.0	(11.3–25.4)
	30	78.0 ± 0.5	
ASA	1000	1.8 ± 1.0	
Indomethacin	100	4.3 ± 1.2	

Each value represents the mean ± S.E.M. of 3 experiments (each in duplicate). IC_{50} value was determined by log-probit analysis using 4 different concentrations. The value of malonyldialdehyde production in control was 183.0 ± 9.1 nmol/g wet tissue (10 experiments). ASA: acetylsalicylic acid.

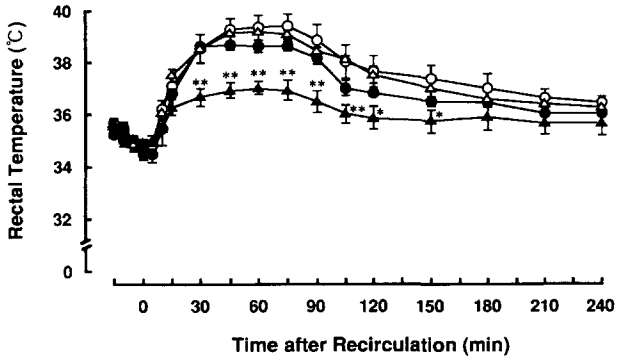


Fig. 2. Effects of KBT-3022, acetylsalicylic acid and indomethacin on rectal temperature after ischemia and recirculation in gerbils. Each point represents the mean and the vertical bar the S.E.M. of 4 animals. (○) Control, (●): KBT-3022 10 mg/kg p.o., (△) acetylsalicylic acid 100 mg/kg p.o., (▲) indomethacin 30 mg/kg p.o. * $P < 0.05$, * * $P < 0.01$, significant difference from control (Dunnett's test).

oxidation in guinea pig brain homogenate are shown in Table 2. KBT-3022 at 3–100 μM partially inhibited lipid peroxidation in brain homogenate. Desethyl KBT-3022 at 3–30 μM dose-dependently inhibited lipid peroxidation with IC_{50} value of 15.9 μM. Neither acetylsalicylic acid nor indomethacin had any effect at 1000 and 100 μM, respectively.

3.3. Ischemic brain edema

The effects of KBT-3022, acetylsalicylic acid and indomethacin on brain water, Na^+ and K^+ contents after 15 min of bilateral carotid occlusion and 4 h of recirculation in gerbils are shown in Table 3. Brain water content in the control animals showed a significant 0.7% increase over that in the sham-operated animals. The Na^+ content increased by 12 mEq/kg and the K^+ content decreased by 12 mEq/kg, but these differences from the sham-operated animals were not significant. In our preliminary study, occlusion for 20 or 30 min caused death in all of 9 animals. The increase in water content reached maximum after recirculation for 4 or 6 h. Therefore, we investigated the effects of the test drugs on ischemic brain edema using the above protocol. Oral administration of KBT-3022 at 1, 3 and 10 mg/kg significantly inhibited the increase in brain water content. Indomethacin at 10 and 30 mg/kg also significantly inhibited the increase in the water content. Treatment at these doses with KBT-3022 and indomethacin slightly but not significantly inhibited the increase in Na^+ and the decrease in K^+ content. Acetylsalicylic acid at 100 mg/kg had no effect on any measurements of brain edema.

Fig. 2 shows the time course of rectal temperature in animals given the highest dose of each drug. Rectal temperature in the control animals fell during ischemia, but rose rapidly after recirculation. During recirculation, rectal temperature increased to more than 2°C above normal temperature for 2 h, and gradually recovered to the normal level by 4 h. The rectal temperature in animals treated with KBT-3022 or acetylsalicylic acid showed the same time course as in the control animals. Indomethacin had no effect on rectal temperature before or during ischemia, but

Table 3
Effects of KBT-3022, acetylsalicylic acid and indomethacin on brain water, Na^+ and K^+ contents after ischemia and recirculation in gerbils

Drug	Dose (mg/kg)	Water content (%)	Na^+ (mEq/kg dry weight)	K^+ (mEq/kg dry weight)
Sham-operated		78.9 ± 0.1 [b]	235 ± 6	481 ± 4
Control		79.8 ± 0.1	247 ± 7	469 ± 4
KBT-3022	0.3	79.7 ± 0.1	240 ± 4	472 ± 7
	1	79.5 ± 0.1 [a]	231 ± 7	475 ± 8
	3	79.3 ± 0.0 [b]	229 ± 7	478 ± 5
	10	79.2 ± 0.1 [b]	232 ± 7	478 ± 7
ASA	100	79.7 ± 0.1	240 ± 6	476 ± 6
Indomethacin	3	79.8 ± 0.1	241 ± 6	470 ± 6
	10	79.4 ± 0.0 [b]	235 ± 7	475 ± 8
	30	79.4 ± 0.1 [b]	237 ± 4	479 ± 4

Brain ischemia was induced by bilateral occlusion of the common carotid arteries for 15 min, and then cerebral circulation restarted for 4 h. KBT-3022, acetylsalicylic acid and vehicle were orally administrated 3 h before occlusion, and indomethacin was 1 h before occlusion. Each value represents the mean ± S.E.M. of 6 animals. [a] $P < 0.05$, [b] $P < 0.01$, significant difference from control (Dunnett's test). ASA: acetylsalicylic acid.

inhibited the elevation of rectal temperature after recirculation.

3.4. Cerebral metabolism after ischemia and recirculation

The effects of KBT-3022 and indomethacin on the changes in the contents of ATP, lactate, pyruvate and glucose in gerbil brain after 15 min of ischemia and 2 h of recirculation are shown in Fig. 3. The levels of ATP in the cortex, hippocampus and striatum in the control animals were markedly lower than those in sham-operated animals. Oral administration of KBT-3022 at 10 mg/kg and indomethacin at 30 mg/kg produced a tendency for the ATP contents of the cortex, hippocampus and striatum to recover. The lactate contents of all regions increased markedly after ischemia and recirculation. KBT-3022 significantly inhibited the increase in the lactate contents of the cortex, hippocampus and striatum. Indomethacin also inhibited the increase in the cortex and striatum. The glucose content of the cortex was also increased significantly after ischemia and recirculation, and KBT-3022 showed a tendency to inhibit the increase in glucose content. The pyruvate content in cortex almost unchanged after ischemia and recirculation. The glucose and pyruvate contents of the hippocampus and striatum could not be determined because of the low sample volume.

4. Discussion

Generally, ischemic brain edema is initially of the cytotoxic type, and soon progresses to the vasogenic type after restoration of the circulation (Katzman et al., 1977; Siesjö, 1992). However, it is difficult to make a clear distinction between the cytotoxic edema and vasogenic edema in experimental models in vivo. Therefore, we investigated the effect of KBT-3022 on cytotoxic edema using the in vitro model described by Chan and Fishman (1978).

In cytotoxic edema, the tissues swell, the intracellular Na^+ content increases and the intracellular K^+ content decreases, as a result of disruption of ion transport systems involving enzymes such as (Na^+,K^+)-ATPase. In the present study, incubation of arachidonic acid with guinea pig cortical slices caused increases in the water and Na^+ contents and a decrease in the K^+ content, as described by Chan and Fishman (1978). In normal slices incubated without arachidonic acid, the Na^+ content was larger and the K^+ content was smaller than those in gerbil brain. These differences might be explained by the fact that slices have a greater degree of damage, resulting in reduced (Na^+,K^+)-ATPase activity during incubation in comparison with brain tissue which was not incubated.

Both KBT-3022 and its metabolite desethyl KBT-3022 significantly inhibited arachidonic acid-induced tissue swelling and changes in the Na^+ and K^+ contents, whereas acetylsalicylic acid and indomethacin showed no effect. This ineffectiveness of acetylsalicylic acid and indomethacin suggests that arachidonic acid-induced cytotoxic edema was not due to arachidonic acid metabolites as proposed by Chan and Fishman (1978). KBT-3022 and desethyl KBT-3022 have been reported to inhibit cyclooxygenase activity of ovine seminal glands with IC_{50}

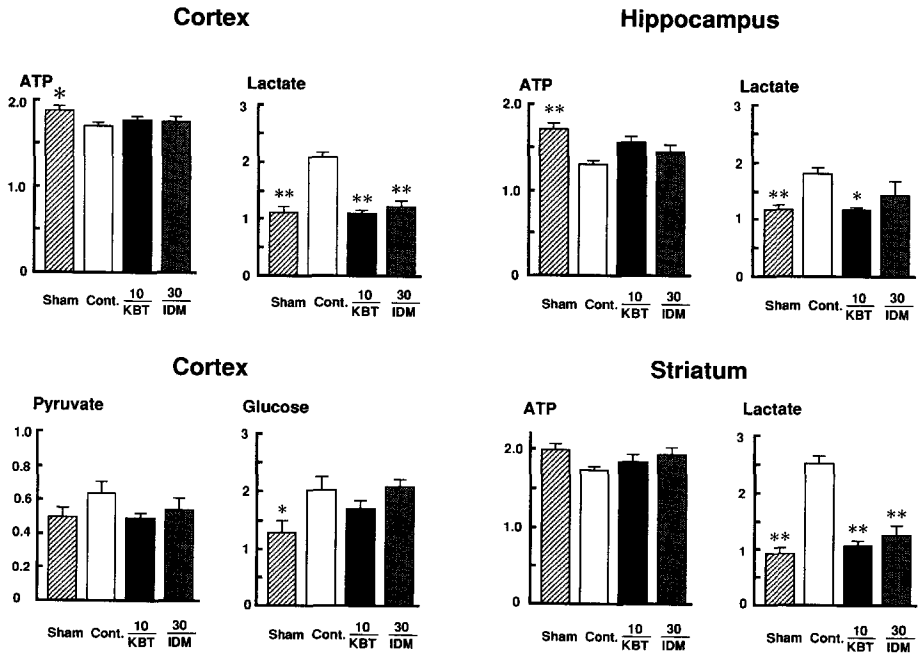


Fig. 3. Effects of KBT-3022 and indomethacin on changes in the content of ATP, lactate, pyruvate and glucose in gerbil brain after ischemia and recirculation. Each column with a vertical bar represents the mean ± S.E.M. in mol/g of brain tissue ($n = 8$–9). * $P < 0.05$, ** $P < 0.01$, significant difference from control (Dunnett's test). Cont.: control, KBT: KBT-3022, IDM: indomethacin.

values of 0.57 and 0.41 μM (Yamashita et al., 1990), but the inhibitory effects of both compounds on arachidonic acid-induced cytotoxic edema may occur through mechanisms other than inhibition of cyclooxygenase. We concluded that one of the mechanisms might be associated with inhibition of lipid peroxidation, since KBT-3022 and desethyl KBT-3022 inhibited lipid peroxidation in guinea pig brain homogenate within a concentration range similar to that producing the inhibitory effect on arachidonic acid-induced tissue swelling. On the other hand, acetylsalicylic acid and indomethacin had no effect on lipid peroxidation. It is possible that KBT-3022 and desethyl KBT-3022 may preserve membrane stability and integrity, but this possibility cannot be confirmed by the present study.

In ischemic brain, the excessive generation of arachidonic acid metabolites by restoration of the circulation may aggravate secondary tissue damage and lead to vasogenic edema. In the present study, recirculation following the occlusion of the common carotid arteries in gerbils produced significant ischemic brain edema and sustained hyperthermia, both of which were inhibited by oral administration of indomethacin. Indomethacin is reported to be useful in ameliorating brain edema in experimental models, presumably as a result of reducing prostaglandin synthesis by inhibiting cyclooxygenase (Matsui et al., 1984; Dempsey et al., 1985).

Recently, the relationship between hyperthermia induced by recirculation following ischemia and cell damage has been studied. It has been reported that hypothermia can stabilize plasma membranes, inhibit the synthesis, release and uptake of neurotransmitters, and reduce postischemic cerebral injury (Young et al., 1983; Chopp et al., 1989). As prostaglandins are well known pyrogens, indomethacin might suppress hyperthermia by inhibition of their production. Hypothermia induced by indomethacin after ischemia may therefore contribute to its beneficial effect on ischemic brain edema.

We found that acetylsalicylic acid had no effects on ischemic brain edema and hyperthermia. The ineffectiveness of acetylsalicylic acid is explained by the fact that acetylsalicylic acid does not penetrate the blood-brain barrier. This is confirmed by the finding that acetylsalicylic acid failed to reduce brain prostaglandin synthesis at a dose which completely inhibited prostaglandin synthesis in peripheral tissue in rats (Abdel-Halim et al., 1978).

Our result demonstrated that KBT-3022 ameliorated ischemic brain edema more potently than indomethacin. This effect of KBT-3022 may be due mainly to inhibition of cyclooxygenase, as well as indomethacin. However, our results confirm that the inhibitory effect of KBT-3022 on ischemic brain edema had a slightly different profile from that of indomethacin, since KBT-3022 did not have any effect on hyperthermia. It is perhaps more likely that KBT-3022 ameliorates ischemic brain edema at a dose which does not affect postischemic hyperthermia through some mechanisms in addition to the inhibition of cyclooxygenase. The inhibition of cytotoxic edema and/or lipid peroxidation by KBT-3022 and its metabolite desethyl KBT-3022 may contribute to prevent the development of vasogenic edema at the first state in vivo.

KBT-3022 has been reported to increase red blood cell deformability and decrease blood viscosity in guinea pigs (Yamamoto et al., 1995). This hemorheological effect may also contribute to the amelioration of ischemic brain edema by improving brain microcirculation. Pentoxifylline, which increases red blood cell deformability and decreases blood viscosity, was demonstrated to reduce cerebral edema in cats (Ganser and Boksay, 1974).

KBT-3022 and indomethacin significantly inhibited the increase in the lactate content of gerbil brain after 15 min of ischemia and 2 h of recirculation. The excessive lactate acidosis and consequential decrease in tissue pH exacerbates the intracellular calcium overload, and triggers free radical formation (Siesjö, 1981, 1988). These metabolic disturbances enhanced ischemic brain edema. These inhibitory effects of KBT-3022 and indomethacin on lactate accumulation may contribute to the prevention of ischemic brain edema, although the mechanism involved is not clear.

In conclusion, KBT-3022 inhibits arachidonic acid-induced cytotoxic edema through inhibition of lipid peroxidation, and prevents the development of vasogenic edema through inhibition of arachidonic acid metabolism among other possible mechanisms. As KBT-3022 has been reported to inhibit experimental thrombus formation (Yokota et al., 1995), our results suggest that pre-administration of KBT-3022 may prevent not only the occurrence of cerebral ischemia due to thrombus formation but also the development of secondary tissue injury, such as brain edema, after ischemia. KBT-3022 may be a more useful agent than other anti-platelet drugs for prophylaxis of ischemic cerebrovascular disease.

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