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KF24345, an adenosine uptake inhibitor, ameliorates the severity and mortality of lethal acute pancreatitis via endogenous adenosine in mice

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Abstract

Adenosine protects against cellular damage and dysfunction under several adverse conditions including inflammation and ischemia. In this study, we examined the effects of 3-[1-(6,7-diethoxy-2-morpholinoquinazolin-4-yl)piperidin-4-yl]-1,6-dimethyl-2,4(1*H*,3*H*)-quinazolinedione hydrochloride (KF24345), an adenosine uptake inhibitor, on experimental acute pancreatitis induced by choline-deficient and ethionine-supplemented diet in mice. KF24345, administered with the diet onset and every 24 h thereafter, prevented hyperamylasemia, acinar cell injury and serum tumor necrosis factor-α elevation and ultimately decreased mortality. Therapeutic treatment with KF24345, which started 32 h after the diet onset, also decreased mortality. The beneficial effect of KF24345 on mortality was abolished by the pretreatment with 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385), a selective adenosine A_{2A} receptor antagonist. An intravenous injection of KF24345 at 48 h after the diet onset increased plasma adenosine concentrations in mice with acute pancreatitis. These results suggest that KF24345 shows anti-pancreatitis effects via endogenous adenosine and adenosine A_{2A} receptors. The adenosine uptake inhibition could be a new therapeutic approach for acute pancreatitis.

Keywords: KF24345; Adenosine; Uptake inhibition; Acute pancreatitis; Mortality; TNF-α (Tumor necrosis factor-α)

1. Introduction

Acute pancreatitis is a disease with high mortality (Steer, 1993). In severe cases, the pancreatic damage may subsequently lead to serious complications including systemic inflammatory response syndrome and multiple organ dysfunction syndrome (Banks, 1993). Although several mediators such as activated pancreatic enzymes (Leach et al., 1991), chemokines and cytokines (Denham et al., 1997; Grady et al., 1997), transcriptional factors (Steinle et al., 1999), platelet-activating factor (Konturek et al., 1992) and free radicals (Niederau et al, 1992) are assumed to be involved in the pathogenesis of acute pancreatitis, the mechanism for the pathogenesis and progression of the disease are complex and not fully understood.

Adenosine, an endogenous purine nucleoside, has been proposed to modulate a variety of physiological responses by stimulating its specific extracellular receptors (Collis and Hourani, 1993). Four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) have been identified (Olah and Stiles, 2000). Under several adverse conditions including inflammation, trauma and ischemia, extracellular concentrations of endogenous adenosine in the jeopardized local tissues are increased after the release of adenosine itself, and/or that of AMP, which is produced from ATP. Increased intracellular AMP is transported to extracellular space, and metabolized to adenosine mainly by ecto-5′-nucleotidase (Cronstein, 1995). Indeed, the activity of 5′-nucleotidase is reported to increase in adverse conditions (Johnson et al., 1999). It is considered that the increased adenosine can protect against cellular damage or dysfunction (Ralevic and Burnstock, 1998), because adenosine and its agonists attenuate ischemic cardiac and cerebral injuries (Liu et al., 1997; von Lubitz et al., 1988), seizures (Murray et al., 1985), pain (Sawynok et al., 1986) and inflammation (Cronstein, 1997) in several animal models. In addition,

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adenosine kinase inhibitors have been shown to exhibit neuroprotective, analgesic and anti-inflammatory effects in vivo (Kowaluk et al., 1998). It is therefore assumed that prevention of adenosine uptake into the cells can enhance extracellular concentrations of endogenous adenosine, resulting in a protective effect against cellular damage and tissue injury.

Adenosine production is also elevated in acute pancreatitis due to the increased demand for energy supplied by ATP and the excessive ATP catabolism to adenosine (Lüthen et al., 1995). Adenosine level in pancreas with acute pancreatitis slightly increases (Satoh et al, 2000), probably resulting from the enhancement of adenosine production. However, the elevation of adenosine level is insufficient to exert its pharmacological effects, because extracellular adenosine usually disappears very fast due to its rapid uptake into the adjacent cells (e.g., erythrocytes and endothelial cells) and subsequent intracellular metabolism (Moser et al., 1989). In fact, when adenosine is added into human whole blood, it disappears with a half-life of less than 30 s (Yeung et al., 1991). If the adenosine uptake inhibitor would increase extracellular adenosine, it would be able to exhibit protective effects in various adverse conditions including acute pancreatitis. These observations led us to examine whether an adenosine uptake inhibitor can attenuate the severity of acute pancreatitis.

3-[1-(6,7-Diethoxy-2-morpholinoquinazolin-4-yl)piperidin-4-yl]-1,6-dimethyl-2,4(1*H*,3*H*)-quinazolinedione hydrochloride (KF24345) is a novel and orally effective adenosine uptake inhibitor (Fig. 1; Noji et al., 2002). In the present study, we examined the effects of KF24345 on experimental acute pancreatitis induced in mice by choline-deficient and ethionine-supplemented (CDE) diet. CDE diet-induced acute pancreatitis is a noninvasive model of severe necrotizing pancreatitis with a high mortality rate (Bhatia et al., 1998). In addition, we examined the effects of selective adenosine receptor antagonists on the pharmacological outcome induced by KF24345 to determine the possible involvement of endogenous adenosine and adenosine receptors.

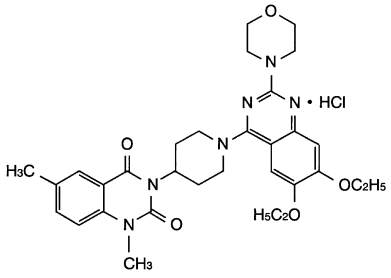

Fig. 1. Chemical structure of KF24345.

2. Materials and methods

2.1. Animals, diets and chemicals

Female CD-1 mice (4 weeks old; weighing about 15 g) were purchased from Charles River Japan (Yokohama, Japan). Mice received standard laboratory diet and water ad libitum and were maintained on 12-h light/dark cycle at 22–23 °C. All experimental procedures conformed to the protocols approved by the Animal Care and Use Committee of Kyowa Hakko Kogyo (Tokyo, Japan).

Choline-deficient diet was obtained from Oriental Yeast (Tokyo, Japan). It was supplemented with 0.5% of DL-ethionine (Sigma, St. Louis, MO) and used as CDE diet.

4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385; Keddie et al., 1996), an adenosine A_{2A} receptor antagonist, was purchased from Tocris Cookson (Bristol, UK). KF24345 and 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902; Shimada et al., 1992), an adenosine A_1 receptor antagonist, were synthesized at Pharmaceutical Research Institute of Kyowa Hakko Kogyo. All other chemicals and solvents were used in their analytical pure form.

KF24345, KW-3902 and ZM 241385 were suspended in 0.5% (w/v) methyl cellulose and these suspensions were orally administered to mice at a volume of 10 ml/kg.

2.2. Induction of acute pancreatitis

Acute pancreatitis was induced in mice according to the previously reported method (Niederau et al., 1986; Silverman et al., 1989) with minor modifications. Mice were fasted for 24 h but given water ad libitum, and then CDE diet was given for 72 h in mortality studies, or for 48 h in other experiments. Normal mice were fasted but given water ad libitum for 24 h, and thereafter given the standard diet throughout the experiment.

2.3. Treatment of KF24345

In the previous study, KF24345 inhibited [^{3}H]adenosine uptake into washed erythrocytes of mice in a concentration-dependent manner (IC_{50} = 130 nM) in vitro. In ex vivo studies, KF24345 inhibited the [^{3}H]adenosine uptake into blood cells sampled from mice. KF24345 showed about 50% inhibition at 3 mg/kg and almost complete inhibition at 10 mg/kg from 2 to 10 h after its oral administration (Noji et al., 2002). Therefore, we used the dosages of 3 and/or 10 mg/kg of KF24345 in the present study.

2.3.1. Mortality

Prophylactic treatment with KF24345 (3 and 10 mg/kg orally) or the vehicle (0.5% methyl cellulose), as control,

was started at the same time with the onset of CDE diet and continued every 24 h thereafter up to 120 h. On the other hand, therapeutic treatment with KF24345 (10 mg/kg orally) was started 32 h after the onset of CDE diet and continued every 24 h thereafter up to 128 h. The starting time of the therapeutic treatment with KF24345 was decided according to the previous reports, in which the pancreatitis is histologically and biochemically well established by 32 h after the onset of CDE diet (Norman et al., 1996).

2.3.2. Pancreas weight, serum amylase and lactate dehydrogenase (LDH)

KF24345 (3 and 10 mg/kg orally) or the vehicle, as control, was treated prophylactically at 0 and 24 h. On the other hand, therapeutic treatment with KF24345 (10 mg/kg orally) was performed at 32 h after the onset of CDE diet.

2.4. Effects of KF24345 on CDE diet-induced acute pancreatitis

2.4.1. Mortality

CDE diet was given to mice for 72 h, and then the surviving animals were placed back on the standard diet and observed for additional 72 h. Each group contained 20–30 animals, and there were no differences in the amount of CDE diet consumption between the control and the KF24345-treated groups.

2.4.2. Pancreas weight, serum amylase and LDH

CDE diet was given to mice for 48 h. At 48 h from the onset of CDE diet, when almost all animals were alive in the present experimental condition, mice were anesthetized with ether and blood samples were collected from the abdominal vein. Thereafter, mice were sacrificed by cervical dislocation, and whole pancreata were rapidly removed and weighed. Pancreatic tissues were histologically examined, and activities of serum amylase and LDH were measured. Each group contained 5–15 animals, and there were no differences in the amount of CDE diet consumption between the control and the KF24345-treated groups.

2.5. Effects of KF24345 on serum tumor necrosis factor-α (TNF-α) concentrations in mice with CDE diet-induced acute pancreatitis

Mice were maintained on CDE diet for 48 h. KF24345 (10 mg/kg) or the vehicle, as control, was orally treated at 0 and 24 h after the onset of CDE diet. At 48 h, blood samples were collected from the abdominal vein and serum TNF-α concentrations were measured. Each group contained 7–8 animals, and there were no differences in the amount of CDE diet consumption between the control and the KF24345-treated groups.

2.6. Effects of selective adenosine receptor antagonists on the pharmacological outcome induced by KF24345 in mice with CDE diet-induced acute pancreatitis

Mice were given CDE diet for 72 h, then placed back on the standard diet and observed for additional 72 h. KF24345 (10 mg/kg) or the vehicle, as control, was orally treated at 0, 24, 48, 72, 96 and 120 h after the onset of CDE diet. KW-3902, a selective adenosine A_1 receptor antagonist, at 0.3 mg/kg or ZM 241385, a selective adenosine A_{2A} receptor antagonist, at 3 mg/kg was orally given to mice 1 h before the onset of CDE diet. It has been reported that KW-3902 has the K_i value of 0.19 nM in rat forebrain adenosine A_1 receptors labelled with [^{3}H]cyclohexyladenosine (Nonaka et al., 1996). ZM 241385 displaced the binding of [^{3}H]5′-*N*-ethylcarboxamidoadenosine (NECA) with the pIC_{50} of 9.52 in rat phaeochromocytoma cell membranes (Poucher et al., 1995). The oral administration of KW-3902 at 0.1–1 mg/kg prominently antagonizes NECA-induced bradycardia without affecting hypotension in rats (Mizumoto et al., 1993). The oral administration of ZM 241385 at 3–10 mg/kg attenuates adenosine-induced hypotension, while its oral administration even at 10 mg/kg did not inhibit the bradycardiac effects of adenosine (Keddie et al., 1996; Poucher et al., 1996). Therefore, the doses of KW-3902 and ZM 241385 used in the present study are assumed to selectively and sufficiently antagonize A_1 and A_{2A} adenosine receptors, respectively. These antagonists were given every 12 h up to 131 h after the onset of CDE diet. Mice were randomly assigned to one of the seven treatment groups as follows: Group 1 (normal): a standard diet + the vehicle; Group 2 (control): CDE diet + the vehicle; Group 3: CDE diet + KW-3902; Group 4: CDE diet + ZM 241385; Group 5: CDE diet + KF24345; Group 6: CDE diet + KW-3902 + KF24345; Group 7: CDE diet + ZM 241385 + KF24345. Each group contained 20–30 animals, and there were no differences in the amount of CDE diet consumption among Groups 2 to 7.

2.7. Effects of KF24345 on plasma adenosine concentrations in mice with CDE diet-induced acute pancreatitis

Mice were maintained on the standard or CDE diet for 48 h, and KF24345 (1.5 mg/kg) was intravenously injected once at 48 h. Immediately before and 10 min after the KF24345 injection, blood samples anticoagulated with 3 mM EDTA were collected from the abdominal vein and plasma adenosine concentrations were measured.

2.8. Assays

Blood sample from each mouse was centrifuged (1200 × *g*,10 min, 4 °C) and serum or plasma was obtained. Activities of serum amylase and LDH were measured by automated chromogenic assays using the commercially available measurement kits with an autoanalyzer (AU-600,

Olympus, Tokyo, Japan). Serum TNF-α concentrations were measured by using the enzyme-linked immunosorbent assay kit from Amersham Biosciences UK (Buckinghamshire, UK) and calculated from a standard curve generated with recombinant mouse TNF-α (Amersham Biosciences UK). Plasma adenosine concentrations were measured by radioimmunoassay with the adenosine assay kit Yamasa (Yamasa, Tokyo, Japan).

2.9. Histology

For light microscopy, a portion of pancreas from each animal was fixed in 10% neutral buffered formalin. Pancreata were pooled in a single paraffin block, cut in 5 μm and stained with hematoxylin and eosin. Each sample was examined and scored blindly for the extent of zymogen granule increase, vacuolation and nuclear degeneration of acinar cells as follows: 0 = no change, 1 = very slight, 2 = slight, 3 = moderate and 4 = severe.

2.10. Statistical analysis

In mortality studies, survival data were analyzed with the Fisher's exact probability test. In other experiments, data are presented as means ± S.E. and statistically analyzed using the Wilcoxon rank sum test when comparing two groups, or by the Kruskal–Wallis test followed by the Steel test for multiple groups. $P < 0.05$ was considered to be statistically significant.

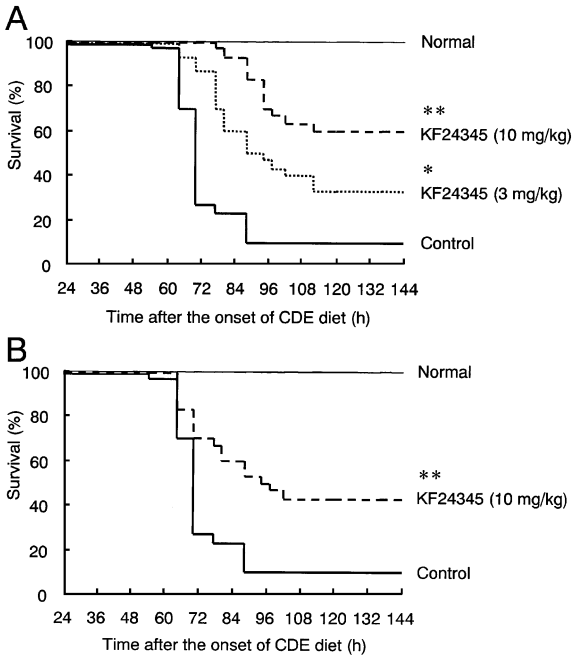

Fig. 2. Effects of prophylactic (A) and therapeutic (B) treatment with KF24345 on the mortality in mice with CDE diet-induced acute pancreatitis. Mice were exposed to CDE diet for 72 h, and observed up to 144 h. Prophylactic treatment with KF24345 (3 and 10 mg/kg orally) was started with the onset of CDE diet, and therapeutic treatment with KF24345 (10 mg/kg orally) was started 32 h after the onset of CDE diet. KF24345 or the vehicle, as control, was administered every 24 h thereafter. Values are percentages of those that survived out of 20–30 animals. *$P < 0.05$, **$P < 0.01$, significantly different from the control group exposed to CDE diet.

3. Results

3.1. Effects of KF24345 on CDE diet-induced acute pancreatitis

3.1.1. Prophylactic treatment with KF24345

3.1.1.1. Mortality. In the control group, 90% mortality was observed at 144 h after the onset of CDE diet. A total of 27 out of 30 mice died until 144 h. Prophylactic treatment with KF24345 (3 and 10 mg/kg orally) significantly improved the survival rate (Fig. 2A). A total of 10 and 18 out of 30 mice survived throughout the experimental period in the 3 and 10 mg/kg-treated groups, respectively. No additional animal died in any of the groups beyond the complete 144-h period of the study up to 288 h. All normal mice receiving standard diet survived throughout the experimental period.

3.1.1.2. Pancreas weight, serum amylase and LDH. Mean pancreas weight and serum amylase activity in the control mice exposed to CDE diet for 48 h increased more than 1.5- and 2-fold, respectively, when compared with those in the normal mice maintained on standard diet. Serum LDH activity, a marker for cell degeneration, also elevated significantly in the control mice. Prophylactic treatment with KF24345 at a dose of 10 mg/kg significantly attenuated the increase of pancreas weight (Fig. 3A), and the elevations of serum amylase (Fig. 3B) and LDH (Fig. 3C) activities. KF24345 at 3 mg/kg attenuated only the elevation of serum LDH activity in the control mice.

3.1.1.3. Histology. Zymogen granule increase and vacuolation as well as nuclear degeneration of acinar cells were observed in the control mice at 48 h after the onset of CDE diet. Prophylactic treatment with KF24345 at 10 mg/kg significantly ameliorated zymogen granule increase (Fig. 4A) and vacuolation (Fig. 4B) of acinar cells. KF24345 at 3 mg/kg had no beneficial effect on these histological findings.

3.1.2. Therapeutic treatment with KF24345

3.1.2.1. Mortality. Therapeutic treatment with KF24345 (10 mg/kg orally), administered 32 h after the onset of CDE diet and every 24 h thereafter, also improved the survival rate significantly (Fig. 2B). A total of 13 out of 30 mice survived throughout the experimental period in the therapeutically KF24345-treated group.

3.1.2.2. Pancreas weight, serum amylase and LDH. Therapeutic treatment with KF24345 (10 mg/kg orally) signifi-

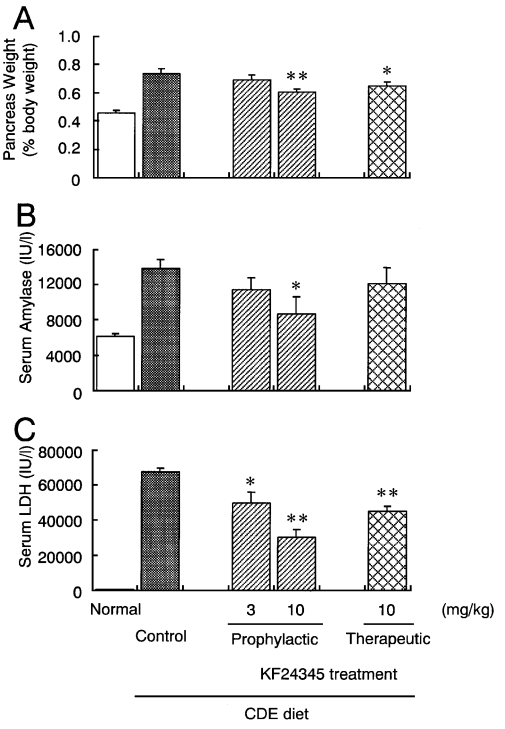

Fig. 3. Effects of KF24345 on pancreas weight (A) and activities of serum amylase (B) and LDH (C) in mice with CDE diet-induced acute pancreatitis. Mice were exposed to CDE diet for 48 h, and KF24345 was orally administered 0 and 24 h after the onset of CDE diet prophylactically (3 and 10 mg/kg), or 32 h after the onset of CDE diet therapeutically (10 mg/kg). Values are means ± S.E. of 5–15 animals. *$P<0.05$, **$P<0.01$, significantly different from the control group exposed to CDE diet.

cantly attenuated the increase of pancreas weight (Fig. 3A) and the elevation of serum LDH activity (Fig. 3C). However, it had no significant beneficial effect on serum amylase activity (Fig. 3B).

3.1.2.3. Histology. Therapeutic treatment with KF24345 (10 mg/kg orally) had no beneficial effect on zymogen granule increase, vacuolation and nuclear degeneration of acinar cells (Fig. 4).

3.2. Effects of KF24345 on serum TNF-α concentrations in mice with CDE diet-induced acute pancreatitis

Serum TNF-α concentrations were elevated in the control mice at 48 h after the onset of CDE diet. Prophylactic treatment with KF24345 (10 mg/kg orally) significantly suppressed the elevation of serum TNF-α concentrations (Fig. 5).

3.3. Effects of selective adenosine receptor antagonists on the pharmacological outcome induced by KF24345 in mice with CDE diet-induced acute pancreatitis

In the control group, 80% mortality was observed at 144 h after the onset of CDE diet. A total of 20 out of 25 mice died until 144 h. KF24345 (10 mg/kg orally) significantly decreased mortality observed in the control mice. A total of 20 out of 30 mice survived throughout the experimental period in the KF24345-treated group. ZM 241385 (3 mg/kg orally), but not KW-3902 (0.3 mg/kg orally), completely reversed the effects of KF24345 on the mortality (Fig. 6B). A total of 16 out of 20 and 1 out of 25 mice survived throughout the experimental period in the KW-3902 + KF24345 and ZM 241385 + KF24345-treated groups, respectively.

KW-3902 itself moderately but significantly decreased mortality induced by CDE diet. On the other hand, ZM 241385 itself tended to increase mortality observed in the control mice (Fig. 6A). A total of 14 out of 30 mice survived throughout the experimental period in the KW-3902-treated group, and all 25 mice died until 144 h in the ZM 241385-treated group.

3.4. Effects of KF24345 on plasma adenosine concentrations in mice with CDE diet-induced acute pancreatitis

At 48 h after the diet onset, the plasma adenosine concentration in the control mice was about 2-fold and significantly higher than that in the normal mice maintained

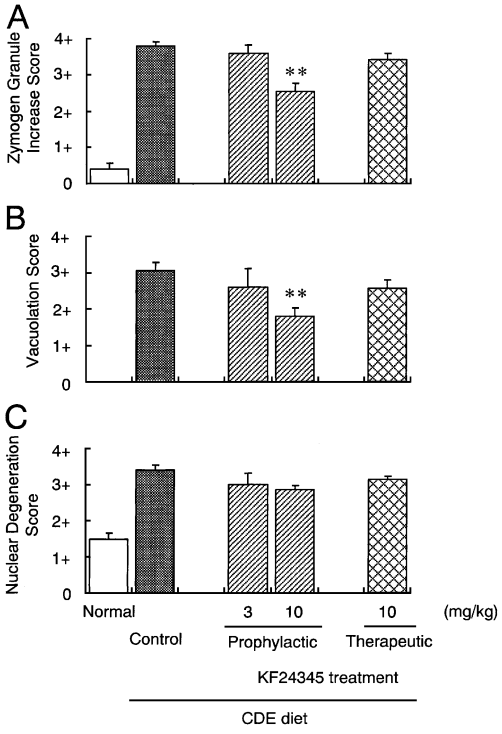

Fig. 4. Effects of KF24345 on zymogen granule increase (A), vacuolation (B) and nuclear degeneration (C) of acinar cells in mice with CDE diet-induced acute pancreatitis. Mice were exposed to CDE diet for 48 h, and pancreas was histologically examined and scored as follows: 0 = no change, 1 = very slight, 2 = slight, 3 = moderate and 4 = severe. Prophylactic or therapeutic treatment with KF24345 was the same as in Fig. 3. Values are means ± S.E. of 5–15 animals. **$P<0.01$, significantly different from the control group exposed to CDE diet.

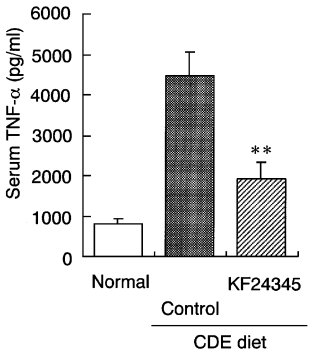

Fig. 5. Effects of KF24345 on serum TNF-α concentrations in mice with CDE diet-induced acute pancreatitis. Mice were exposed to CDE diet for 48 h, and serum TNF-α concentrations were measured as described in the text. KF24345 (10 mg/kg) was orally administered 0 and 24 h after the onset of CDE diet. Values are means ± S.E. of 7–8 animals. $^{**}P<0.01$, significantly different from the control group exposed to CDE diet.

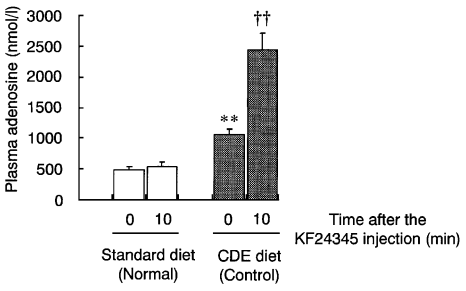

Fig. 7. Effects of KF24345 on plasma adenosine concentrations in mice with or without CDE diet-induced acute pancreatitis. Mice were exposed to the standard or CDE diet for 48 h, and then KF24345 (1.5 mg/kg) was intravenously injected. Blood samples were collected from the abdominal vein immediately before and 10 min after the injection of KF24345, and were processed for measurement of plasma adenosine concentrations. Values are means ± S.E. of 5 animals. $^{**}P<0.01$, significantly different from the normal group exposed to the standard diet; †† $P<0.01$, significantly different from the value immediately before the KF24345 injection in mice with CDE diet-induced acute pancreatitis.

on the standard diet. In the normal mice, an intravenous injection of KF24345 (1.5 mg/kg) had no effect on the plasma adenosine concentration, whereas in the control mice, it significantly elevated plasma adenosine concentrations 10 min after the injection (Fig. 7).

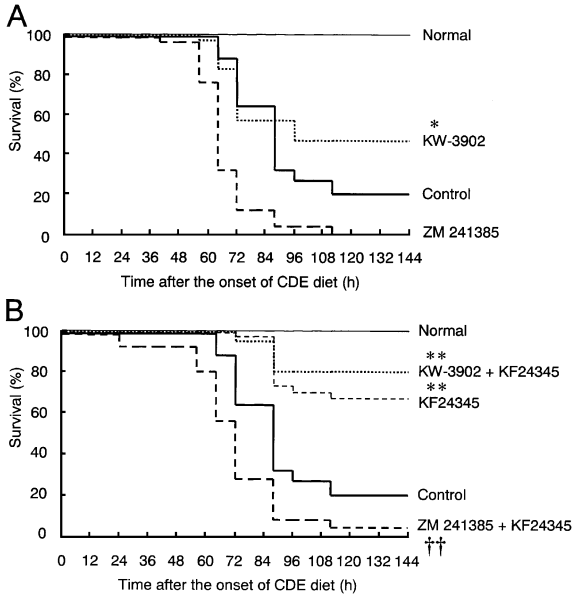

Fig. 6. Effects of selective adenosine receptor antagonists on the mortality (A), and effects of selective adenosine receptor antagonists on the attenuation of the mortality by KF24345 (B) in mice with CDE diet-induced acute pancreatitis. Mice were exposed to CDE diet for 72 h, and observed up to 144 h. KF24345 (10 mg/kg) or the vehicle, as control, was orally administered with the onset of CDE diet, and every 24 h thereafter. KW-3902 (a selective adenosine A_1 receptor antagonist; 0.3 mg/kg) or ZM 241385 (a selective adenosine A_{2A} receptor antagonist; 3 mg/kg) was orally administered 1 h before the CDE diet onset and every 12 h thereafter. Values are percentages of those that survived out of 20–30 animals. $^{*}P<0.05$, $^{**}P<0.01$, significantly different from the control group exposed to CDE diet; †† $P<0.01$, significantly different from the KF24345-treated group with CDE diet-induced acute pancreatitis.

4. Discussion

In the present study, prophylactic treatment with KF24345 significantly decreased mortality in mice with CDE diet-induced acute pancreatitis, and the effects of KF24345 were completely blocked by ZM 241385, an adenosine A_{2A} receptor antagonist, but not by KW-3902, an adenosine A_1 receptor antagonist. These results suggest that KF24345 could ameliorate the lethality via endogenous adenosine and adenosine A_{2A} receptors. The endogenously increased adenosine by uptake inhibition of KF24345 is assumed to act mainly to adenosine A_{2A} receptors in this model.

KW-3902 moderately but significantly decreased mortality, and ZM 241385 tended to increase mortality in this study. These results suggest that endogenous adenosine is involved in the mortality of mice in this model. Stimulation of adenosine A_1 receptor pathways and adenosine A_{2A} receptor pathways is assumed to have deleterious effects and beneficial effects, respectively. Neither KW-3902 nor ZM 241385 caused death or any changes of serum parameters in normal mice (unpublished observation); therefore, the effects of these antagonists on the survival rate in this study are supposed to be due to their effects on A_1 and A_{2A} adenosine receptors, respectively. Since the protective effects of KF24345 against mortality were blocked by ZM 241385, the beneficial effects of enhanced endogenous adenosine via adenosine A_{2A} receptor pathways are likely to have played a major role in the amelioration of mortality by KF24345. However, it cannot be denied that reversal of KF24345 effects by ZM 241385 was mediated via some other deleterious effect of ZM 241385 itself, since it increased mortality to nearly 100% suggesting enhanced

and irreversible pancreatitis caused by this compound itself. Further examination for the effects of adenosine receptor antagonists (e.g., dose–response evaluation, other adenosine receptor subtypes involved) is needed to determine a role of endogenous adenosine and the receptor subtypes involved in this model.

In addition to the attenuation of mortality, prophylactic treatment with KF24345 attenuated the pancreatic damage such as zymogen granule increase and vacuolation of acinar cells. These results suggest that endogenous adenosine inhibits the pathogenesis and progression of CDE diet-induced acute pancreatitis. The activation of adenosine A_1 receptors stimulates inflammatory cells (Cronstein, 1997; Rosengren and Firestein, 1997) and decreases pancreatic blood flow via vasoconstriction (Satoh et al., 2000). On the other hand, the activation of adenosine A_2 receptors inhibits the inflammatory cell (e.g., leukocytes and macrophages) activation (Cronstein, 1997; Rosengren and Firestein, 1997), increases pancreatic blood flow via vasodilation (Quéré et al., 1997) and stimulates pancreatic exocrine secretion (Yamagishi et al., 1985). The inflammatory response, the decrease of pancreatic blood flow and the impairment of pancreatic exocrine secretion seem to be involved in the pathogenesis of acute pancreatitis (Steer, 1998). Therefore, endogenous adenosine is assumed to have a protective role via adenosine A_2 receptors and an aggravating role via adenosine A_1 receptors in the pathogenesis and progression of acute pancreatitis. Since KF24345 attenuated the pancreatic damage in the present study, it is likely that the increased endogenous adenosine also acts to limit the pathogenesis and progression of acute pancreatitis through adenosine A_2 receptors.

In contrast with prophylactic treatment, therapeutic treatment with KF24345 did not improve the pancreatic degeneration in this study. However, therapeutic treatment with KF24345 decreased mortality in CDE diet-induced acute pancreatitis as well as prophylactic treatment. These results suggest that KF24345 decreases mortality and attenuates pancreatic damage by different mechanisms. In severe cases, the patients with acute pancreatitis die from serious complications including systemic inflammatory response syndrome and multiple organ dysfunction syndrome. Systemic inflammatory response syndrome and multiple organ dysfunction syndrome, being mediated by activated macrophages and neutrophils, are assumed to be relevant to the high mortality rate (Niederau et al., 1991; Ogawa, 1998). Adenosine has been reported to suppress the activation of macrophages and neutrophils (Cronstein, 1997; Rosengren and Firestein, 1997). In addition, KF24345 suppressed the elevation of serum TNF-α concentrations in mice with acute pancreatitis in this study. These observations and results raise the possibility that KF24345 can suppress systemic inflammatory response syndrome and multiple organ dysfunction syndrome by the enhancement of endogenous adenosine. Although further studies are necessary to clarify the mechanism of the effects of KF24345 on acute pancreatitis, the suppressing effects of KF24345 on systemic inflammatory response syndrome and multiple organ dysfunction syndrome are assumed to result in the attenuation of the mortality ultimately.

In the control mice with acute pancreatitis, the plasma adenosine concentration was about 2-fold and significantly higher than that in normal mice. The sources of adenosine seem to be the damaged organs or tissues, including the pancreas, in which adenosine production is enhanced due to the increased demand for energy supplied by ATP and the increased ATP catabolism to adenosine (Lüthen et al., 1995). Indeed, in rats with acute pancreatitis, the pancreatic tissue level of adenosine is reported to be slightly but significantly higher than that in non-pancreatitis rats (Satoh et al, 2000). In addition to the pancreatic damage, we observed lung injury (neutrophil infiltration and microvascular hyperpermeability) and liver injury (vacuolation and necrosis of hepatocyte) in CDE diet-induced acute pancreatitis in mice (unpublished observation), suggesting that the damaged organs or tissues are probably the source of increased adenosine in this model. An injection of KF24345 significantly elevated plasma adenosine concentrations in the control mice with acute pancreatitis, but not in normal mice. These results suggest that increased adenosine in plasma is due to the inhibition of adenosine uptake of adjacent cells in damaged tissues, not of erythrocytes. In the normal mice, basal level of extracellular adenosine surrounding the cell is low, and an injection of KF24345 did not affect plasma adenosine concentrations. Collectively, the inhibitory effects of KF24345 on adenosine uptake into adjacent cells in damaged organs and tissues seem to have contributed to the elevation of plasma adenosine in the control pancreatitis mice. Such inhibition could elevate adenosine level not only in plasma but also in damaged organs or tissues, and also lead to the preservation of cell integrity of the damaged organ. Further examination is needed for the organ adenosine level.

In our previous study, KF24345 inhibited the increases in serum amylase and lipase activity, and ameliorated pancreatic pathologic changes such as interstitial edema, polymorphonuclear cell infiltration and acinar cell necrosis in cerulein-induced acute pancreatitis in mice (Noji et al., in press). This observation and our present results suggest that KF24345 could ameliorate both mild and severe forms of acute pancreatitis. In the rat cerulein pancreatitis, 2-[*p*-(2-carboxyethyl)-phenethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680), an adenosine A_{2A} receptor agonist, has been reported to decrease leukocyte infiltration in the pancreas (Satoh et al., 2002). The adenosine A_{2A} receptor agonist (CGS21680) and the adenosine uptake inhibitor (KF24345) seem to share a common mechanism in the anti-inflammatory effects shown in acute pancreatitis. On the other hand, CGS21680 is also reported to aggravate pancreatic edema, while such aggravation does not occur following the KF24345 administration. The edema aggravation caused by CGS21680 is likely to be due to systemic,

rather than local, effects following the administration of an adenosine receptor agonist. Indeed, the application of adenosine and its agonists has been reported to cause hypotension, bradycardia, sedation and so on (Williams, 1996), while KF24345 does not induce such side effects (unpublished observation). Accordingly, the adenosine uptake inhibitor may have some advantage over the adenosine A_{2A} receptor agonist for the treatment of acute pancreatitis.

In conclusion, we have shown that KF24345 decreases the severity and improves survival in CDE diet-induced acute pancreatitis in mice. These are the first demonstrations that the adenosine uptake inhibitor ameliorates the progression and complications of experimental lethal acute pancreatitis. The present data suggest that the adenosine uptake inhibition may be effective in the treatment of severe acute pancreatitis.

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