Effects of potassium channel opener KRN4884 on human conduit arteries used as coronary bypass grafts

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Aims The effects of a new potassium channel opener KRN4884 on human arteries have not been studied. This study was designed to investigate the effects of KRN4884 on the human internal mammary artery (IMA) in order to provide information on possible clinical applications of KRN4884 for preventing and relieving vasospasm of arterial grafts in coronary artery bypass grafting.

Methods IMA segments ($n=140$) taken from patients undergoing coronary surgery were studied in the organ chamber. Concentration-relaxation curves for KRN4884 were established in the IMA precontracted with noradrenaline (NA), 5-hydroxytryptamine (5-HT), angiotensin II (ANG II), and endothelin-1 (ET-1). The effect of glibenclamide (GBC) on the KRN4884-induced relaxation was also examined in NA or 5-HT-precontracted IMA. Concentration-contraction curves for the four vasoconstrictors were constructed without/with pretreatment of KNR4884 (1 or 30 μM) for 15 min.

Results KRN4884 induced less relaxation ($P<0.05$) in the precontraction induced by ET-1 ($72.9 \pm 5.5\%$) than by ANG II ($94.2 \pm 3.2\%$) or NA ($93.7 \pm 4.1\%$) with lower EC_{50} ($P<0.05$) for ANG II ($-8.54 \pm 0.54 \log \text{M}$) than that for NA ($-6.14 \pm 0.15 \log \text{M}$) or ET-1 ($-6.69 \pm 0.34 \log \text{M}$). The relaxation in the IMA pretreated with GBC was less than that in control ($P<0.05$). KRN4884-pretreatment significantly reduced the contraction ($P<0.05$) induced by NA ($151.3 \pm 18.4\%$ vs $82.7 \pm 8.7\%$), 5-HT ($82.7 \pm 12.2\%$ vs $30.1 \pm 7.3\%$), and ANG II ($24.3 \pm 6.3\%$ vs $5.4 \pm 1.6\%$), but did not significantly reduce the contraction induced by ET-1 ($P>0.05$).

Conclusion KRN4884 has marked vasorelaxant effects on the human IMA contracted by a variety of vasoconstrictors and the effect is vasoconstrictor-selective.

Keywords: human conduit arteries, KRN4884, potassium channel opener, vasorelaxation

Introduction

Use of vasodilators in coronary artery bypass grafting has recently become an important clinical issue regarding vasospasm of the bypass graft [1–3]. Many vasodilators such as calcium (Ca^{2+}) channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, long-lasting nitrates,

and phosphodiesterase (PDE) inhibitors, etc. have been used in patients undergoing coronary artery bypass grafting and the effects of some of these on coronary bypass grafts have been extensively investigated [1–4]. However, little has been reported with regard to the vasorelaxant effects of potassium (K^+) channel openers (KCOs) on human conduit arteries used as coronary artery bypass grafts [5]. The effects of KRN4884, a novel ATP-sensitive potassium channel (K_{ATP}) opener, on human arteries have not been studied.

K_{ATP} exists in a wide range of cells, particularly in smooth muscle, skeletal muscle, endocrine cell, neurone, and cardiac cell [6]. KCOs mainly deactivate voltage-

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dependent Ca^{2+} channels by hyperpolarization of cell membrane through activation of K^+ channels [7], decrease intracellular free Ca^{2+} via the $\text{Na}^+/\text{Ca}^{2+}$ exchange pathway [8] and inhibition of inositol-1,4,5-trisphosphate (IP3) production [9], and reduce sensitivity of contractile proteins to Ca^{2+} [10]. These mechanisms result in vascular dilatation. It has been suggested that vascular smooth muscle is particularly sensitive to KCOs [6]. KRN4884, a novel pyridinecarboxamide type K_{ATP} opener, has sensitivity for the coronary vasculature greater than that of Ca^{2+} channel blockers such as nifedipine and nilvadipine [11], and its potency for opening K^+ channels exceeds that of other related compounds such as Ki3005, Ki5624, and Ki1796 [12].

The present study was designed to investigate the effects of KRN4884 on the human internal mammary artery (IMA), the most commonly used arterial graft for coronary artery bypass surgery in order to provide information on possible clinical applications of KRN4884 for preventing and releasing vasospasm of the arterial graft in coronary artery bypass surgery.

Methods

General

One hundred and forty-two human IMA segments were collected from 40 patients undergoing IMA graft surgery. There were 32 males and 8 females with a mean age of 64.4 ± 1.1 years. Approval to use discarded IMA tissue was given by the Institutional Review Board of St. Vincent Hospital. The discarded distal IMA segments were collected and placed in a container with oxygenated, physiological solution (Krebs') maintained at 4°C and transferred to the laboratory. The IMA was placed into a glass dish and dissected out from its surrounding connective tissue. The vessels were cut into 3 mm long rings and suspended on wires in organ baths [13, 14] filled with Krebs' solution. The rings taken from each patient varied from 2 to 6. The Krebs' solution had the following composition (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, H_2PO_4^- 1.2, and glucose 11. The solution was aerated with a gas mixture of 95% O_2 and 5% CO_2 at 37°C throughout the experiment.

Organ-bath technique

A technique that allows normalization of the vascular rings to be studied under physiological pressure in the organ bath was used. This sets the vascular rings at a pressure comparable with that *in vivo* [13, 14]. The rings were stretched in progressive steps to determine the length-tension curve for each ring. A computer iterative fitting

program (VESTAND 2.1, Yang-Hui He, Princeton University, NJ) was used to determine the exponential line, pressure and the internal diameter. When the transmural pressure on the rings reached 100 mmHg, determined from their own length-tension curves, the stretching procedure was stopped and the rings were released to 90% of its internal circumference at 100 mmHg. This degree of passive tension was then maintained throughout the experiment.

Protocol

After the normalization procedure, the IMA rings were equilibrated at least for 45 min.

Relaxation

KRN4884-induced relaxation was studied in IMA rings contracted with noradrenaline (NA, $3 \mu\text{M}$, $n=8$), 5-hydroxytryptamine (5-HT, $3 \mu\text{M}$, $n=8$), angiotensin II (ANGII, 3 nM , $n=8$), and endothelin-1 (ET-1, 10 nM , $n=8$). The concentrations of these vasoconstrictors were submaximal as determined from the logistic-curve fitting equation [13]. These concentrations are equal to EC_{50} – EC_{80} for the NA, 5-HT, ANGI, and ET-1-induced contraction in the human IMA from previous studies [2, 3, 5, 13, 14]. Cumulative concentration-relaxation curves to KRN4884 were then established. Only one concentration-relaxation curve was obtained from each IMA ring. From eight rings (taken from 6 to 8 patients), a mean concentration-relaxation curve was constructed. The concentration-relaxation curve to KRN4884 was also established in rings pretreated with glibenclamide (GBC, $3 \mu\text{M}$), a K_{ATP} blocker, for 30 min before the contraction-relaxation curve was induced in either NA or 5-HT-precontracted IMA rings ($n=6$ or 7 in each group).

Depression of contraction by pretreatment with KRN4884 After equilibration, 100 mM K^+ was added into the organ bath and the contraction force was recorded. Rings were discarded if their contraction force to 100 mM K^+ was less than 1 g. The ring was frequently washed to restore the baseline. To determine whether pretreatment with KRN4884 would alter the contraction response to various vasoconstrictors (NA, 5-HT, ANGI, and ET-1), cumulative concentration-contraction curves were constructed in IMA ring segments. These rings were equilibrated for 15 min with 0 (control), -6 and $-4.5 \log \text{ M}$ KRN4884. The contraction was expressed as percentage of the contraction force induced by 100 mM K^+ .

Data analysis

The effective concentration of the constrictor (or dilator)

agent that caused 50% of maximal contraction (or relaxation) was defined as EC_{50} . The EC_{50} was determined from each concentration-contraction (or relaxation) curve by a logistic, curve-fitting equation: $E = MA^p / (A^p + K^p)$ where E is response, M is maximal contraction (or relaxation), A is concentration, K is EC_{50} concentration, and p is the slope parameter [13]. A computerized program was used for the curve-fitting. From this fitted equation, the mean EC_{50} value $\pm$ s.e. mean was calculated in each group. Data were expressed as mean $\pm$ s.e. of the mean with 95% confidence intervals for differences (95% CI) where appropriate. Unpaired t -test or analysis of variance (ANOVA) were used to test statistical significance among different constrictors and dilators regarding the maximal response or EC_{50} . Scheffe's F -test was used as a *posthoc* test between groups. $P < 0.05$ was considered significant.

Materials

Drugs used in this study and their sources were: noradrenaline (Sigma, St Louis, MO); 5-HT (Sigma); Angiotensin II (Sigma); Endothelin-1 (Peptides International, Louisville, Kentucky). KRN4884 was a generous gift by Pharmaceutical Research Laboratory of Kirin Brewery Co. Ltd, Japan. Stock solution of endothelin-1 and angiotensin II was held frozen until required.

Results

Resting vessel parameters

The mean internal diameter of the 140 rings at an equivalent transmural pressure of 100 mmHg (D 100) was 2.7 ± 0.1 mm as determined from the normalization procedure. When the IMA rings were set at a resting diameter of $0.9 \times D100$, the equivalent transmural pressure was 75.2 ± 0.6 mmHg and the resting force was 4.5 ± 0.1 g.

Relaxation by KRN 4884 in the IMA precontracted by ET-1, ANGII, NA or 5-HT

The precontraction force was 2.8 ± 0.3 g for ET-1 (10 nM), 1.4 ± 0.3 g for ANG II (3 nM), 1.8 ± 0.3 g for NA (3 μ M), and 1.7 ± 0.6 g for 5-HT (3 μ M) ($P = 0.09$, ANOVA). KRN4884 induced marked relaxation in a concentration-dependent manner in human IMA precontracted by the four constrictors. However, among the four groups, there was a significant difference in the relaxation ($P = 0.0057$, ANOVA). KRN4884-induced relaxation at the concentration of 100 μ M was significantly less in the IMA precontracted by ET-1 ($72.9 \pm 5.5\%$) than in that by ANG II ($94.2 \pm 3.2\%$, $P = 0.02$, 95% CI: 2.4, 40.1%, Scheffe's F -test), or by NA ($93.7 \pm 4.1\%$, $P = 0.03$,

Figure 1 Concentration-relaxation curves for KRN4884 in the human internal mammary artery precontracted by ET-1 (●, 10 nM, $n = 8$), 5-HT (○, 3 μ M, $n = 8$), ANG II (△, 3 nM, $n = 8$); or NA (▲, 3 μ M, $n = 7$). Values are mean $\pm$ s.e. of the mean. $P = 0.0057$, ANOVA among the four groups. * $P < 0.05$, compared with the relaxation in ET-1-precontracted ring (Scheffe's F -test).

95% CI: 1.3, 40.3%), but not significantly different from that by 5-HT ($80.8 \pm 4.9\%$, $P = 0.67$, 95% CI: -10.9, 26.8%) (Figure 1).

With regard to sensitivity, there was a significant difference among the four groups in EC_{50} ($P = 0.0004$, ANOVA). The EC_{50} value for KRN4884-induced relaxation was significantly lower in the ANG II (-8.54 ± 0.54 log M) than in the NA (-6.14 ± 0.15 log M, $P = 0.001$, Scheffe's F -test, 95% CI: -3.9, -0.9 log M), or in the ET-1 (-6.69 ± 0.34 log M, $P = 0.008$, 95% CI: -3.3, -0.4 log M) -induced precontraction. However, the difference compared to the 5-HT-induced precontraction did not reach statistical significance (-7.1 ± 0.2 log M, $P = 0.053$, 95% CI: -2.9, 0.01 log M).

The relaxation in IMA pretreated with glibenclamide (GBC) was remarkably less than in the control for the NA- ($71.3 \pm 1.9\%$ vs $93.7 \pm 4.1\%$, $P = 0.036$, 95% CI: 11.8, 33.1%; EC_{50} : -3.61 ± 0.82 log M vs -6.14 ± 0.15 log M, $P = 0.043$, 95% CI: -4.2, -0.8 log M; unpaired t -test, Figure 2a) and for the 5-HT- ($56.1 \pm 2.2\%$ vs $80.8 \pm 4.9\%$, $P = 0.02$, 95% CI: 12.5, 36.9%; EC_{50} : -3.91 ± 0.38 log M vs -7.1 ± 0.2 log M, $P = 0.01$, 95% CI: -4.1, -2.3 log M; unpaired t -test, Figure 2b) induced contraction.

Depression of contraction in KRN4884-pretreated IMA

The pretreatment with KRN4884 (1 or 30 μ M) for 15 min significantly depressed the contraction induced by NA, 5-HT, or ANG II compared with control (Figure 3a,b,c. Table 1). In NA-induced contraction, compared with $151.3 \pm 18.4\%$ in the control, the maximal contraction was $95.0 \pm 15.9\%$ ($P = 0.047$, Scheffe's F -test, 95% CI: 0.6, 111.9%) and $82.7 \pm 8.7\%$ ($P = 0.014$, 95% CI: 12.9, 124.2%) with the pretreatment of KRN4884 at

Figure 2 Concentration-relaxation curves for KRN4884 in the human internal mammary artery precontracted by NA ($3 \mu\text{M}$, a), or 5-HT ($3 \mu\text{M}$, b) with (●) or without (○) pretreatment by glibenclamide (GBC, $3 \mu\text{M}$). Values are mean $\pm$ s.e. of the mean. Unpaired *t*-test, * $P < 0.05$.

the concentration of 1 and $30 \mu\text{M}$ ($P = 0.009$, ANOVA, among the three groups), respectively. Similarly, in 5-HT-induced contraction, compared with $82.7 \pm 12.2\%$ in the control, the maximal contraction was $34.9 \pm 7.2\%$ ($P = 0.005$, 95% CI: 13.7, 81.9%) or $30.1 \pm 7.3\%$ ($P = 0.002$, 95% CI: 18.6, 86.8%) in the IMA pretreated with KRN4884 at 1 or $30 \mu\text{M}$ ($P = 0.001$, ANOVA, among the three groups). Again, in ANG II-induced contraction, compared with $24.3 \pm 6.3\%$ in the control, at the maximal concentration of ANG II ($-6 \log \text{M}$) the contraction was $5.7 \pm 1.5\%$ ($P = 0.009$, 95% CI: 4.3, 32.9%) or $5.4 \pm 1.6\%$ ($P = 0.008$, 95% CI: 4.6, 33.2%) in the IMA pretreated

with KRN4884 at 1 or $30 \mu\text{M}$ ($P = 0.003$, ANOVA, among the three groups, Figure 3c). However, the pretreatment with KRN4884 (1 or $30 \mu\text{M}$) did not significantly depress the contraction induced by ET-1, compared with the control ($P > 0.05$, 95% CI: -62.4 , 54.9% . Figure 3d, Table 1).

With regard to sensitivity, the EC_{50} values for NA, and 5-HT were significantly increased after pretreatment with KRN4884 (Table 1). However, no significant changes of EC_{50} to KRN4882 were observed in the IMA pretreated with ET-1 and ANG II ($P > 0.05$, 95% CI: -0.9 , $0.7 \log \text{M}$. Table 1).

Figure 3 Concentration contraction (% of 100 mM K^+ -induced contraction) curves to the constrictors of NA (a), 5-HT(b), ANGII(c) and ET-1(d) in the human internal mammary artery. Control (●, $n = 8$): without KRN4884-pretreatment; KRN4884 $1 \mu\text{M}$ ($-6 \log \text{M}$, ○, $n = 8$) or $30 \mu\text{M}$ ($-4.5 \log \text{M}$, ▲, $n = 8$) was added into the organ bath 15 min before the concentration-contraction curve was established. Values are mean $\pm$ s.e. of the mean. $P < 0.01$, ANOVA among the three groups in the experiments for all four vasoconstrictors. * $P < 0.01$, compared with the control (Scheffé's *F*-test).

Table 1 Inhibition of KRN4884 on the contraction induced by various vasoconstrictors in human internal mammary artery.

Pretreatment	n	Contraction at maximal concentration (% of 100 mM K ⁺ -induced contraction)	EC ₅₀ (log M)
<i>Noradrenaline contraction</i>			
Control	8	151.3 ± 18.4 ^{††}	-6.89 ± 0.20 [†]
KRN4884 (1 µM)	8	95.0 ± 15.9 [*]	-6.11 ± 0.27
KRN4884 (30 µM)	8	82.7 ± 8.7 [*]	-5.99 ± 0.18 [*]
<i>5-hydroxytryptamine contraction</i>			
Control	8	82.7 ± 12.2 ^{††}	-6.51 ± 0.14 [†]
KRN4884 (1 µM)	8	34.9 ± 7.2 [*]	-6.30 ± 0.11
KRN4884 (30 µM)	8	30.1 ± 7.3 [*]	-5.67 ± 0.32 [*]
<i>Angiotensin II contraction</i>			
Control	8	24.3 ± 6.3 ^{††}	-8.91 ± 0.11
KRN4884 (1 µM)	8	5.7 ± 1.5 [*]	-8.89 ± 0.08
KRN4884 (30 µM)	8	5.4 ± 1.6 [*]	-8.41 ± 0.33
<i>Endothelin-1 contraction</i>			
Control	8	132.2 ± 15.6	-8.11 ± 0.24
KRN4884 (1 µM)	8	136.9 ± 15.6	-7.97 ± 0.14
KRN4884 (30 µM)	8	134.8 ± 15.1	-7.85 ± 0.15

[†] $P < 0.05$, ^{††} $P < 0.01$, ANOVA among three groups, Scheffé's F -test, ^{*} $P < 0.05$ compared with the control.

Discussion

Graft spasm during the perioperative or postoperative period reduces the luminal blood flow [15] and may cause hypoperfusion [16]. The mechanism of graft spasm is not well understood. However, increased plasma levels of ET-1, NA, 5-HT, ANG, and thromboxane A₂ have been measured, probably due to injury to vascular endothelium and platelet or activation of renin-angiotensin system during or after coronary artery bypass surgery [17–19]. It is therefore necessary to search for effective antispastic agents for arterial grafts.

KRN4884, a new and specific K_{ATP} opener, activates cardiac K_{ATP} in single ventricular cells of guinea pig through not only decreasing the sensitivity of the channel to ATP but also directly stimulating the opening of the channel [20]. In the rat aorta, it may produce concentration-dependent relaxation by deactivation of Ca²⁺ channels and decrease in the Ca²⁺ sensitivity of contractile elements [21]. KRN4884 has selective effect on coronary vasculature greater than that of Ca²⁺ channel blockers such as nilvadipine and nifedipine [11] and its vasorelaxant potency exceeds that of other pyridinecarboxamide derivatives [12, 22]. However, all these reports are based on animal experiments. The present study, for the first time, investigated the effects of KRN4884 on human arteries. The effect of KRN was studied in the human IMA contracted by endothelium-derived contracting factor ET-1, α₁-adrenoceptor agonist NA, a platelet-derived factor 5-HT, and a vasoconstrictor related to renin-angiotensin system ANG II. These spasmogenic

agents induce contractions of vascular smooth muscle by increasing Ca²⁺ influx or Ca²⁺ mobilization from intracellular stores and consequently leading to an increase in intracellular free Ca²⁺ level [16, 23].

KRN4884 directly stimulates the opening of K_{ATP} and causes hyperpolarization. This prevents Ca²⁺ entry through inhibiting voltage-dependent Ca²⁺ channel, reduces intracellular free Ca²⁺, and decreases the Ca²⁺ sensitivity of contractile elements [11, 20, 21]. Subsequently, the vessel is relaxed. In this study, KRN4884 relaxed markedly the human IMA contracted by all of the four vasoconstrictors. In the IMA pretreated by glibenclamide (a specific blocker of K_{ATP}), the relaxation induced by KRN4884 was significantly inhibited in either NA or 5-HT-induced contraction. These results demonstrate that KRN4884 has marked relaxant effect on human IMA and relaxation is mainly related to the opening of K_{ATP}.

The present study further demonstrates that the vasorelaxant effect of KRN4884 is vasoconstrictor-selective in the human artery. The maximal relaxation was significantly less in the IMA contracted by ET-1 than in that by NA or ANG II. Further evidence for the vasoconstrictor-selective effect of KRN4884 is the selective inhibition of contraction in IMA pretreated by KRN4884. The pretreatment of KRN4884 (either 1 or 30 µM) significantly depressed the contraction elicited by NA, ANG II, and 5-HT but not ET-1.

Endothelin is the most potent vasoconstrictor known [24]. ET-1 is a potent and sustained constrictor of resistance and capacitance vessels *in vivo* in man [25].

The mechanisms of ET-1-induced vasoconstriction, it was initially considered to be activation of voltage-dependent (L -type) Ca^{2+} channels [26]. Other studies demonstrated that removal of extracellular calcium or addition of the calcium antagonist nicardipine attenuates but does not abolish the response to ET-1 [27–29], and therefore, suggest that contraction of ET-1 depends only in part on Ca^{2+} entry through voltage-dependent calcium channels. In electrophysiological studies of porcine coronary artery smooth muscle cells, ET-1 has been shown to block K_{ATP} and depolarize smooth muscle cells [30], leading to vasoconstriction by mechanisms additional to opening of voltage-operated Ca^{2+} channels [31]. Other ion transport mechanisms and activation of protein kinase C may also play a role in the contraction induced by ET-1 [29, 32]. Indeed, the activation of protein kinase C has been suggested to inhibit the K^+ channel opening [33, 34]. Thus, ET-1 appears to cause vasoconstriction through several mechanisms. This may at least partially explain the limited depress effect of KCOs on the ET-1 induced contraction in the present study. Others have demonstrated that the pretreatment with levcromakalim (a benzopyran KCOs) significantly depressed the maximal contractile responses to NA and U46619 but not ET-1 in the human IMA and gastroepiploic artery [36]. Our observations on the interaction between KRN4884 and ET-1 are in accordance with the above reports.

We have previously reported that some vasodilators have more significant effects when used to relax precontraction than used prior to the contraction [2, 3, 14]. For example, the effect of nitroglycerin is more potent when it is used to relax the ET-1-mediated contraction but it is less potent if it is added before the contraction induced by ET-1 [2]. This phenomenon is also observed in the present study. This may be related to the state of the smooth muscle [2, 3].

KCOs have several clinical applications for which the findings of this study may have relevance. Apart from its vasorelaxant effect, pharmacological K_{ATP} openers have been shown to protect the myocardium from ischaemia-reperfusion injury [37, 38], to improve myocardial preservation for heart transplantation [39], to have use in hyperpolarizing cardioplegia [40, 41], and to benefit lipid metabolism [42].

In conclusion, the present study suggests that KRN4884 has marked vasorelaxant effects in human IMA contracted by a variety of vasoconstrictors, although the antispastic effects are vasoconstrictor-selective with greater potency to NA, ANG II or 5-HT than to ET-1. The vasorelaxant effect of KRN4884 may be useful in coronary artery bypass surgery for prevention and treatment of vasospasm in the graft.

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