A New ATP-Sensitive Potassium Channel Opener Reduces Blood Pressure and Reverses Cardiovascular Remodeling in Experimental Hypertension

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ABSTRACT

Some potassium channel openers (KCOs) are potent vasodilators that mainly target the ATP-sensitive potassium channels in vascular smooth muscle cells. Their lack of tissue selectivity limits their clinical use in hypertension therapy. Iptakalim [2,3-dimethyl-N-(1-methylethyl)-2-butylamine], which belongs to a novel chemical type of KCO, possesses unique pharmacological characteristics. In vitro experiments have shown that iptakalim could limit its vasorelaxing actions to resistance vessels. In this study, we investigate the antihypertensive effects of iptakalim on two different experimental hypertensive models: stroke-prone, spontaneously hypertensive rats (SHRsps) and two-kidney with one-clip renal hypertensive dogs (2K1C RHD).

In acute hypotensive tests, iptakalim showed stable, long-lasting antihypertensive effects in SHRsps and 2K1C RHDs. Meanwhile, it had little effect on heart rate when compared with pinacidil, nifedipine, captopril, or bisoprolol. In experimental therapeutic tests, repeated doses in SHRsps for 30 days or in 2K1C RHDs for 14 days produced consistent antihypertensive effects without causing tolerance. In separate experiments, chronic administration of iptakalim resulted in reversing hypertensive vascular remodeling in spontaneously hypertensive rats and hypertensive cardiac remodeling in SHRspes. These results suggest that iptakalim is a promising antihypertensive drug.

Hypertension is a substantial public health problem, affecting about 25% of the adult population in the world, and it is an important risk factor for death from stroke, myocardial infarction, congestive heart failure, and renal failure (Lifton et al., 2001). According to various pharmacological targets in cardiovascular and nervous-humeral system, more than 200 distinct chemical entities have been developed and used in the clinical management of this disease (Mancia et al., 1999; Burnier, 2000). Among these drugs, vasodilators are able to reduce blood pressure by relaxing excessive contractions of arteries. Potassium channel openers (KCOs) belong to a relatively new type of vasodilators that have been previously regarded as calcium antagonists. It is now revealed that ATP-sensitive potassium (K\textsubscript{ATP}) channels are the main target proteins for this kind of agent (Fujita and Kurachi, 2000).

By opening K\textsubscript{ATP} channels in vascular smooth muscles, KCOs would induce membrane hyperpolarization and a decrease of intracellular Ca\textsuperscript{2+} levels, thereby relaxing the vessels and suppressing blood pressure.

KCOs exhibit extreme chemical diversity and comprise a number of different structural classes, such as benzopyrans, cyanoguanidines, thioformamides, and pyrimidines (Mannhold, 2004). Some of them have been developed for clinical use and have several benefits, such as strong and long-term action and the ability to decrease blood lipids, in contrast with other antihypertensive agents. The shortfall of this kind of agent is a lack of tissue selectivity. In addition to smooth muscle cells, the pancreas and the heart also contain large concentrations of K\textsubscript{ATP} (Noma, 1983; Cook and Hales, 1984; Standen et al., 1989). Therefore, the antihypotensive effects of KCOs are usually accompanied by other side effects, such as hyperglycemia and cardiotoxicity (Nielsen-Kudsk et al., 1996). Although the structural modification of current chemical types has produced some good results, these are still not optimal as antihypertensive drugs (Wright, 2000). Therefore, new structural forms of KCOs need to be developed.

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ABBREVIATIONS: KCO, potassium channel opener; K\textsubscript{ATP}, ATP-sensitive potassium; iptakalim, 2,3-dimethyl-N-(1-methylethyl)-2-butylamine; SHR, spontaneously hypertensive rat; SHRsp, stroke-prone spontaneously hypertensive rat; 2K1C RHD, two-kidney with one-clip renal hypertensive dog; SBP, systolic blood pressure; HR, heart rate; DBP, diastolic blood pressure; NTR, normotensive rat; M, media thickness; L, lumen diameter; LV, left ventricle; S, septum; BW, body weight; MAP, mean blood pressure; bpm, beats per minute.
After screening 10 chemical structure types, which included nearly 1000 compounds, we discovered a novel chemical type of KCO, aliphatic amines, that were completely different from those previously reported (Wang et al., 2002). A representative compound is named iptakalim [2,3-dimethyl-\(n\)-(1-methylethyl)-2-butanamine]. Besides a difference in structure, iptakalim also possesses unique characteristics when compared with pinacidil, the classical KCO that has been used extensively in clinical settings. First, iptakalim has selectivity, relaxing the small arteries in vitro. Second, the vascular relaxing effects of iptakalim are selective for blood pressure and act more strongly in hypertensive states. Third, iptakalim and pinacidil respond differently on \(K_{ATP}\) gene expression levels (Jia et al., 2004). The excessive contractions of resistance vessels mainly contribute to the pathological change of hypertension. Current antihypertensive drugs, lacking the selective effects for small arteries, may cause many adverse effects. Since iptakalim could limit its vasorelaxing actions to small arteries in the hypertensive state, it is possible that this compound will exhibit better effects than current KCOs and other antihypertensive agents. Here, we validate the hypotensive effects of iptakalim on two different experimental hypertensive models, spontaneously hypertensive rats (SHRs) or stroke-prone SHRs (SHRsp)s and two-kidney with one-clip renal hypertensive dogs (2K1C RHDs). Iptakalim showed stable, antihypertensive effects, with very little effect on heart rate. During long-term administration, no tolerance to iptakalim developed, and it was found that iptakalim could be administered repeatedly without any decrease in its effects. In addition, we also investigated whether this compound could be efficacious at ameliorating target organ damage, which is always the main cause of death or disability in hypertensive patients. It was found that iptakalim reversed hypertensive cardiovascular remodeling in SHRs or SHRsp s. These data indicate that this new compound has potential for hypertension therapy.

### Materials and Methods

#### Animals

SHR, SHRsp, and Wistar rats were obtained from the Institute of Cardiovascular Diseases, Chinese Academy of Medical Sciences (Beijing, China). Mongrel dogs were purchased from the Academy of Military Medical Sciences (Beijing, China). All animal procedures were in accordance with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication 85-23, revised 1985).

#### Chemical Compounds

Iptakalim was synthesized by the Beijing Institute of Pharmacology and Toxicology (Beijing, China). Nifedipine, captoril, and pinacidil were purchased from Sigma-Aldrich (St. Louis, MO). Lisinopril was purchased from Merck (Whitehouse Station, NJ). Bispromol was purchased from Beijing Four-Rings Pharmaceutical Science and Technology Co., Ltd. (Beijing, China). All other chemicals and materials were obtained from local commercial sources.

#### Acute Hypotensive Test in Conscious Stroke-Prone SHRs and in Conscious Two-Kidney with One-Clip RHDs

**Conscious SHRsp s.** Male SHRsp s (5–7 months old; 250–300 g) were used in this study. The rats were housed in groups of five with a 12-h light/dark cycle at a temperature of 24 ± 1°C, relative humidity of 56 ± 10%, and free access to food and water. The compound or vehicle was administered by oral lavage in a volume of 2 ml/kg. For experiments involving indirect recording of blood pressure and heart rates, rats were prewarmed at 38°C for 10 min. Systolic blood pressure (SBP) and heart rate (HR) were recorded indirectly by the standard tail-cuff technique (BP recorder, RBP-I; China-Japan Friendship Hospital, Beijing, China). Measurement of parameters was performed at 1, 3, 5, 9, 12, and 24 h after administration.

**Conscious Two-Kidney with One-Clip RHDs.** 2K1C RHDs were made as previously described (Shuai and Wang, 2003). Briefly, the male dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.), and the left renal artery was exposed through a retroperitoneal flank incision. The renal artery was constricted to reduce the blood flow to 20 to 30% of baseline value with nylon threads. The incision was sutured, and an antibiotic was given. The dogs were fed a standard diet twice per day (7:00 AM and 4:00 PM) and were allowed free access to water. Experiments were performed on the established phase of hypertension (>150 mm Hg systolic blood pressure) 1 month after the operation. The animals were randomly sorted into groups and fed the indicated drug-filled capsules. The control group was administered empty capsules. When the blood pressure of the dogs remained stable during the 5-day control period, the experiments were begun. The SBP, diastolic blood pressure (DBP), and HR were measured as described previously (Shuai and Wang, 2003). In brief, the blood pressure of the common carotid artery was measured by stethoscope, and HR was measured by palpation. An electrocardiogram was recorded simultaneously.

#### Experimental Therapeutic Test in Conscious SHRsp s and in Conscious Two-Kidney with One-Clip RHDs

**Conscious SHRsp s.** Male SHR (10 weeks old; 100–200 g) were used in this experiment. Iptakalim was administered orally at 1, 2, and 4 mg/kg, and vehicle was given orally at 2 ml/kg. Pinacidil and nifedipine were given orally at 3 and 10 mg/kg, respectively. Drugs were administered at 9:00 AM every day for 30 days. The SBP and HR were measured 3 h after administration of iptakalim or 1 h after administration of pinacidil and nifedipine. The whole experiment was divided into three periods: the control period, the treatment period, and the drug withdrawal period. During a 1-week control period, the SBP and HR were measured every 3 days. During the 1-month treatment period, the SBP and HR were measured at 3 h after administration of iptakalim every 3 days. During a 1-week drug withdrawal period, the blood pressure returned to control levels.

**Conscious Two-Kidney with One-Clip RHDs.** 2K1C RHDs, made as described above, were randomly divided into three groups. Iptakalim was administered orally in capsule once a day for 14 days (9:00 AM). The SBP, DBP, and HR were measured before and after administration 2 and 4 h every day, using the same method as mentioned above. After drugs were withdrawn, the blood pressure and HR were measured continuously for 3 days.

#### Studies on Hypertensive Target Organ Injuries on SHRs or SHRsp s

**Hypertensive Vascular Remodeling.** SHRs (3 months old, 200–300 g) of either sex were orally administered with 3 mg/kg iptakalim, 2 mg/kg pinacidil, 12 mg/kg lisinopril, or 10 mg/kg water once a day for 4 weeks. Normotensive rats (NTRs) and age-matched Wistar rats were used as normal control. During the treatment period, the blood pressure and HR were measured once a week using the same method as mentioned above. After treatment for 4 weeks, the animals were killed, and the structural changes of aorta and mesenteric arteries were investigated using a light microscope. The media thickness (M) and lumen diameter (L) of aorta and mesenteric arteries were recorded under a light microscope, and the ratio of M/L was calculated.

**Hypertensive Cardiac Remodeling.** SHRsp s (10 weeks old, 100–200 g) of either sex were randomly divided into four groups, which were orally treated with 0.25, 1, and 4 mg/kg iptakalim or 10 mg/kg nifedipine, at 200–300 g) were used in this study. The rats were housed in groups of five with a 12-h light/dark cycle at a temperature of 24 ± 1°C, relative humidity of 56 ± 10%, and free access to food and water. The compound or vehicle was administered by oral lavage in a volume of 2 ml/kg. For experiments involving indirect recording of blood pressure and heart rates, rats were prewarmed at 38°C for 10 min. Systolic blood pressure (SBP) and heart rate (HR) were recorded indirectly by the standard tail-cuff technique (BP recorder, RBP-I; China-Japan Friendship Hospital, Beijing, China). Measurement of parameters was performed at 1, 3, 5, 9, 12, and 24 h after administration.
mg/kg water, respectively, once a day for 12 weeks. Age-matched Wistar rats were used as normal control. To accelerate the onset of stroke, animals were given 1% NaCl solution as drinking water every day. During the treatment period, the blood pressure and HR were measured once a week using the same method as mentioned above. After treatment for 12 weeks, the animals were sacrificed by cervical dislocation. The hearts were exposed, and the atria were removed. The weight of the right ventricle and the weight of the left ventricle (LV) and septum (S) were measured, and the ratio of LV/S/body weight (BW) was calculated.

### Statistical Analysis
The results were expressed as mean ± S.E. Student’s paired t test, Student’s two-sample t test, or analysis of variance were performed to analyze data using the SAS Software Program (SAS Institute, Cary, NC). Statistical significance was accepted at *P* < 0.05.

### Results

#### Acute Antihypertensive Effects in Conscious SHRs.
Oral administration of iptakalim at the doses of 0.75, 1.5, 3, and 6 mg/kg produced dose-dependent reductions in SBP. The maximal ∆SBP were 19, 23, 31, and 49 mm Hg, respectively. The antihypertensive actions of iptakalim commenced at 1 to 3 h and lasted for 5 to 12 h, whereas the HR remained unchanged (Figs. 1A and 2).

Under the same experimental conditions, oral administration of antihypertensive drugs, including the potassium channel opener pinacidil (3 mg/kg), the calcium channel blocker nifedipine (10 mg/kg), the angiotensin-converting enzyme inhibitor captopril (40 mg/kg), or the β receptor blocker bisoprolol (60 mg/kg), also decreased SBP. The maximal ∆SBP were 34, 56, 36, and 31 mm Hg, respectively. The total time of the induced hypotensive effects were 2, 2, 5, and 1 h, respectively, shorter than iptakalim. Unlike iptakalim, these four agents significantly changed HR. Among them, pinacidil, nifedipine, and captopril increased the HR and bisoprolol decreased the HR (Figs. 1B and 2).

#### Acute Antihypertensive Effects in Conscious 2K1C RHDs.
Oral administration of iptakalim at the doses of 0.125, 0.25, 0.5, and 1 mg/kg produced dose-dependent reductions in blood pressure in conscious RHD (Fig. 3). The antihypertensive actions of iptakalim commenced at 0.5 h and were maximal 2 to 3 h after administration. The maximal decreases in ∆SBP/∆DBP were 13 ± 4/13 ± 5, 23 ± 6/15 ± 6, 25 ± 7/27 ± 13, and 36 ± 12/36 ± 16 mm Hg, respectively. The total time of the hypotensive effects were 4, 6, 6, and 12 h, respectively. The ED₃₀ value, the dose required to reduce MAP by 30 mm Hg, was 0.65 mg/kg. The HR was increased significantly and the effects were maximal at 2 to 3 h after administration of iptakalim at 0.125, 0.25, 0.5, and 1 mg/kg. The maximal increases in ∆HR were 5 ± 3, 15 ± 11, 22 ± 15, and 31 ± 14 beats per minute (bpm), respectively. The ED₅₀ value, the dose required to increase HR by 50 bpm, was 2.17 mg/kg. The rate of separation between ED₅₀ bpm and ED₃₀ mm Hg was 3.338 (Fig. 4). This figure reflects the efficacy of drugs. Higher value of this figure means less side effects of increased HR induced by drugs. Under the same experimental conditions, oral administration of pinacidil at the doses of 0.125, 0.25, 0.5, and 1 mg/kg also decreased the MAP in conscious RHD in a dose-dependent manner. The rate of separation [ED₅₀ (beats per minute)/ED₃₀ (mm Hg)] was 0.775, which is about one-quarter of iptakalim (Fig. 4). The results suggested that the side effects of increased HR induced by iptakalim are less than those of pinacidil.

#### Experimental Therapeutic Actions in Conscious SHRsp.
SHRsp were given 1, 2, or 4 mg/kg iptakalim daily at 9:00 AM every day for 30 days. The SBP in the control group increased continuously during the experimental period, and the HR did not change significantly. Under the same experimental conditions, 1, 2, and 4 mg/kg iptakalim decreased SBP by around 20, 25, and 30 mm Hg, respectively, whereas HR remained unchanged (Fig. 5A). It suggested that SBP could be decreased in a dose-dependent manner by administering iptakalim repeatedly, as iptakalim
had very little effect on HR. After a single dose of iptakalim administration per day, the total time for the hypotensive effects was 12 h, and no obvious effects on HR were observed (Fig. 5B). These results were similar with those of acute antihypertensive experiment of iptakalim (Fig. 1A).

During the experimental therapeutic period, iptakalim had no effects on body weight. We also found that iptakalim did not affect the appetite, the central nerve system, or the behavior of animals.

Under the same experimental conditions, pinacidil (4 mg/kg) and nifedipine (10 mg/kg) decreased SBP significantly by around 37 and 34 mm Hg, respectively. At the same time, HR was increased markedly by around 83 and 112 bpm. These results were similar with those of acute antihypertensive experiment (Fig. 1B).

Experimental Therapeutic Actions on Conscious RHDs. 2K1C RHD were given 0.25, 0.5, or 1 mg/kg iptakalim daily at 9:00 AM every day for 14 days. MAP decreased significantly after drug administration. SBP decreased by around 37 and 34 mm Hg, respectively, and DBP decreased by around 10, 19, and 30 mm Hg, respectively. Mean-while, the HR increased by 2, 5, and 22%, respectively (Fig. 6). After withdrawal of iptakalim, the blood pressure returned to the baseline value.

Effects against Hypertensive Vascular Remodeling. The SBP of the SHR were 165 to 175 mm Hg before treatment, which were much higher than those of the normal (124 ± 9 mm Hg). During the 4-week treatment period, the blood pressure of NTR remained stable, whereas the blood pressure of SHR in the control group increased continuously. After 3 weeks, SBP were stable at the level of 197 ± 10 mm Hg. After treatment with iptakalim (3 mg/kg) or lisinopril (12 mg/kg), SBP decreased by around 40 to 50 mm Hg.

The HR of the SHR was 472 ± 21 bpm before treatment, which were higher than those of the NTR (389 ± 30 bpm). During the 4-week treatment period, the HR of the NTR remained unchanged, whereas the HR of the SHR in the control group increased continuously. After a 4-week treatment, lisinopril had no obvious effects on HR (553 ± 27 bpm) compared with those of the control group (563 ± 32 bpm), whereas iptakalim inhibited the tendency to increase HR (498 ± 27 bpm).

 Compared with NTR, in mesenteric arteries of untreated SHR, the M increased, the L decreased, and the ratio of M/L increased. After treatment with iptakalim, pinacidil, and lisinopril, the media thickness decreased to the normal level, the lumen diameter did not change significantly, and the M/L ratios were lower than that of untreated SHR (Fig. 7A). These results suggested that the hypertensive remodeling of
mesenteric arteries could be reversed by iptakalim and lisinopril. Compared with NTR, in the aorta of untreated SHR, the media thickness and the ratio of M/L increased. After treatment with iptakalim, pinacidil, and lisinopril, the media thickness decreased to the normal level, and the M/L ratios were lower than that of untreated SHR (Fig. 7A). These results suggested that the hypertensive remodeling of aorta could be reversed by iptakalim and lisinopril.

**Studies on Hypertensive Cardiac Remodeling.** During the 12-week experimental period, the SBP of the untreated stroke prone SHR were increased progressively. Iptakalim (0.25, 1, and 4 mg/kg) could decrease the SBP effectively. Iptakalim at doses of 0.25 and 1 mg/kg had no effect on HR, but in the 4th week after administration of iptakalim (4 mg/kg), HR was significantly decreased.

Compared with NTR, the weight of the LV/H11001S and the ratio of LV/H11001S/BW in untreated SHRsp were elevated significantly. Iptakalim at 0.25, 1, and 4 mg/kg could decrease LV + S and (LV + S)/BW significantly (Fig. 7B). There was no difference in the weight of the right ventricle among the five groups.

**Discussion**

The antihypertensive actions of iptakalim, a new type of aliphatic amine KCO, were evaluated in different hypertensive animal models. This new chemical entity belongs to the aliphatic secondary amine derivatives, having a general structure of 1,1,2-trimethylpropylamine with side chain and several substituted structural modifications. A representative compound, with the isopropyl substituted in the N-side chain, is named iptakalim (Wang et al., 2002). In vitro experiments demonstrated its ability to induce outward potassium currents in vascular smooth muscle cells and relax small arteries (Jiang et al., 2003; Jia et al., 2004). This study showed that, in the animal models, iptakalim had obvious antihypertensive therapeutic advantages: good absorption, low dose, and the ability to commence its antihypertensive effect quickly and produce long-lasting hypotensive effects. Iptakalim showed a stable antihypertensive effect and had few effects on the heart. No tolerance developed to it, and it could be administered repeatedly without any decrease in its effects. In addition to reducing blood pressure, iptakalim could reverse hypertensive vascular cardiac remodeling and exhibit protective effects on the hypertensive brain (Wang et al., 2004). From available preclinical observations, the future of iptakalim seems promising for hypertension therapy.

SHRsp are regarded as a good model of malignant hypertension and whether various agents can reduce blood pressure effectively (Okamoto et al., 1974). Of the acute antihypertensives tested here, we have taken several drugs as positive controls, including KCO, calcium channel blocker, angiotensin-converting enzyme inhibitor, and β-receptor antagonist. Comparatively, the onset time of iptakalim to decrease blood pressure is shorter than other drugs but persists longer than others, with the effect continuing for 5 to 9 h. As
for effects on the heart, pinacidil, nifedipine, and captopril all increased the HR and bisoprolol significantly decreased it, whereas iptakalim affected HR only slightly. In addition, in a chronic experiment, we discovered that iptakalim could decrease blood pressure effectively. This action was long-lasting, with less effect on HR. When drug administration was stopped, the blood pressure returned to normal levels; no rebound phenomenon has been observed. The data demonstrated that iptakalim has a therapeutic advantage over other agents.

The two-kidney with one-clip animal model is a commonly used model for renal hypertension. Its mechanism involves kidney ischemia, which causes the rennin-angiotensin-aldosterone system to be excessively activated. Vasodilators such as pinacidil, nifedipine, and iptakalim can lower the blood pressure of model animals effectively and have no obvious influence on cardiac rhythms and electrocardiographic waves. According to the rate of separation figure, the efficacy of iptakalim was 4 times higher than that of pinacidil, which suggests that the side effects of increased HR induced by iptakalim are less than those by pinacidil.

Organ damage in hypertensive sufferers is the main cause of death or disability, and the development of new antihypertensive agents should be aimed at conserving target organs.
as well as controlling pressure. Current first line agents, β-adrenergic receptor blockers and diuretics, cannot alleviate heart, brain, or kidney injuries caused by hypertension. According to a separate investigation in our laboratory, iptakalim has been shown to protect from brain damage in hypertensive rats (Wang et al., 2004). In the current study, decreasing pressure simultaneously, iptakalim can also prevent hypertensive cardiovascular remodeling, which is one of its important therapeutic characteristics. Besides high blood pressure, excessive endothelin level is also a risk factor for hypertensive cardiovascular injury. Iptakalim has been shown to reduce endothelin release from endothelial cells against endothelin-induced arterial contraction and decrease endothelin-induced hypertension in rats (Wang 2003). Therefore, the protective profile of iptakalim may not only be due to the controlling about blood pressure but may also relate to its effects in the endothelium system.

According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, a person with a systolic pressure of 120 to 139 mm Hg has already been included in a new category of hypertension: prehypertension (Chobanian et al., 2003). Such concern for chronic effects has driven the search for prevention of and a cure for this disease. Because of the side effects of some recommended first line drugs and associated detrimental effects on the quality of life, it is essential for us to discover and develop new mechanisms and structurally novel antihypertensive drugs (Julius, 2000). The agents developed should not only depress the blood pressure indefinitely but also reverse cardiovascular remodeling and improve quality of life for patients by avoiding the occurrence of arteriosclerosis, stroke, and coronary heart disease. As mentioned in this article, iptakalim has a selective antihypertensive efficacy with steady and long-lasting characteristics and produces less side effects and toxicity under the effective doses (Wang et al., 2004). It has the virtue for hypertension treatment by reversing hypertensive cardiovascular remodeling and protecting the target organs. Therefore, this agent may be developed as a promising candidate in clinical application. Having completed all preclinical studies, this drug is now undergoing clinical tests.

References


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