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Research Article

Electrophysiology and Cardiovascular Pharmacology of Mokuboito (Formula Aristolochiae): Cardiotonic and Cardioprotective Actions for Chronic Heart Failure

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Abstract

Cardiovascular actions of Mokuboito (Formula aristolochiae), a kind of Kampo formulations, the ingredients and the phytochemicals, were investigated. In electrophysiological experiments, Mokuboito and its main ingredient (an herb), *Sinomeni Caulis* et Rhizoma (SCR), enhanced the L-type Ca^{2+} current (I_{Ca}) and the delayed rectifier K^+ current (I_{Krec}), and inhibited the inwardly rectifying K^+ current (I_{K1}). Simultaneously they affected the action potential configurations, and decreased the maximum rate of depolarization. On the other hand, the phytochemicals such as sinomenine and tetrandrine inhibited the ionic currents concentration-dependently. But magnoflorine had less or no effect. In addition, sinomenine abolished dysrhythmias under Ca^{2+} overload conditions. In vascular experiments, sinomenine greatly dilated NE-induced vasoconstriction in 10-weeks old rats, but the vasodilatation became weaker age-dependently. SCR showed almost the similar behaviors. On the other hand, Mokuboito constantly produced the vasodilatation in all aged rats. Thus, Mokuboito as a mixture exhibit the active cardiotonic and cardioprotective actions, independent of advance in ages. Additional prescription to the standard recommended therapy for chronic heart failure may expect to improve clinical manifestations as a complemental and alternative medicine.

Keywords: Cardiotonic Action; Complemental and Alternative Medicine; Mokuboito; Phytochemicals; *Sinomeni Caulis* et Rhizoma; Sinomenine; Vasodilatation

Introduction

Mokuboito (Formula aristolochiae), a kind of Kampo formulations (Japanese herbal medicine), has been used clinically for centuries in Japan. Mokuboito has been traditionally used as an indication for the patients with the symptoms including wheezing, dark complexion and deep tight pulse. It has been reported that Mokuboito improves heart failure symptoms accompanied by the decline of plasma Brain Natriuretic Peptide (BNP) concentration, and reduces the rank of NYHA classification [1]. The main ingredient (herb) of Mokuboito is *Sinomeni Caulis* et Rhizoma (SCR) containing the phytochemicals such as sinomenine, tetrandrine and magnoflorine, and has been applied for disturbance of body fluids and Rheumatic diseases as a pain killer [2,3].

Chronic heart failure occurs more frequently in elderly persons. Advance with age produces various physiological and pathological deleterious changes such as plaque formation in vascular systems. Simultaneously, the age-dependent alterations of receptors, ion channels and cellular signal transduction pathways cause in the endothelium and smooth muscle cells. As a result, the pharmacological effects of the drugs on cardiovascular system may be necessarily modulated along with ageing. The endothelium-dependent relaxation has been reported to be attenuated in aged rat aorta [4,5]. In direct in situ measurement of Nitric Oxide (NO) in rat aorta, the NO-release diminishes further in aged rats. The relaxation induced by ACh is impaired in the aortic rings obtained from old female rats [6]. NO-synthesis inhibitor (L-NAME) attenuates the age-dependent ACh-induced vasodilatation. Thus, the NO-dependent vasodilatation decreases with ageing.

Satoh [7] has already shown that Mokuboito, SCR, and sinomenine (the main phytochemical), have the electropharmaco-

logical actions and exert the electrophysiological effects on cardiomyocytes. As well, Mokuboito and the ingredients dilate rat aorta, due to not only endothelium-dependent mechanisms related with the activations of NO and PGI_2, but also endothelium-independent mechanisms related with the inhibitions of Ca^{2+} channel, Ca^{2+}-activated K^+ channel and PK-C activities [8,9]. Thus, Mokuboito regulates the tone of blood vessels and adjusts the blood pressure and flow, possibly resulting in the improvement of the cardiac functions under the chronic heart failure [1,10].

Now the characteristics of cardiovascular pharmacology of Mokuboito, the ingredients and the phytochemicals are examined, and their ameliorative pharmacological actions on chronic heart failure are discussed.

Mokuboito (Formula aristolochiae)

Mokuboito is composed of four herbal drugs

1. SCR (or *Sinomenium acutum* Rehder et Wilson)
2. Cinnamoni cortex [11]
3. Ginseng radix [12]
4. Gypsum

SCR is comprised of some phytochemicals such as tetrandrine, sinomenine and magnoflorine [13]. SCR has been well-known as a modulator of body's fluid, and has been used for fluid's disturbance and Rheumatic diseases [2,14]. Sinomenine, a phytochemical of SCR, is an alkaloid [15], and exhibits anti-inflammatory [16] and immuno-modulative actions [3]. Accordingly, Mokuboito has usually been also used for clinical treatment of Rheumatoid arthritis [1,17].

Cardiovascular pharmacological experiments for Mokuboito have demonstrated the effective actions for (a) hypertension [18,19] and (b) proximal supra ventricular tachycardia [20-22]. Furthermore, it possesses (c) an antioxidant action [23] and (d) an anti-thrombogenic action [24]. Most recent studies demonstrate that application of Mokuboito is so effective for chronic heart failure [25,26]. Advance in ages alters the physiological and pharmacological functions. Mokuboito has been found to cause no or less age-related pharmacological effect, because of the multifunction and the complicated interactions induced by mixed drug [27]. The multiple pharmacological actions would compensate and maintain the lack of some responses with ageing. The actions induced by Mokuboito are exhibited as a net of the complicated interactions among numerous ingredients and phytochemicals. In our laboratory, Mokuboito possesses and maintains a more potent vasodilating action as compared with the ingredient and phytochemical alone [25].

Electropharmacology of Mokuboito

On the action potential of guinea pig hearts

In guinea pig ventricular cardiomyocytes, the current-clamp experiments were performed to examine the modulation of the action potential configuration. The modified Tyrode solution was used: NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.3, glucose 5.0, and HEPES 5.0 (in mM). The herbal medicines were dissolved with DMSO, and bath solutions with the desired concentrations were superfused at 36^0C.

Mokuboito affected the action potential configurations. The maximum rate of depolarization (V_{max}) and the amplitude (APA) decreased, and the Action Potential Duration (APD) increased, as shown in Figure 1A & B. SCR (1 mg/ml) decreased the V_{max}. In the phytochemicals contained in SCR, sinomenine (300 μM) caused the similar effects; the inhibitions of APA and V_{max}, and the prolongation of APD. Tetrandrine at 30 and 100 μM prolonged the 75% repolarization of APD (APD_{75}) by 24.8 ± 2.7 % (n = 7, P<0.01) and by 41.7 ± 2.9 % (n = 7, P<0.001), respectively. The APA was reduced by 16.3 ± 2.7 % (n = 6, P<0.05) at 100 μM tetrandrine. On the other hand, magnoflorine (another phytochemical) just at 1 mM also prolonged APD_{75} by 16.7 ± 4.3 % (n = 5, P<0.05), but failed to affect other action potential parameters. These effects on the action potential configuration are roughly summarized in Figure 2.

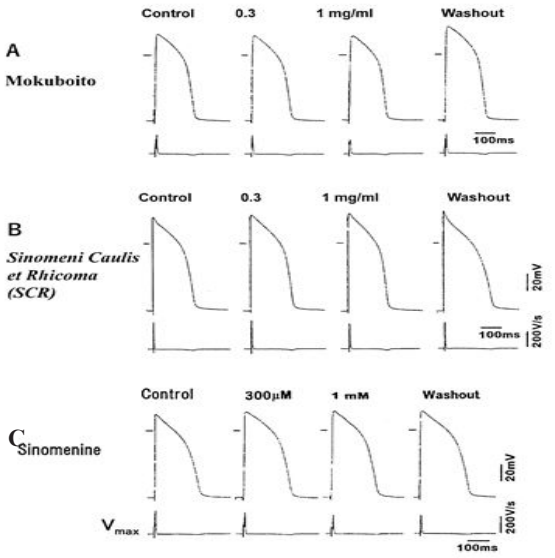

Figure 1: A: Effects of Mokuboito, B: Sinomeni caulis et rhizoma (SCR) and C: sinomenine on the action potentials in guinea pig ventricular cardiomyocyte. Upper panel: Action potential configurations. Lower panel: Vmax. Short lines at the left of action potential recordings represent zero mV level.

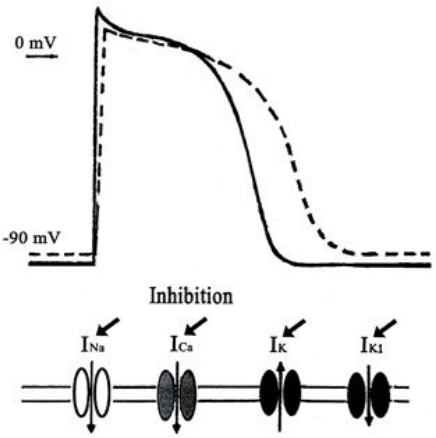

Figure 2: The superimposed action potentials in the absence and the presence of Mokuboito. The electrophysiological effects are summarized. The horizontal line indicates zero mV.

Thus, major action of these drugs is the V_{max} inhibition. Mokuboito has the slight effects on the other action potential parameters, and other ingredients also produce almost the similar responses. Simultaneously, the APA was markedly inhibited. Both the V_{max} and APA inhibitions are due to the inhibitory action on the fast Na^+ current (I_{Na}), indicative of a class I antiarrhythmic action. Both also lead to an inhibition in the conduction velocity and a suppression of excitability. As well, the I_{Na} inhibition leads to greater decline of the cellular Ca^{2+} concentration ($[Ca^2]_i$). Then, the cellular Ca^{2+} overload under heart diseases would be reduced.

To examine the effects on the action potential configuration of papillary muscles (multicellular preparations) in guinea pig heart, conventional microelectrode experiments were carried out. The preparations were stimulated at 1 Hz. Mokuboito at 1 mg/ml decreased the V_{max} by 13.6 ± 2.4 % (n = 6, P<0.05). The other action potential parameters were unaffected. SCR (0.3 and 1 mg/ml) had no or less effects on the action potential configuration. At 1 mg/ml, only the V_{max} decreased by 10.0 ± 2.1 % (n = 5, P<0.05). The 50% and 90% repolarizations of APD (APD_{50} and APD_{90}) at 1 mg/ml decreased by 5.4 ± 3.6 % (n = 6) and 9.2 ± 2.8 % (n = 6), respectively, but not significantly. The APA was also tended to decrease, and the resting potential (RP) was unaffected. A washout for 15-20 min recovered to almost 90 % of control values.

Sinomenine (100 μM to 1 mM) showed the inhibitory effects on the action potentials of papillary muscles Figure 1C. The V_{max} was inhibited by 20.0 ± 2.4 % (n = 6, P<0.05) at 300 μM and by 32.1 ± 3.3% (n = 6, P<0.01) at 1 mM. Sinomenine (1 mM) also decreased the APA, but did not cause to significant extent (by 0.9 ± 2.1 %, n = 5). Sinomenine at 300 μM increased the APD_{75} by 24.9 ± 3.5 % (n = 6, P<0.05) and at 1 mM by 43.7 ± 3.3 % (n = 6, P<0.001). The RP was unaffected. Tetrandrine at 10 to 300 μM caused the concentration-dependent inhibitory effects on the action potentials. The APA decreased by 18.3 ± 2.2 % (n = 7, P<0.05) at 300 μM, but not at the lower concentrations. Simultaneously the V_{max} significantly decreased at higher concentrations than 30 μM; by 35.8 ± 2.4 % (n = 7, P<0.001) at 300 μM. Tetrandrine tended to decrease both the APD_{50} and the APD_{90}. The RP was also unaffected. Magnoflorine had less or no effect on any action potential parameters.

As a result, Mokuboito and the ingredients at even acute administrations exert the potent electropharmacological actions and produce the cardioprotections. However, there are some different actions in myocyte and multicellular preparations. Significant effects on the APD are not caused (but are tended to shorten) in the papillary muscles (multicellular preparations), whereas the APD is prolonged in the cardiomyocytes. In both specimens, the great V_{max} inhibition is produced. Mokuboito as a mixture should exhibit the APD shortening due to the V_{max} inhibition, but the responses are controversial. The phenomena are consistent with our previous reports [28,29]. Several possibilities for this discrepancy may exist. One is no intercellular space in the cardiomyocytes, and thereby the physiological relationship between cell and cell (including such as a gap junction) is absent [30-32]. The existence of intercellular space would be a key for whole heart to maintain the functions. Another may be a difference of sensitivity to the drugs. The effective responses were produced at relatively lower concentrations in the myocytes than multicellular preparations. Using higher concentrations to the myocytes, there might be some limitations for the occurrence of cell damages.

Interestingly, application of 300 μM sinomenine suppressed and completely abolished the abnormal action potentials (the dysrhythmias) induced by cellular Ca^{2+} overload Figure 3. In high extracellular Ca^{2+} solution (5.4 mM), the abnormal action potentials elicited by triggered activities occurred irregularly, despite the fact that the papillary muscle of guinea pig heart was constantly stimulated at 1 Hz. At even acute administrations, thus, these drugs greatly exhibit the electropharmacological actions and cardioprotections. Therefore, Mokuboito and its ingredients may improve the cardiac functions such as the antiarrhythmic and cardiotonic actions under chronic heart failure.

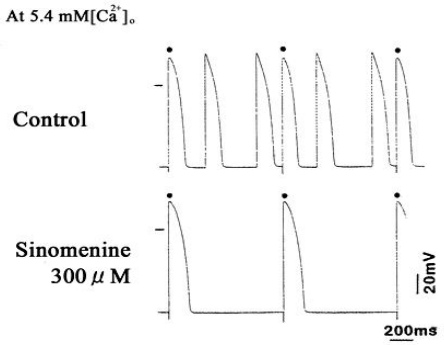

Figure 3: Antiarrhythmic action of sinomenine. Dysrhythmias occurred at 5.4 mM $[Ca^{2+}]_o$. Dots above the action potential recordings are represented the regular rhythms induced by 1 Hz stimulation. The horizontal line indicates zero mV.

On the ionic channel currents of cardiomyocytes

In the voltage-clamp experiments using guinea pig ventricular cardiomyocytes, Figure 4 shows the modulation by Mokuboito of the ionic currents. Test pulses were applied from -120 to 60 mV from a holding potential of -30 mV. The average capacitance was 84.1 ± 2.4 pF (n = 23). Mokuboito at 1 mg/ml enhanced the L-type Ca^{2+} current (I_{Ca}) at 0 mV by 157.3 ± 5.5% (n = 6, P<0.001) and the delayed rectifier K^+ current (I_{Krec}) at 60 mV by 262.1% ± 7.3% (n = 6, P<0.001), and inhibited the inwardly rectifying K^+ current (I_{K1}) at -120 mV by 9.7 ± 2.1% (n = 6, P<0.05). The enhancement is responsible for the stimulation of β-adrenergic receptor [33]. SCR at 1 mg/ml had almost the similar responses; the increases in I_{Ca} current at 30 mV by 158.3 ± 7.1% (n = 6, P<0.01) and I_{Krec} at 60 mV by 257.6 ± 7.1% (n = 6, P<0.01), and the inhibition of I_{K1} at -120 mV by 10.3 ± 2.4% (n = 6, P<0.05) Figure 5.

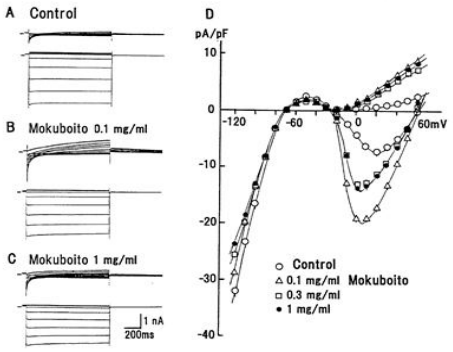

Figure 4: Effect of Mokuboito on the ionic channel current. **A:** Current traces in control. **B-C**: Current traces in Mokuboito 0.1 and 1 mg/ml. Test pulses are applied from 60 mV to -120 mV. The holding potential is -30 mV. The horizontal line indicates zero current level. **D:** Current-voltage relationships as the current density for the Ca^{2+} current, the delayed rectified K^+ current and the inwardly rectifying K^+ current. Symbols used are control (open circles), 0.1 mg/ml (triangles), 0.3 mg/ml (squares) and 1 mg/ml (filled circles) of Mokuboito.

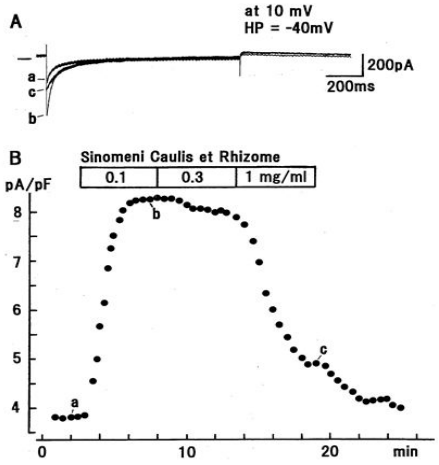

Figure 5: Effect of *Sinomeni caulis* et rhizoma (SCR) on the L-type Ca^{2+} channel current. A: Superimposed current traces. B: Time-dependent changes in the current traces in SCR 0.1, 0.3 and 1 mg/ml. Test pulses are applied to 10 mV. The holding potential is -40 mV. The horizontal line indicates zero current level.

Sinomenine (a phytochemical) at 300 μM inhibited the I_{Ca} at 0 mV by 12.3 ± 2.5% (n = 6, P<0.05), and at 1 mM by 18.2 ± 2.1 % (n = 6, P<0.05). Similarly, at 1 mM, the I_{Krec} at 60 mV was inhibited by 16.2 ± 2.6 % (n = 6, P<0.05), and the I_{K1} at –120 mV was inhibited by 47.2 ± 3.8 % (n = 6, P<0.01). Tetrandrine (30-100 μM) also inhibited markedly the ionic currents concentration-dependently [34]. The I_{Ca} at 10 mV decreased by 28.1 ± 2.5 % (n = 6, P<0.05) at 100 μM and by 55.9 ± 2.7 % (n = 6, P<0.01) at 300 μM. Simultaneously, the I_{Krec} at 60 mV also decreased by 8.6 ± 1.4 % (n = 6, P>0.05) at 100 μM and by 13.2 ± 2.5 % (n = 6, P<0.05) at 300 μM, but not significantly at lower concentrations. Liu et al. [35] have shown that tetrandrine inhibits both L- and T-type Ca^{2+} channel currents. The I_{Ca} inhibitions strongly decline the $[Ca^{2+}]_i$. The V_{max} inhibition means the depression of the I_{Na} current, leading to the $[Ca^{2+}]_i$ reduction and also generally the APD shortening. But the I_{Krec} decrease might greatly lead to the APD prolongation. Magnoflorine had less or no effect on the action potential parameters, and all the ionic currents did not also affect significantly; at 1 mM, the I_{Ca} at 10 mV by -7.8 ± 2.8 % (n = 5), the I_{Krec} at 60 mV by -5.6 ± 2.2 % (n = 5), and the I_{K1} at -120 mV by -2.9 ± 2.0 % (n = 5). At 20 min after washout, these responses were recovered to almost the control.

Accordingly, the cardiac electropharmacological actions of both Mokuboito and SCR are the I_{Ca} and I_{Krec} enhancements, and the I_{K1} inhibition Figure 2. However, the ingredients (the phytochemicals) inhibit all the currents (the I_{Ca}, I_{Krec} and I_{K1}). At even acute administrations, thus, Mokuboito and the ingredients exert the marked electropharmacological actions, and thereby produce the cardiotonic and cardioprotective actions. Therefore, Mokuboito may improve the cardiac functions under chronic heart failure.

Vascular pharmacology of Mokuboito

The comparative vasodilating actions among Mokuboito, SCR and sinomenine were examined using different aged rats. The ring strips of rat aorta were pretreated with 5 μM NE. The modified Krebs-Henseleit solution was, in mM: 118 NaCl, 4.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 27.2 $NaHCO_3$, 0.03 ethylne glycol-O, O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), and 1.8 $CaCl_2$. The drugs used were dissolved with DMSO. The chamber solution was kept at 36.5)^{0}C and oxygenated with 95% O_2 and 5% CO_2.

Mokuboito exerted the vasodilatation in all aged rats, but inversely at low concentrations exhibited the vasoconstriction in higher aged rats Figure 6. Mokuboito at 3 mg/ml dilated the NE-induced constriction by 98.9 ± 2.8 % (n = 7, P<0.01) in 10-weeks and by 97.5 ± 13.5% (n = 6, P<0.01) in 65-weeks old rats. Sinomenine at 100 μM dilated the vasoconstriction by 68.8 ± 5.2% (n = 6, P<0.01) in 10-weeks old rats, but only by 18.6 ± 1.5% (n = 6, P<0.01) in 65-weeks old rats. SCR at 0.3-3 mg/ml showed only vasodilatations in 10- and 35-weeks old rats, and at 3 mg/ml by 96.7 ± 4.8% (n = 7, P<0.01) in 10-weeks old rats. In 65-weeks old rats, however, SCR at low concentrations (0.03-0.3 mg/ml) also constricted the aorta. The vasodilatation induced by 3 mg/ml SCR was attenuated to 46.0 ± 5.7% (n = 6, P<0.01). The dilatation is not only due to the endothelium-dependent mechanisms (due to the activations of NO and PGI_2), but also due to the modulation of Ca^{2+} channel, β-adrenoceptor and PK-C Activity Table 1 [8,9]. Thus, Mokuboito regulates the tone of blood vessels and adjusts the blood pressure and flow.

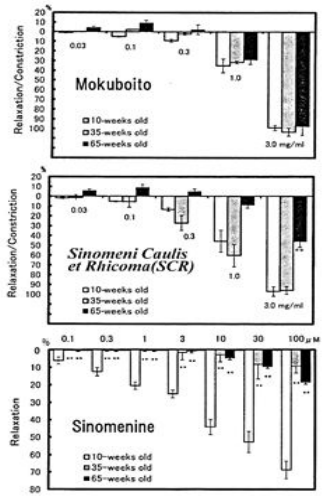

Figure 6: Age-related vasodilatations induced by Mokuboito, *Sinomeni caulis* et rhizoma (SCR) and sinomenine. The upper part from zero line on each panel indicates the contraction, and the lower indicates the relaxation. 10-weeks old rats are represented by white column, 35-weeks old rats by grey column and 65-weeks old rats by black column. Values (%) represent mean ± S.E.M. **: P<0.01, with respect to the values of 10-weeks old rats.

	NOS stimulation	**PGI_2 synthesis stimulation**	**Ca^{2+} channel inhibition**	PK-C inhibition	**α-stimulation**	**β-stimulation**
Mokuboito	+	+	+	+	+	+
Sinomeni Caulis et Rhizoma I (SCR)	+	+	+	+	+	±
Sinomenine	+	+	+	+	-	+

Table 1: Vascular pharmacology of Mokuboito and the ingredients.

Sinomenine produces the endothelium-dependent vasorelaxation via NO and PGI_2 releasing from endothelium. NOS activation and PGI_2 release are caused via the $[Ca^{2+}]_i$ elevation in endothelium cells [5,6,36]. Thus, sinomenine would increase $[Ca^{2+}]_i$ in endothelium cells and then, activate NOS activity and PGI_2 release, as reported previously [30]. For the endothelium-independent mechanisms, sinomenine causes the great vasorelaxation via modulation of Ca^{2+} channels and PK-C activity in vascular smooth muscle cells. The contraction/relaxation systems are regulated by the ion channels and the receptors, and also by the intracellular signal conductions [37-40]. Actually, sinomenine adjusts the contraction/relaxation systems mediated through the modulation of Ca^{2+} and Na^+ channels, delayed rectifier K^+ channels, and Ca^{2+}-activated K^+ (K_{Ca}) channel, accompanied with the activation of PK-C [9]. In addition, the β-adrenoceptor stimulation induced by Mokuboito and SCR also potentiates the dilatation of vascular smooth muscle cells.

Advance in ages causes numerous physiological and pathological deleterious changes such as plaque formation in vascular systems. Simultaneously, the age-dependent alterations of ion channels and signal transduction pathways may be caused in both the endothelium and the smooth muscle cells. It has been reported that the inhibitory effect of a Ca^{2+} channel blocker on the NE-induced constriction decreases along with ageing of 3- to 10-weeks old, whereas it increases with ageing of 10- to 40-weeks old. The endothelium-dependent relaxation is reduced with ageing [4]. L-NAME (a NO-synthesis inhibitor) attenuates the ACh-induced vasodilatation with ageing, indicative of the age-related reduction of the NO-dependent vasodilatation [8,9]. Also, the vasorelaxation mediated by β-adrenoceptor stimulation decreases with ageing in rat aorta and various vascular beds of many species [41,42]. In our

studies, Mokuboito, SCR and sinomenine by themselves had the potent vasodilating actions. SCR- and sinomenine-induced vasodilatations decreased along with ageing, whereas Mokuboito as a mixture had less or no effect on the age-dependent attenuation in any aged rats [27]. Herbal drugs (mixter-mixter) including plenty ingredients maintain more effective actions for higher aged rats [30,43]. Chronic heart failure occurs more frequently in elderly persons. Therefore, the marked vasodilatation supports the cardiotonic and cardioprotective actions, and contributes to the reductions both after load and preload under the chronic heart failure.

Cardiovascular effects on chronic heart failure

The clinical therapeutic methods of chronic heart failure are (a) reducing the workload of heart, (b) protection of cardiomyocytes and (c) restriction and control of waters and sodium. The therapeutic effects of Mokuboito are produced by the intricate mechanisms induced by the large number of herbs (ingredients) and phytochemicals containing in Mokuboito. It has been generally considered that a single dose of Kampo medicine does not produce any pharmacological activities, but the repeated doses for long period of administration produce ameliorative effects. However, our studies indicate that even acute single administration can produce the marked actions on the ionic channel currents and the action potentials. In cardiac pharmacological experiments, Mokuboito increases the sinus rate and enhances the developed tension in isolated canine heart [44]. In our voltage-clamp experiments, Mokuboito and SCR markedly enhanced the I_{Ca} and the I_{Krec} in ventricular cardiomyocytes, and also in isolated sino-atrial nodal cells. Possibly these enhancements are produced by the stimulation of β-adrenoceptors on cardiac cell membrane [33]. Mokuboito contains further Cinnamomi cortex and Gypsum fibrosum which may release cathecholamines [13].

Mokuboito and the ingredients produce the marked effects on the action potential and the underlying ionic channel currents, resulting in clinical modulation of Electrocardiogram (ECG). As a kind of phytochemicals, tetrandrine has been reported to possess the cardiac electropharmacological actions [45,46]. Tetrandrine inhibits the voltage-dependent Ca^{2+} channel on cardiac cell membrane, and depresses the heart rate and the contractility [34,35,47]. Also, sinomenine has been found to decline the blood pressure [48]. Sinomenine and magnoflorine also exert anti-allergic and anti-histamine actions [16,49-51].

Actually, sinomenine blocked the dysrhythmias induced by triggered activities under the Ca^{2+} overload. Tetrandrine also exhibits antiarrhythmic actions and protects ischemic-reperfusion injury [52,53]. Furthermore, the I_{K1} inhibition induced by Mokuboito and the ingredients depolarizes the membrane potential and stabilizes the cell membrane, which plays a role for antiarrhythmic actions. The RP is not modified by Mokuboito and the ingredients. The change in RP may not have been apparent because the RP (at around -80 mV) is quite close to the E_K of cardiac cells. Anyways, tetrandrine and sinomenine possess potent cardioprotective actions on heart cells, due to the active modulations of the ionic channel currents (such as I_{Ca}, I_{Krec}, I_{K1} and I_{Na}) under the disease conditions.

Under the ischemia and heart failure, the cellular Ca^{2+} overload of heart muscles has been well known to elicit some arrhythmias and dysfunctions [28,29]. Sinomenine possesses the suppressive actions for dysrhythmias. The regulation of Ca^{2+} influx reduces the Ca^{2+} overload in cardiomyocytes and produces the cellular protection [54]. Therefore, sinomenine restrains the cell damages of heart muscles by means of the decline of $[Ca^{2+}]_i$, and as a result, can exert the great cardioprotective actions. The cardioprotective actions of sinomenine on acute myocardial ischemia have already been demonstrated. Reperfusion injury is induced by ligating the rat left coronary artery for 15 min and reopening [52]. Under the conditions, sinomenine inhibits the incidence of arrhythmias and reduces $[Ca^{2+}]_i$ concentration [48,55] quite consistent with our results.

Not only Mokuboito and SCR (comprised of multiple phytochemicals), but also sinomenine (a phytochemical) exhibit the strong vasodilating actions. The vascular pharmacological effects of just single phytochemical are greatly attenuated in advance with ageing. On the other hand, Mokuboito and SCR decrease the age-dependent attenuation of vasodilating action. Sinomenine has various vasodilating mechanisms above mentioned, contributing to the regulation of the preload and afterload of cardiovascular systems. The vasodilating action is one of the great useful tools for heart failure. Therefore, sinomenine-induced vasodilating action can improve cardiac functions by the reduction of workload under heart failure. Moreover, Mokuboito (a mixture) maintains the pharmacological actions with ageing. The plenty ingredients and phytochemicals contribute to the Mokuboito-induced vasodilatation via their interactions. Increasing the number of herbal ingredients (and phytochemicals) can maintain the ameliorative effects in elder rats, presumably due to the compensation each by each of the decay of pharmacological sensitivity. For elder persons, therefore, the mixture drugs comprised of many herbs (ingredients) such as a Kampo medicine exert more effective actions [56,57].

On the other hand, main electropharmacological effect of Mokuboito and SCR is the I_{Ca} enhancement, but that of phytochemicals such as sinomenine and tetrandrine is the I_{Ca} inhibition [56]. The interactions among the ingredients and phytochemicals play an important role for the pharmacological actions of herbal medicines. Therefore, the complex interactions produce more effective pharmacological actions of Mokuboito, according with less or no age-dependent alterations. Addition of Gypsum exaggerates the vasodilatation in elder rats.

Under the Ca^{2+} overload conditions such as ischemia and heart failure, Mokuboito exhibits the cardiotonic and cardiopro-

tective actions and the vasodilatation. Clinical application of Mokuboito decreases plasma BNP concentration from approximately 800 to 200 pg/ml significantly and reduces the afterload of right heart [25,26,58,59]. Mokuboito has been known effective against hypertension [18-20]. Mokuboito-induced ameliorative actions have also been observed in our experiments. The vasodilating mechanisms would be due to the inhibition of Ca^{2+} channel and the modulation of excitation-contraction coupling, possibly mediated through many cellular signal transductions. The pharmacokinetics and tissue distribution of sinomenine in rats have been reported [55,60]. Sinomenine achieves high bioavailability (about 80%) by oral administration of 90 mg/kg. At 45min later, sinomenine is found widely in internal organs such as kidney, liver, lung, spleen, heart, brain and testis. Sinomenine is metabolized and eliminated by kidney and liver. T_{max} is 39.5 ± 8.49 min, C_{max} is 13.89 ± 4.29 μg/ml, $T_{1/2A\ phase}$ is 61.28 ± 53.62 min, AUC_{0-t} is 2331.53 ± 1172.77 μg-min/ml, and C_L is 42.95 ± 14.4 ml/min per kg.

Clinically, therefore, when the symptoms fail to be well improved by standard recommended therapies, addition of Mokuboito would be much expected to improve the symptoms. Moreover, when the thrapeutic limitation by several side effects induced by western medicines exist, Kampo medicines such as Goreisan (Pulvis quinque-hystericorum) and Saireito (Formula hoelen et bupleuri) as well as Mokuboito may also be more effective for chronic heart failure.

Conclusion

The pharmacological characteristics of Mokuboito are mainly the cardiotonic and the protective actions, and are also the great vasodilating action. These profitable actions are responsible for the modulation of not only ionic channel currents, β-adrenoceptor receptor and PK-C, but also endothelial cells and vascular muscle. Furthermore, SCR possesses a diuretic action, and can reduce both the preload and the afterload. SCR has been clinically applied for disturbance of body fluids and Rheumatic diseases [12]. Along with ageing, Mokuboito does not decrease and maintain the vasodilating action, although sinomenine (phytochemical) strongly declines it. Moreover, addition of Gypsum produces stronger vasodilatation in elder rats.

The age-related alterations would be responsible for physiological and anatomical reductions such as receptors, ionic channels and cellular signal transductions. Also, advanced ages elicit the decline in the sensitivity to drugs. However, Kampo medicine (mixter-mixter) maintains the pharmacological effects with ageing (but not fully). From our studies, Kampo medicine keeps the balance, and can produce either vasoconstriction or vasodilatation, according to the pathophysiological conditions [i.e., the initial (previous) tension of vessels]. In final, additional prescription of Mokuboito to the standard recommended therapy for chronic heart failure may expect to improve clinical manifestations as a complemental and alternative medicine. Extensive experiments are needed to elucidate the unknown and detailed mechanisms for pharmacology of Mokuboito.

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