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Influences of ginsenosides Rb_1, Rb_2, and Rb_3 on electric and contractile activities of normal and damaged cultured myocadiocytes

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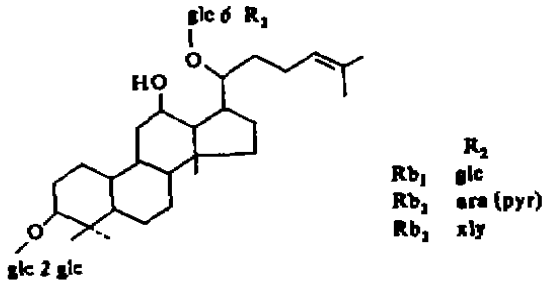
ABSTRACT Free radical damage to the cultured myocardiocytes of Wistar rat was induced by adding xanthine 0.42 mmol · L^{-1} and xanthine oxidase 5.3 nmol · L^{-1} to the culture medium. 30 $\mu g \cdot ml^{-1}$ of Rb_1, Rb_2 or Rb_3 extracted from the leaf and stem of *Panax ginseng* C A Meyer restored the action potentials (AP) of free radical damaged cells to normal, indicating their antioxidative action. On normal myocardial cells, Rb_1, Rb_2, Rb_3 20 $\mu g \cdot ml^{-1}$ inhibited the AP and spontaneous contractility, (suggesting the Ca channel blockade action of panaxadiol saponins). The degrees of their inhibitory effects were found to be $Rb_1 > Rb_2 > Rb_3$. Their effects against X-XO were basically the same.

KEY WORDS cultured cells; free radicals; action potentials; ginseng; saponins; myocardium

The active components of ginseng are ginsenosides, which have widespread influence on immunoregulation[1], enzyme activity[2], antioxidative damage[3], panaxadiol saponins (PDS)[4], and *Panax notoginseng* saponin Rb_1[5] are Ca channel blockers. This work compared the PDS monomers Rb_1, Rb_2, and Rb_3 from the view-point of their antioxidative actions and Ca channel blockade actions, taking the electric and contractile activities of cultured myocardiocytes as indices.

MATERIALS AND METHODS

Drugs Dulbecco's modified Eagle medium (DMEM) (Life Technologies, USA); fetal bovine serum (FBS) (Department of Animals, Norman Bethune University of Medical Sciences). Xanthine (X) (Dong Hai Pharmaceutical Factory, Shanghai); xanthine oxidase (XO) (Shanghai Institute of Biochemistry, Chinese Academy of Sciences). Rb_1, Rb_2, Rb_3 (purity >95%) were extracted from the leaf and stem of *Panax ginseng* C A Meyer by the Department of Organic Chemistry in our university, who first extracted the total ginsenosides in 1982[6]. Then, the saponins were separated into 2 groups — panaxadiol saponins (PDS) and panaxatriol saponins. Rb_1, Rb_2, and Rb_3 are monomers in the PDS group. All of the ginsenosides are tetracyclic triterpenoid saponins.



Ginsenosides

Myocardiocyte culture Myocardiocytes were cultured with routine culture medium, consisting of 80% DMEM and 20% FBS[7]. Recording of action potentials (AP) and counting of myocardiocyte clusters were made in the same way as in our previous work[3,7].

Rb_1, Rb_2 or Rb_3 0.1 ml (2 mg · ml^{-1}) was added into 10 ml culture medium separately to make final concentrations at 20 $\mu g \cdot ml^{-1}$.

In the antioxidative experiments, xanthine 0.42

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mmol · L^{-1} plus xanthine oxidase 5.3 nmol · L^{-1} (X–XO), or X–XO plus Rb_1, Rb_2, or Rb_3 30 μg · ml^{-1} were added to the culture medium 16 h previously.

RESULTS

Effects on spontaneous contractility When the myocardiocytes were cultured for 4 d, before and after adding PDS saponins 125, 250, 500, 1000, or 2000 μg · ml^{-1} to the medium, the numbers of beating clusters were counted and plotted against the lg concentrations of PDS (Fig 1). The slops of the 3 lines indicated the inhibitory effects on the beating clusters: $Rb_1 < Rb_2 < Rb_3$.

Effects on AP On 4–7 d of culture, the dosage–dependent effect of Rb_1 on the AP was examined. Then the concentration of 20 μg · ml^{-1} was selected to compare the effects of Rb_1, Rb_2, and Rb_3. Immediately after adding any of the 3 saponins, all of the action potential amplitude (APA), overshoot (OS), threshold (TP), and maximal rate of depolarization (V_{max}) decreased significantly without variation in maximal diastolic potential (MDP). APA, OS, V_{max}, and APD differed significantly (t–test) among the 3 saponin groups: $Rb_1 >$ $Rb_2 >$ Rb_3 in efficacy. Rb_1 was taken as a representative monomer to test the effect of washing. Using nimodipine as the positive calcium channel blocker. Raising $[Ca^{2+}]_o$ turned all the

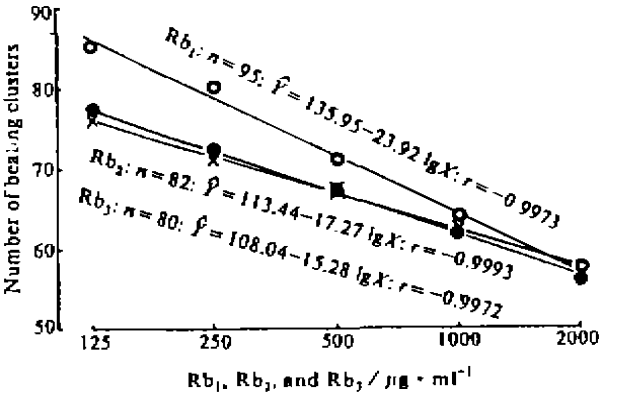

Fig 1. Relationship between number of beating clusters and concentrations of Rb_1 (○), Rb_2 (●), and Rb_3 (×). n = number of beating clusters before exposure.

Tab 1. Dosage–dependent action potential inhibit action of Rb_1 (upper part) and comparison of the action potential inhibit actions of Rb_1, Rb_2, and Rb_3 on cultured myocardiocytes (lower part). $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control; $^{-}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* Rb_1 5 (upper part) 20 (lower part) μg · ml^{-1}; $^{\S}P>0.05$, $^{\S\S}P<0.05$, $^{\S\S\S}P<0.01$ *vs* Rb_2; Rb_1, Rb_2, Rb_3 (20 μg · ml^{-1}); Ca^{2+} (40 μg · ml^{-1}); Nimodipine (0.2 μg · ml^{-1}).

Rx / μg · ml^{-1}	n	APA / mV	OS / mV	MDP / −mV	V_{max} / V · s^{-1}	APD_{50} / ms	APD_{90} / ms	F / bpm
Control	27	65 ± 5	21 ± 4	42 ± 7	15 ± 3	80 ± 5	128 ± 11	198 ± 11
Rb_1 5	28	60 ± 8*	18 ± 5**	42 ± 7*	12 ± 4***	80 ± 7*	128 ± 9*	200 ± 14*
Rb_1 10	31	57 ± 7***++	16 ± 7***+	41 ± 6*−	7 ± 3***+++	78 ± 7*+	124 ± 9*+	209 ± 13***++
Rb_1 20	26	51 ± 6***+++	12 ± 7***+++	39 ± 7**	6 ± 3***+++	77 ± 8*+	124 ± 8*+	210 ± 17***++
Rb_1 40	25	44 ± 5***+++	9 ± 5***+++	35 ± 7***—	5 ± 4***+++	76 ± 6**++	121 ± 11**++	220 ± 16***+++
Rb_1 80	29	44 ± 6***+++	8 ± 5***+++	36 ± 7***—	5 ± 4***+++	78 ± 9*+	122 ± 8**+−	215 ± 17***+++
Control	27	64 ± 6	20 ± 8	45 ± 9	16 ± 6	82 ± 9	130 ± 8	168 ± 18
Rb_1 20	30	51 ± 7**	9 ± 6***	42 ± 6*	6 ± 5***	80 ± 14*	123 ± 10***	190 ± 22***
Wash out	27	62 ± 7+++	20 ± 4*+++	42 ± 7*+	16 ± 8*+++	83 ± 8*+	129 ± 9*++	192 ± 15*+
Rb_2 20	27	56 ± 7***++	13 ± 6***—	43 ± 8*+	9 ± 4***++	81 ± 12*+	125 ± 5***+	187 ± 20***−
Rb_3 20	27	61 ± 5***+++§§§	16 ± 5***+++§§	45 ± 7*+§	12 ± 4***+++§§	84 ± 11*+§§	127 ± 5***+§§	179 ± 20***+§
Rb_1 20 + Ca^{2+}	27	63 ± 7−−	21 ± 6*+++	42 ± 6*+	14 ± 6*+++	80 ± 6*−	122 ± 5***+	214 ± 19***+
Nimodipine	32	45 ± 3***	13 ± 4***	33 ± 7**	4 ± 4***	78 ± 6*	123 ± 7**	207 ± 18**

parameters back to normal (Tab 1 and Fig 2).

Antioxidative action During the period of 4–7 d explantation, the AP were recorded.

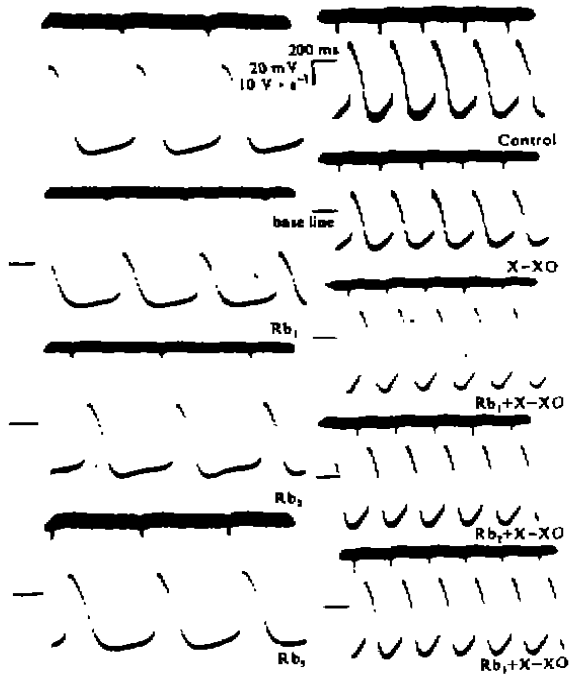

Fig 2. Typical action potentials of the effect against X–XO and the Ca channel inhibitory action of Rb_1, Rb_2, and Rb_3. Upper tracing: dV/dt; Lower tracing: action potential; Horizontal bar: 200 ms and base line: vertical bar: 20 mV.

Compared with the control group, all of the electrical parameters of X–XO group diminished significantly. Rb_1, Rb_2, and Rb_3 resumed the inhibited AP to normal with basically the same efficacy (Tab 2 and Fig 2).

DISCUSSION

The damage of the heart cell membrane induced by X–XO[8,9] led to a disorder in the ion distribution across the membrane and hence a decrease in MDP, which in turn reduced the rate and amplitude of 0 phase and thus decreased V_{max}, OS, APA, TP in this experiment. R–dioxyls form a kind of non–specific ionic channel whose permeability for Ca^{2+} is much higher than that of the other ions[10]. The consequent increase in $[Ca^{2+}]_i$ in the present experiment inactivated the Ca channel, and thus the APD was shortened. Therefore, Rb_1, Rb_2, and Rb_3 resumed the X–XO–inhibited AP, pointing to a membrane protecting action against X–XO and suggesting their antioxidative action against free radicals.

The AP of slow–response cultured myocardiocytes are determined by Ca current[11], so both the damage of cardiac cell membrane[12] and the blockade of Ca

Tab 2. Comparison of protective actions of Rb_1, Rb_2, and Rb_3 (30 $\mu g \cdot ml^{-1}$) on cultured myocardiocytes. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control. $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* X–X0 (0.42 mmol · L^{-1}–5.4 nmol · L^{-1}), $^{\S}P>0.05$, $^{\S\S}P<0.05$ *vs* Rb_1. $^{\|}P>0.05$ *vs* Rb_2.

Group	Control	Rb_1 + X–X0	Rb_2 + X–X0	Rb_3 + X–X0	X–X0
n	27	30	27	27	27
APA / mV	68 ± 7	66 ± 6^{+++}	67 ± 7$^{+++\S}$	76 ± 8$^{***+\S\|}$	49 ± 8***
OS / mV	20 ± 6	19 ± 4^{*+++}	20 ± 7$^{+++\S}$	19 ± 7$^{***\S\|}$	13 ± 9***
MDP / mV	47 ± 7	47 ± 7^{*+++}	47 ± 7$^{+++\S}$	48 ± 5$^{***\S\|}$	35 ± 8***
TP / mV	37 ± 8	37 ± 7^{*+++}	36 ± 7$^{+++\S}$	38 ± 8$^{***\S\|}$	23 ± 9***
V_{max} / V · s^{-1}	14 ± 2	12 ± 2^{**+}	14 ± 2*§§	14 ± 3$^{*\S\|}$	12 ± 3**
APD_{10} / ms	19 ± 2	18 ± 3^{**+++}	17 ± 3***§	17 ± 3$^{*+++\S\|}$	11 ± 2***
APD_{50} / ms	78 ± 8	82 ± 7^{**+++}	83 ± 8***§	76 ± 8$^{*+++\S\|}$	66 ± 12***
APD_{90} / ms	123 ± 9	120 ± 5***	120 ± 6$^{***+\S}$	120 ± 8$^{*+++\S\|}$	102 ± 7***
F / bpm	209 ± 20	216 ± 17^{**+}	219 ± 17^{***+}	224 ± 18$^{***+\S\|}$	277 ± 25***

channel[13] decreased the AP parameters. As the course of membrane damage is slow, while the Ca channel blockade is an immediate reaction, thus the immediate inhibition of APA, OS, TP, and V_{max} by Rb_1, Rb_2, and Rb_3 suggested their Ca channel blockade actions. In addition, the unchanged MDP excluded the damage to the cell membrane. As to the reason why APD did not show shortening, we thought it might be the net result of the primary Ca channel blockade and the secondary decrease in $[Ca^{2+}]_i$, which agonizes the blokade of Ca channel. The Rb_1–dosage–dependent variation of AP, reversion taking place immediately after washing out Rb or raising the $[Ca^{2+}]_o$, and the nimodipine control further supported the Ca channel blockade action. The contractile behavior was consistent with that of the AP, supporting the Ca channel blockade action of Rb_1, Rb_2, and Rb_3.

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人参皂甙 Rb_1, Rb_2 和 Rb_3 对正常与致损的培养心肌细胞电位与收缩活动的影响

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提要 向培养基中加黄嘌呤 0.42 mmol · L^{-1} 和黄嘌呤氧化酶 5.3 nmol · L^{-1} 诱发培养心肌细胞的自由基损伤. 自吉林人参茎叶提取的 Rb_1, Rb_2, Rb_3 30 μg · ml^{-1} 使自由基损伤心肌细胞动作电位(AP)恢复正常，表明其抗氧化损伤作用. Rb_1, Rb_2, Rb_3 20 μg · ml^{-1} 抑制正常培养心肌细胞 AP 及自发性搏动(提示钙通道阻滞作用)，其钙通道阻滞效力为 $Rb_1 > Rb_2 > Rb_3$,抗氧化作用基本相同.

关键词 培养的细胞; 自由基; 动作电位; 人参; 皂甙; 心肌