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Review

Cardiac toxicity of resibufogenin: electrophysiological evidence

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KEY WORDS bufanolides; digitalis; electrophysiology; arrhythmia; Purkinje fibers

ABSTRACT

Resibufogenin (RBG) is a single compound isolated from Chansu, a traditional Chinese medicine obtained from the skin venom gland of the toad. Formulations of Chansu have been widely applied in China, Japan, and other Asian countries for a long time and are currently used as alternative medicines. However, there have been several reports about the toxicity of Chansu and its medical formulations in the United States recently. As digitalis, RBG possesses both pharmacologic and toxicologic effects. According to our study results, RBG, one of major ingredient of Chansu, induced delayed afterdepolarization and triggered arrhythmias both in cardiac fiber *in vitro* and in beating heart *in vivo* at the high concentrations. The electrophysiologic toxic effects of RBG, the possible mechanism of toxicity, and treatment possibilities are discussed in the present review.

INTRODUCTION

Resibufogenin (RBG) is an isolated compound of Chansu, a traditional Chinese medicine obtained from the skin venom gland of the toad. Chansu is dried toad venom in English, and also called "toad cake" in China, "Senso" in Japan, and "Somso" in Korea[1-3]. Chansu is often found in traditional Chinese formulations of medicine, such as Jiuxin (kyushin in Japan), Yixin, Liushen wan, Laryngitis, Huoxin, and Shexiang baoxin wan[4]. These Chinese medications have long been widely applied in China, Japan, Korea, and other Asian countries and are currently used as alternative medicines. Presently, Chinese medicines are available without prescription in health food stores in the United States[5]. As an over-the-counter Chinese medicine, Chansu is used as a cardiotonic agent[6]. Several lines of studies using modern technology and methods demonstrated that Chansu has a series of pharmacologic functions. For example, the crude venom from the parotid gland of the *Bufo marinus* toad increased myocardial contractility, inhibited ouabain-sensitive Na^+, K^+-ATPase, and cross-reacted with various antidigoxin antibodies[7,8]. Another report[9] suggested that RBG, bufalin, and cinobufagin do not have a cumulative nature. There were reports in the United States recently, however, that toxicities were induced by these Chinese medicines containing Chansu[10-13]. Morb Mortal Wkly Rep[14] indicated that during 1993 February - 1995 May, the New York City Poison Control Center was informed about poisoning in five men, four of whom died after developing cardiac dysrhythmias. The purported substance contains bufadienolides, naturally occurring cardioactive steroids that have digoxin-like effects. The other report suggested that the purported substance was identical to Chansu[11]. RBG is a major chemical component of Chansu. According to our research, RBG induced delayed afterdepolarization (DAD) and triggered arrhythmias both in cardiac fiber *in vitro* and in heart *in vivo*. The present article reviews the electrophysiologic toxic effects of RBG, the possible mechanism of toxicity, including DAD and TA, and the possible treatment of RBG intoxication.

THE MAJOR CHEMICAL CONSTITUENT OF CHANSU

Chansu contains multiple biological active substances, dozens of which have been isolated. Some ex-

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amples are genin derivatives and alkaloids. The cardiac genin group includes resibufogenin ($C_{24}H_{32}O_4$), bufalin ($C_{24}H_{34}O_4$), bufotalin ($C_{26}H_{36}O_6$), cinobufagin ($C_{26}H_{34}O_6$), desacetylcinobufagin, telocinobufagin, gamabufotalin, arenobufagin ($C_{24}H_{32}O_6$), resibufogin ($C_{24}H_{30}O_3$), cinobufotalin ($C_{26}H_{34}O_7$), and bufotalinin and so on. The alkaloid group has bufotenine and bufotenidine. The structure of bufalin is similar to that of other digitalis glycosides[7]. RBG (3-hydroxy-14,15-epoxy-20,22-dienolide glycoside, $C_{24}H_{32}O_4$) is a cardiac glycoside and its chemical structure is similar to digitoxigenin[15-17] (Fig 1). Because toads have permeable skin, RBG is also found in high concentrations in the blood[18]. Previous reports indicated that RBG exhibited pharmacological actions, and electrophysiologic and toxicologic effects in both clinic and animal experiments. The pharmacologic activity of RBG in Chansu is discussed in the present review.

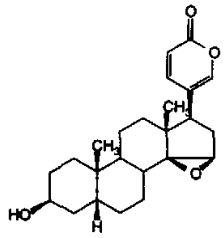
CONDENSED PHARMACOLOGIC FUNCTIONS OF RBG

Basically, RBG exhibits the following three major pharmacological effects: cardiotonic, vasopressor, and respiratory stimulator[6,10,19].

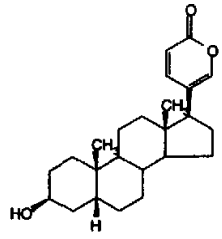
1) Cardiotonic effect: A number of animal experiments demonstrated that RBG increased ventricular contractile force by 34 % in rabbits, and 36 % in cats, 32 % or 50 % in adult mongrel dogs[20-22] (Tab 1). Just as digitalis does, RBG increases the contractility of cardiac muscle in a dose-dependent manner, a positive inotropic effect. This is why Chansu formulations are a cardiotonic in clinic.

2) Vasopressor action: Several reports have indicated that in the hemorrhanged animal model, there was a significant increase in mean system arterial pressure following the administration of RBG. The reason was thought to be due to an increase in cardiac output without a significant change in heart rates[20-24]. The vasopressor effect of RBG appeared to be predominantly due to its peripheral vasoconstrictor action, and partly due to its cardiotonic effect.

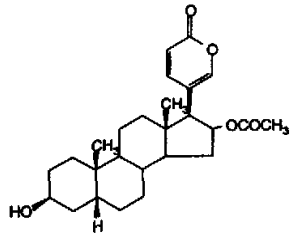
3) Effect on the respiratory center: Uniquely, RBG was demonstrated to be an efficacious respiratory stimulator[7,19,21,22]. In animal experiments, respiratory amplitude, tidal volume, and minute volume, were increased significantly by RBG. The mechanism of the effect on



Resibufogenin



Bufalin



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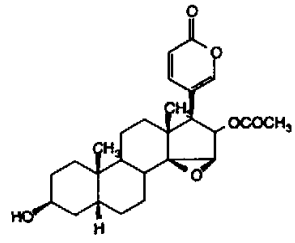
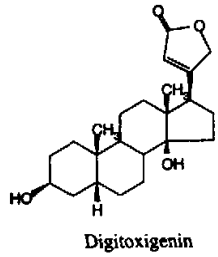


Cinobufagin



Digitoxigenin

Fig 1. The chemical structures of Chansu (cardiac genin derivatives) and digitoxigenin.

Tab 1. Effect of RBG (0.2 mg/kg, iv) on increasing percentage heart contractile force. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **control (100 %).**

Animals	*n*	Increasing percentage of heart contractile force/%				
		RBG for 1′	3′	5′	10′	30′
Rabbits	12	27.0±2.3[c]	34.0±3.0[c]	23.0±2.0[c]	25.0±1.9[c]	21.0±1.7[c]
Cats	4	13.0±1.5	36.0±3.3[c]	36.0±3.3[c]	27.0±2.5[b]	–
Dogs	4	14.0±0.8	32.0±2.8[c]	22.0±1.8[b]	26.0±2.4[b]	–

respiration was considered to be excitation of the respiratory center[21-23]. The excitatory effect of RBG on respiration was not abolished by the administration of procaine, suggesting that this effect was mediated through the central nervous system. Some results demonstrated that RBG was a central respiratory stimulant, the action of which was not inhibited after intravenous injection of procaine or the removal of the carotid sinus nerve and the ganglion nodosum, which differed from nicotine and lobeline, the actions of which were blocked after the same procedure[21-23].

THE ELECTROPHYSIOLOGIC EFFECTS OF RBG

Electrophysiologic experiments were performed on dog, sheep, rabbit, guinea pig, and human heart tissues *in vitro* using standard glass microelectrode techniques and on the intact beating heart *in vivo* monophasic action potential techniques[25] were performed. The studies have demonstrated that RBG is very similar to digitalis[26-31]. The basic effects of RBG on transmembrane and monophasic action potentials showed that there were progressive decreases in all parameters of action potential, including action potential amplitude (APA), action potential duration at 50 %, 75 %, and 90 % of repolarization ($APD_{50,75,90}$), the maximum rate of rise of action potential phase 0 (V_{max}), and resting potential (RP). The typical data with RBG doses of 0.6 and 0.9 μmol/L were summarized in Tab 2. Concisely, there were three major effects on the electrophysiological parameters: 1) RBG decreased the absolute values of RP and V_{max}, 2) RBG shortened APD both in membrane potential and in monophasic potential, and 3) RBG decreased action potential amplitude both *in vitro* and *in vivo*.

In addition, Tab 2 also showed that the concentration-dependent manner of RBG on electrophysiologic effects[31]. A comparison study between RBG and acetylstrophanthidin was performed on sheep Purkinje fibers by microelectrode and extracellular electrogram techniques[26]. The results indicated that the electropharmacologic characteristics of RBG were similar to those of acetylstrophanthidin, including all parameters of action potentials, electrograms, and time course of the effect, *etc*. This suggests that RBG belongs to this family of digitalis-like drugs.

Tab 2. The electrophysiological characteristics of residbufogenin (RBG) on canine Purkinje fibers. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* **0 min.**

Concentration	Time/min	APA/mV	APD_{75}/ms	RP/mV	V_{max}/V·s^{-1}
0.6 μmol/L	0	118±6	296±62	88±8	312±39
	10	114±4[a]	296±46[a]	84±8[a]	305±24[a]
	20	113±5[a]	291±49[a]	83±7[a]	282±38[b]
	30	106±4[b]	266±45[b]	81±6[b]	222±32[b]
	40	102±9[b]	254±37[b]	74±10	200±52[b]
0.9 μmol/L	0	102±5	311±81	90±4	536±38
	10	97±4[b]	327±99[a]	89±4[b]	537±60[a]
	20	94±6[b]	307±71[a]	87.0±2.9[b]	498±78[b]
	30	93±6[b]	275±70[b]	85.0±2.0[b]	440±80[b]
	40	89±8[b]	260±83[b]	83±3[b]	428±83[b]

APA: Action potential amplitude; APD_{75}: Action potential duration at 75 % repolarization; RP: Resting potential; V_{max}: The maximum rate of rise of action potential.

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THE ELECTRO-TOXIC EFFECTS OF RBG

RBG-inducing delayed afterdepolarizations (DAD) As with digitalis, numerous animal experimental studies showed that RBG induced DAD and triggered arrhythmia at high concentrations of the drug both *in vitro* and *in vivo*[27-31]. Triggered arrhythmia occurs when DAD reach sufficient amplitude to "trigger" extra or spontaneous action potentials[32-34]. DAD are low-frequency (around 4 – 5 Hz) and low-amplitude depolarizing oscillations in membrane voltage that are triggered by one or more preceding action potentials. Afterdepolarizations arise either before or after complete repolarization of the action potential. They are named early afterdepolarizations (EAD) and DAD, respectively. When DAD or EAD is of sufficient amplitude, it may initiate an action potential that can propagate into the surrounding tissues and the remainder of the heart, thereby producing a triggered arrhythmia. According to previous reports, RBG caused DAD in sheep and canine Purkinje fibers, human atrial fibers and guinea pig papillary fibers at high concentrations of 0.52 and 0.60 μmol/L. Fig 2 illustrates a typical example of DAD induced by RBG (0.52 μmol/L) both in intracellular and extracellular recordings of sheep Purkinje fibes at the stimulation basic cycle lengths of 990 ms (left hand) and 690 ms (right hand).

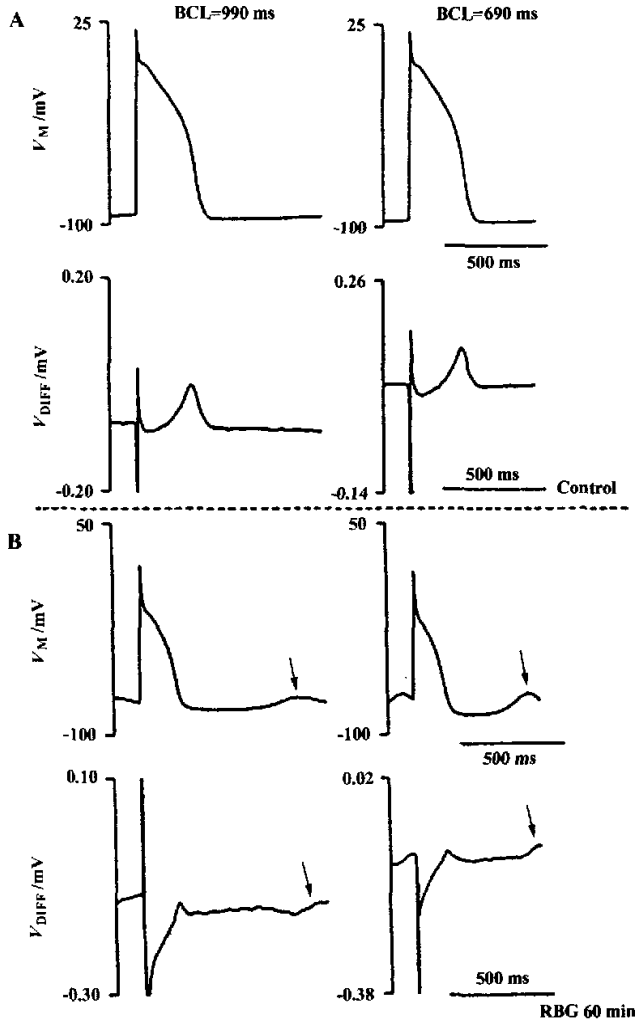

Fig 2. Resibufogenin (0.52 μmol/L)-induced both DAD on the transmembrane action potentials (upper traces) and DAD-E on extracellular electrograms (lower traces) in isolated sheep heart Purkinje fiber at the pacing cycle length of 990 ms (legt hand) and 690 ms (right hand). Top panels: control conditions. Bottom panels: following superfusion with resibufogenin for 60 min, DAD and DAD-E were presented in these recordings. BCL: basic cycle length. RBG: resibufogenin. From reference 29.

For the control condition (Fig 2A), no DAD and DAD-E were present in these recordings. Following exposure to toxic concentrations of RBG for 60 min, DAD (Fig 2B, top trace with an arrow) on transmembrane action potential recording and DAD-E (Fig 2B, bottom trace with an arrow) on intracellular electrogram developed obviously and simultaneously. On the other hand, the rate-dependent effect of RBG was examined in the experiments. The intracellular recordings showed that DAD amplitudes depended on the stimulation cycle length. Namely, the shorter pacing cycle length DAD amplitudes were greater than those induced by longer stimulation. This result suggested that DAD and triggered arrhythmia might be easier induced at higher heart rates. In this experiment, however, the DAD amplitudes did not reach threshold, and no triggered arrhythmia was observed. The intra- and extracellular recordings recovered to control condition after drug washout for 60 min (not shown in this figure).

On the other hand, unequivocal evidence of RBG induced-DAD was obtained from the intact animal heart[28]. DAD were elicited by RBG in rabbit left ventricle *in vivo* using monophasic action potential technique[25]. DAD appeared at about 0.5 – 1.0 min after injection of RBG at 0.3 mg/kg and lasted 20 – 30 min. The amplitudes of DAD were not large enough to reach the threshold, therefore, the triggered arrhythmias did not appear in these experiments.

RBG-induced triggered arrhythmias Afterdepolarization could be an important mechanism for the generation of arrhythmias. Triggered arrhythmias were evoked from DAD by RBG in sheep heart Purkinje fibers[28-29,31]. Fig 3 showed that RBG induced DAD

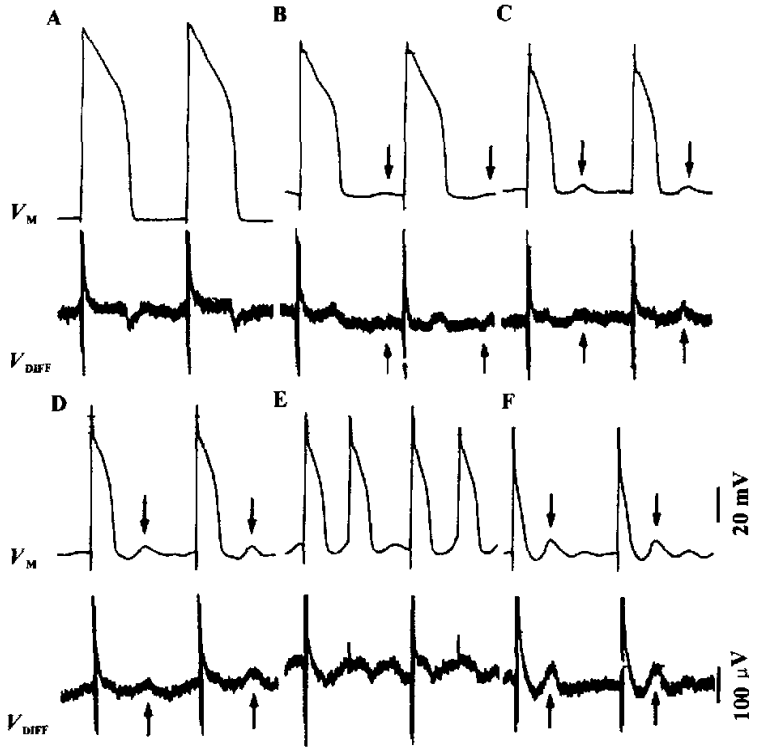

Fig 3. Resibufogenin-induced DAD, DAD-E, and triggered arrhythmias in a sheep heart Purkinje fiber at pacing cycle length of 1000 ms. Upper and lower tracings show transmembrane action potentials (V_M) and extracellular electrograms (V_{DIFF}) in all panels, respectively. A: Control. B – D: Following exposure to resibufogenin (2.6 μmol/L) for 10, 15, and 20 min. Both DAD (down arrow) in action potentials and DAD-E (up arrow) in electrograms were induced simultaneously. E: Following exposure to resibufogenin for 25 min. The premature action potentials appeared from the peak of DAD. F: Washing out of resibufogenin for 60 min, No recovery was observed in this experiment. From reference 29.

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and triggered arrhythmias in a Purkinje fiber at a pacing cycle length of 1000 ms. The transmembrane action potentials (upper traces in each panel) and extracellular electrograms (lower traces in each panel) were simultaneously recorded in the experiments. Panel A showed the control condition and no DAD and DAD-E (shown by the up arrows) in electrograms (V_{DIFF}) were induced. Following exposure to RBG for 10, 15, and 20 min (Fig 3, Panel B, C, and D), both DAD (down arrows) and DAD-E (up arrows) were induced simultaneously. After exposure to the drug for 25 min (Panel E in Fig 3), DAD amplitudes were increased sufficiently and initiated extra or premature action potentials in intracellular recording and sustained oscillations in extracellular recordings. The mechanism for RBG-induced arrhythmias is triggered activity based on DAD. This kind of arrhythmia induced by DAD was considered to belong to triggered arrhythmia. No recovery was seen, however, after washout of 60 min in this experiment (Fig 3, Panel F).

Another typical example of RBG-induced DAD and TAs in canine Purkinje fibers at the pacing cycle length of 900 ms is shown in Fig 4. Panel A is the control condition, and no DAD and TA appeared after repolarization in action potential. Following superfusion with RBG (0.6 μmol/L) for 50 min (Panel B), DAD was induced after the completion of repolarization. During the pause in stimulation, an oscillatory coupling to the DAD occurred. Panel C showed following exposure to RBG for 70 min, DAD amplitude increased sufficiently and reached the threshold potential, giving rise to a sustained spontaneous firing at a cycle length of 167 ms. Additionally, spontaneous rhythms, sustained bigeminies, paroxysmal tachycardias, and other tachyarrhythmias were also induced by RBG in the different experiments.

It must be pointed out that Chansu not only possesses arrhythmogenic action as described above, but also has an antiarrhythmic effect. Morishita *et al*[10] reported that "kyushin", a drug containing Chansu, inhibited the aconitine-induced arrhythmia significantly after intraduodenal administration with 80 mg/kg, and the thyroxin-induced arrhythmia with 40 mg/kg, respectively. They suggested that the antiarrhythmic effects of "kyushin" might be attributable to a possible inhibitory effect on the conduction system and a potential effect on the parasympathetic nervous system.

THE POSSIBLE MECHANISM FOR TRIGGERED ACTIVITIES BY RBG

Theoretically, one has to consider that digitalis-induced arrhythmia may be based on reentry, afterdepolarizations, or abnormal automaticity. However, the cellular mechanism for DAD induced by cardiac steroids has remained controversial up to the present[35]. On the basis of previous reports, DAD is thought to be induced by a transient inward current (i_{TI}). Several lines of evidence suggest that i_{TI} is the current that underlies DAD and that it is closely linked to a rise in intracellular Ca^{2+}[36-38]. And i_{TI} is generated by either: 1) a nonselective cationic current[39-42]; or 2) the activation of an electrogenic Na^+/Ca^{2+} exchanger[40-44]. The two

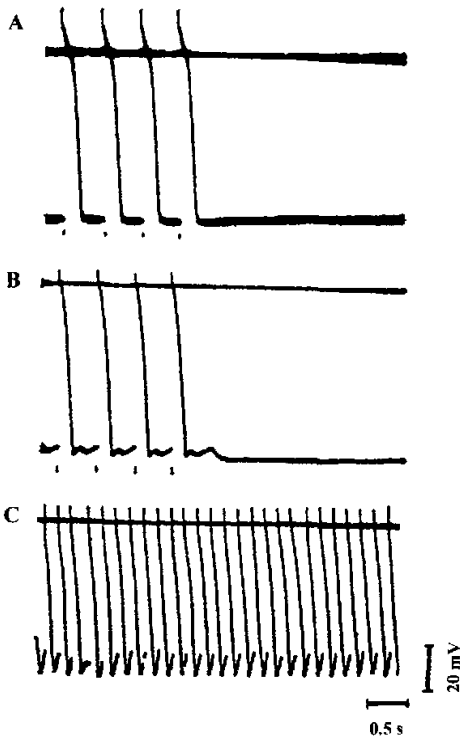

Fig 4. Resibufogenin (RBG) induced delayed afterdepolarizations (DAD) and triggered arrhythmias (TA) in an isolated canine heart Purkinje fiber at the pacing cycle length of 900 ms. A: Control of transmembrane action potentials. No DAD and TA appeared after each repolarization. B: following superfusion with RBG (0.6 μmol/L) for 50 min, DAD was induced after the completion of repolarization. During the pause in stimulation, an oscillatory coupling to the DAD occurred. C: After superfusion with RBG for 70 min, DAD amplitude increased enough and reached the threshold potential giving rise to a sustained firing spontaneously at a cycle length of 167 ms. From reference 30.

above hypotheses for DAD and i_{TI} depend on a process that is sensitive to intercellular Ca^{2+}. It has been reported that both RBG and acetylstrophanthidin can poison the Na^+-K^+ pump[15,45-49], and there is an elevation in intracellular Na concentration. In turn, this induces a shift in the Na^+-Ca^+ exchange, such that more Na^+ is extruded and Ca^{2+} conserved, resulting in a rise in intracellular Ca^{2+}. This induces further intracellular Ca^{2+} elevation via its extrusion from the mitochondrial store and release from the sarcoplasmic reticulum. Finally, the resultant elevation in Ca^{2+} induces a current carried by monovalent cations, referred to as the i_{TI} or DAD. The hypothesis of the mechanism for DAD induced by RBG should be demonstrated by further study.

THE POSSIBLE TREATMENT OF CHANSU INTOXICATION

At the present, nonstandard therapies can be used for those patients poisoned by Chansu. Like other intoxication, the routine treatment of Chansu poisoning includes gastric lavage with 0.2 % to 0.5 % potassium permanganate solution and subcutaneous injection of atropine in a dose of 0.5 to 1.0 mg[7]. Chem *et al*[13] (1991) reported that a 31-year old male labor worker was poisoned by consuming a bowl of toad soup. After treating the patient, they concluded that atropine or pacemaker therapy appeared to be a reasonable approach when symptoms were primarily due to bradycardia. They also suggested that propranolol was contraindicated in the patient because of sinus arrest and high-grade atrioventricular block. Isoproterenol is not advised because neurologic symptoms and ventricular fibrillation may be potentiated by catecholamines. Another clinic report showed that a five-year-old boy was poisoned after ingestion and mouthing of some toads. The patient did well on high-dose hydrocortisone sodium succinate and phenobarbital[50].

On the other hand, because toxicity from Chansu poisoning is similar to digoxin toxicity, the antibodies used in the assays of digoxin react with Chansu, which contains cardiotonic steroids with a chemical structure similar to digoxin[11,51]. A method of enzyme immunoassay was developed to measure antiresibufogenin IgG reactive substances in plasma and urine, as well as a high-performance liquid chromatographic (HPLC) method for the simultaneous determination of RBG and cinobufagin in traditional Chinese medicine[7,52,53]. Dasgupta and Emerson[54] studied neutralization of cardiac toxins by digibind. The results showed that digibind can bind cardiac toxins, such as bufalin and cinobufotalin, *in vitro*, thus reducing the free concentrations. Because this neutralization effect may occur *in vivo*, the digibind may be useful for treating patients suffering from poisoning. Bagrov *et al*[8] have shown that antidigoxin antibodies not only bind to toad venom, but also block the ability of toad venom to inhibit Na^+, K^+-ATPase. According to the possible mechanism of triggered arrhythmias by Chansu described above, the inhibition of Na^+, K^+-ATPase may be a very important mechanism for the resolution of ECG abnormalities of this digibind. Brubacher *et al*[11] have studied the treatment of toad venom poisoning with digoxin-specific Fab fragments clinically. The clinical courses of patients 5 and 6 also suggest that digoxin Fab fragments might be effective treatment for poisoning by this product. Patient 5 had a resolution of vomiting and an improvement in his heart rate after digibind administration. Patient 6 also had improvement of symptoms and resolution of ECG abnormalities following digibind. Fig 5 shows the effect of digoxin-specific Fab fragment on ECG II. There was Mobitz one atrioventricular block before treatment of antibody. Following treatment with digoxin Fab fragments (20 vials of digibind), Mobitz block disappeared and heart rate recovered. Therefore, they recommend administration of large doses of digoxin Fab fragments (10 vials) to all patients with suspected cardioactive steroid overdose who have hyperkalemia or any abnormal cardiac rhythms. However, the treatment of Chansu or RBG intoxication with antibodies was successful only in a few patients. Further clinical evidence is needed. Recently, they (1999) used digibind in the treatment of toad venom poisoning of mice and obtained similar result[55].

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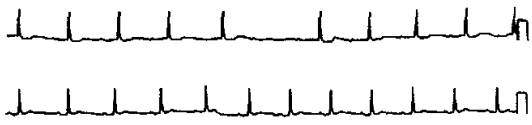

Fig 5. Effect of digixin-specific Fab fragment on ECG (II) of a patient poisoned by Chansu. Top: Pretreatment rhythm from a patient. There was Mobiz one atrioventricular block with a heart rate of 55 beats/min. Bottom: Rhythm from patient 6 following digoxin Fab fragments (20 vials of Digibind). The heart rate was 70 beats/min. The PR interval has narrowed to 200 ms and all beats were conducted. From Ref 11.

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脂布福吉宁的心脏毒性作用：电生理学证据

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关键词 蟾酥甾类；洋地黄类；电生理学；心律失常；浦肯野氏纤维

脂布福吉宁是从传统中药蟾酥中分离的单一化合物．长期以来，蟾酥制品广泛应用于中国、日本及亚洲其他国家．但是，近来在美国出现一些有关蟾酥及其药物制剂引起中毒的报告．如洋地黄一样，脂布福吉宁也具有药理和毒理作用．根据我们的实验结果，高浓度脂布福吉宁，蟾酥的主要成分之一，可以引起离体心肌细胞和在体心脏的延时性后去极化及触发性心律失常．本文对引起毒性的可能机制及治疗心律失常的可能性也进行了讨论．

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