OPEM

Oriental Pharmacy and Experimental Medicine 2003 **3(2)**, 51-55 **Review**

Resibufogenin induces cardiac arrhythmia

Jing-Tian Xie[1,2], Sangeeta R. Mehandale[1,2], Spring A. Malechar[1,2] and Chun-Su Yuan[1,2,3]*

[1]*Tang Center for Herbal Medicine Research;* [2]*Department of Anesthesia & Critical Care;* [3]*Committee on Clinical Pharmacology, The Pritzker School of Medicine, University of Chicago, Chicago, Illinois, U.S.A.*

SUMMARY

Resibufogenin is a single compound isolated from the skin venom gland of the toad (Bufo bufo gargarizans cantor). Formulations containing toad venom have been widely used as complementary and alternative medicines. However, like digitalis, resibufogenin possesses both pharmacological and toxicological activities. Our previous data indicated that resibufogenin induces electro-toxicity, including delayed afterdepolarization and triggered arrhythmias at high concentration, both in cardiac fiber *in vitro* and in beating heart *in vivo.*

Key words: Resibufogenin; Toad venom; Digitalis; Arrhythmia

INTRODUCTION

Toad venom (called Chan su in Chinese) is a traditional Chinese medicine obtained from the skin venom gland of the toad (Suga, 1973; Tsukada, 1974; Xie, 1987). It contains multiple biological active substances, dozens of which have been isolated. Some examples 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 ($C_{26}H_{34}O_6$) and so on. Resibufogenin (3-hydroxy-14, 15-epoxy-20, 22-dienolide glycoside) is a cardiac glycoside and its chemical structure is similar to digitoxigenin (Sondheimer *et al.*, 1968; The First Tianjin Factory for Chinese Herbal Medicine, 1974; Lichtstein *et al.*, 1986).

Resibufogenin, an isolated compound from toad venom, is often found in traditional Chinese medicine formulations, which have been used as cardiotonic agents (Dasgupta *et al.*, 2000). Toad venom can increase myocardial contractility, inhibit ouabain-sensitive Na^+, K^+-ATPase, and cross-react with various antidigoxin antibodies (Bagrov *et al.*, 1993; Huang, 1999). Resibufogenin is a major chemical component of toad venom. Previous studies showed that resibufogenin induced both delayed afterdepolarization and triggered arrhythmias in cardiac fiber *in vitro* (Xie *et al.*, 1988a; Xie *et al.*, 1994) and in heart *in vivo* (Xie *et al.*, 1988b).

*Correspondence: Chun-Su Yuan, MD, PhD, Department of Anesthesia and Critical Care, The University of Chicago Medical Center, 5841 S. Maryland Avenue, MC 4028, Chicago, IL 60637, USA. Tel: 773-702-1916; Fax: 773-834-0601; E-mail: cyuan@midway.uchicago.edu

MAJOR PHARMACOLOGICAL FUNCTIONS

According to previous reports, resibufogenin possesses multiple pharmacological actions (Xie, 1987; Hayashi *et al.*, 2002; Xie *et al.*, 2002) including cardiotonic, vasopressor, and respiratory stimulatory effects (Suga, 1973; Moreshita *et al.*, 1992; Dasgupta *et al.*, 2000).

Cardiotonic effect

A number of animal experiments have demonstrated that resibufogenin increases ventricular contractile force in different animals, (e.g. rabbits, cats, and adult mongrel dogs) (Iwatsuki *et al.*, 1965; The First Tianjin Factory for Chinese Herbal Medicine, 1975; Herbal Medicine Section of Dept of Biology, 1977). Similar to the effects of digitalis, resibufogenin increases the contractility of cardiac muscle in a dose-dependent manner, a positive inotropic effect.

Vasopressor effects

Several reports have indicated that in the

hemorrhaged animal model, there is a significant increase in mean systemic arterial pressure following the administration of resibufogenin. It is believed to be due to an increase in cardiac output without a significant change in heart rate (Okada *et al.*, 1960; Iwatsuki *et al.*, 1965; Leigh and Caldwell, 1969; The First Tianjin Factory for Chinese Herbal Medicine, 1975; Herbal Medicine Section of Dept of Biology, 1977). The vasopressor effect of resibufogenin appears to be predominantly due to its peripheral vasoconstrictor action, with a lesser contribution from its cardiotonic effect.

Respiratory stimulatory effect

Resibufogenin was demonstrated to be an efficacious stimulator of respiration (Okada *et al.*, 1960; Suga, 1973; The First Tianjin Factory for Chinese Herbal Medicine, 1975; Herbal Medicine Section of Dept of Biology, 1977). In animal experiments, respiratory amplitude, tidal volume, and minute volume, increased significantly with resibufogenin. The mechanism of the effect on respiration is thought to be excitation of the respiratory center. The excitatory effect of resibufogenin on respiration was not abolished by the administration of procaine, suggesting that this effect was mediated through the central nervous system.

ELECTROPHYSIOLOGICAL AND ELECTRO-TOXIC EFFECTS

Electrophysiological effect

Electrophysiological experiments 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* using monophasic action potential techniques were performed in our labs (Xie and January, 1993). The studies have demonstrated that resibufogenin is very similar to digitalis (Xie *et al.*, 1983; Xie *et al.*, 1985; Xie *et al.*, 1988a; 1988b; Xie *et al.*, 1994; Xie *et al.*, 2000; Xie *et al.*, 2002). The basic effects of resibufogenin 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). Concisely, there were three major effects on the electrophysiological parameters: (1) resibufogenin decreased the absolute values of RP and V_{max}; (2) resibufogenin shortened APD both in membrane potential and in monophasic potential; (3) resibufogenin decreased action potential amplitude both *in vitro* and *in vivo*.

Resibufogenin-inducing delayed afterdepolarizations (DADs)

As with digitalis, a number of animal experimental studies demonstrated that resibufogenin induced DADs and triggered arrhythmia at high concentrations of the drug both *in vitro* and *in vivo* (Xie *et al.*, 1985; Xie *et al.*, 1988a; 1988b; Xie *et al.*, 1994; Xie *et al.*, 2000; Xie *et al.*, 2002). Triggered arrhythmia occurs when DADs reach sufficient amplitude to "trigger" extra or spontaneous action potentials (Cranefield and Aronson, 1988; Wit and Rosen, 1991; January *et al.*, 1992). Figure 1 shows a typical example of DADs induced by resibufogenin (0.52 μM) in sheep Purkinje fiber at the basic stimulation cycle length of 690 msec. For control conditions, no DAD was present in the intracellular recordings (Fig. 1, top trace). During exposure to resibufogenin for 60 min, DADs occurred after repolarization of each action potential (Fig. 1, middle trace). In this experiment, however, the DAD amplitudes did not reach threshold, and no triggered arrhythmia was observed. The action potentials recovered to control conditions after washout of the drug for 60 min (Fig. 1, bottom trace). Our data also indicated that DADs are low-frequency (approximately 4-5 Hz) and low-amplitude depolarizing oscillations in membrane voltage that are triggered by one or more preceding action potentials.

Resibufogenin-induced triggered arrhythmias

Afterdepolarization is an important mechanism for the generation of arrhythmias. Triggered arrhythmias were evoked from DADs by resibufogenin in sheep heart Purkinje fibers (Xie *et al.*, 1988a; 1988b; Xie *et al.*, 1994). The mechanism for resibufogenin-induced arrhythmias is triggered activity based on DADs. Figure 2 indicated that resibufogenin induced triggered arrhythmia in canine Purkinje fiber. Under control conditions, DADs and

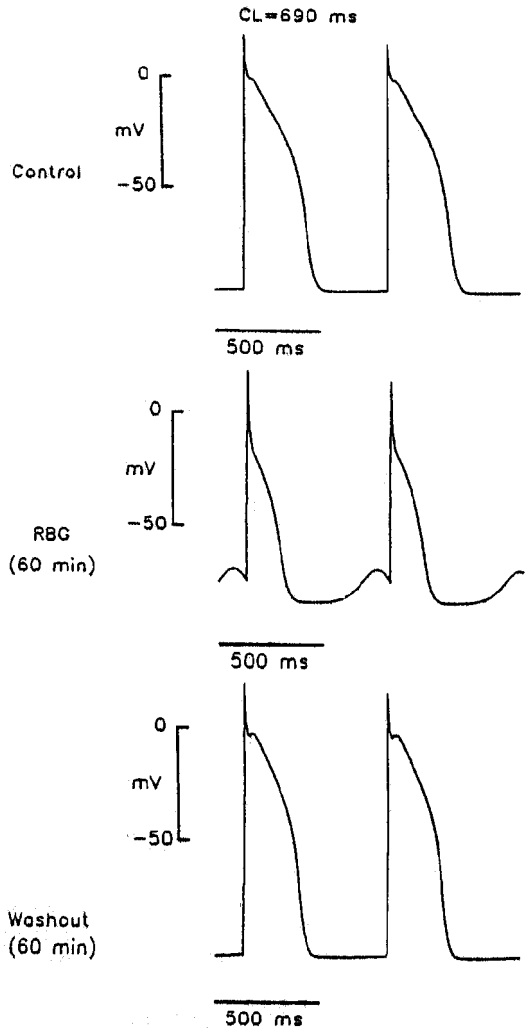

Fig. 1. Effects of resibufogenin (RBG) on the transmembrane action potentials in sheep heart Purkinje fiber at the stimulation cycle length of 690 msec. Top trace: control. Middle trace: after superfusion of resibufogenin (0.52 μM) for 60 min, showing delayed afterdepolarizations. Bottom trace: 60 min after washout.

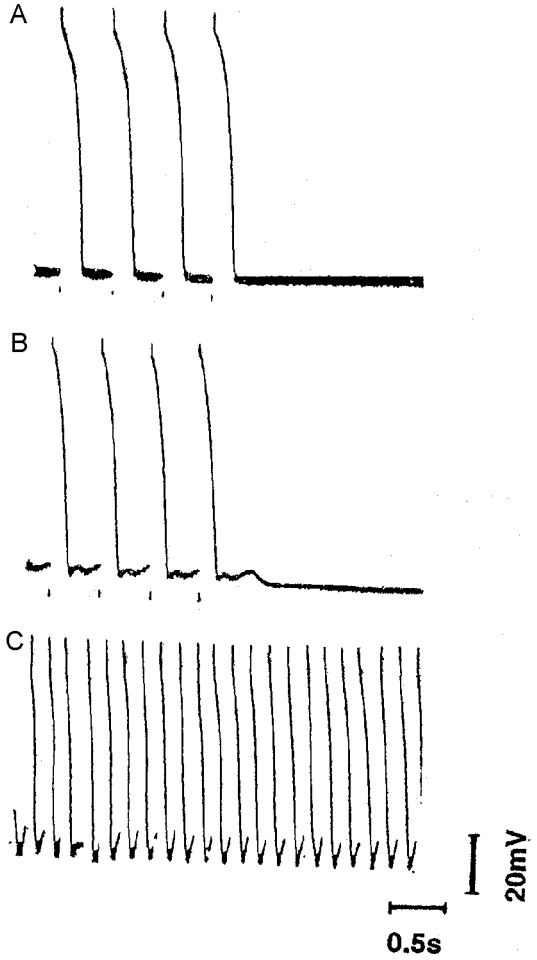

Fig. 2. Resibufogenin induced delayed afterdepolarizations (DADs) and triggered arrhythmia in a canine Purkinje fiber at the pacing cycle length of 900 msec. A was a control condition of action potentials. No DAD and triggered arrhythmia appeared after repolarizations. B showed superfusion with resibufogenin (0.6 μM) for 50 min. DADs occurred after the completion of each repolarization. During the pause in stimulation, an oscillatory coupling to the DAD occurred. C showed superfusion with resibufogenin for 70 min, DADs reached the threshold giving rise to a spontaneous, sustained firing at a cycle length of 167 msec.

triggered arrhythmias were not observed (Fig. 2, top trace). DADs were induced after every completion of action potential during superfusion with resibufogenin for 50 min (Fig. 2, middle trace). After a pause in stimulation, an oscillatory afterdepolarization was coupled to the DAD. During exposure with resibufogenin (0.6 mM) for 70 min, DADs reached a threshold potential and induced triggered arrhythmias. The arrhythmias were evoked from DADs induced by resibufogenin and spontaneously sustained firing at a cycle length of 167 msec without any simulation (Fig. 2, bottom trace).

POSSIBLE TREATMENT OF TOAD VENOM INTOXICATION

At the present, nonstandard therapies can be used for those patients poisoned by toad venom or resibufogenin. Like other intoxication, the routine treatment of toad venom poisoning includes gastric lavage and subcutaneous injection of atropine

(Huang, 1999). Chern *et al.* (1992) reported that a 31-year old man was poisoned by consuming a bowl of toad soup. After treating the patient, they concluded that atropine or pacemaker therapy appears to be a reasonable approach when symptoms are 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 case report showed that a five-year-old boy was poisoned after placing a toad in his mouth for ten minutes. The patient did well with high-dose hydrocortisone sodium succinate and Phenobarbital treatment (Hitt and Ettinger, 1986).

Toxicity from toad venom poisoning is similar to digoxin toxicity. The antibodies used in the assays of digoxin react with toad venom, (Fushimi *et al.*, 1990; Brubacher *et al.*, 1992). Dasgupta and Emerson (1998) studied neutralization of cardiac toxins by digibind, which may be useful for treating patients suffering from toad venom poisoning. Digibind was used in mice with toad venom poisoning and obtained similar results (Brubacher *et al.*, 1999). Brubacher *et al.* (1996) studied the treatment of toad venom poisoning with Digoxin-Specific Fab fragments, and their case report suggested that digoxin Fab fragments might be effective treatment for toad venom poisoning.

CONCLUSIONS

Resibufogenin, which is obtained from the skin venom gland of the toad (Bufo bufo gargarizans Cantor) has multiple pharmacological effects, such as cardiotonic, vasopressor, and respiratory stimulatory effects. Like digitalis, resibufogenin also possesses toxic activities. Our previous studies demonstrated that resibufogenin at high concentrations induced cardiac electrophysiological changes and the stimulated triggered activities (including delayed after depolarizations and cardiac arrhythmias) both in cardiac fiber *in vitro* and in beating heart *in vivo*. At present, nonstandard therapies are available for patients poisoned by toad venom.

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