

## Review

# Active ingredients in many Chinese medicines promoting blood circulation are Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitors

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The positive inotropic effect of cardiac glycosides lies in their reversible inhibition on the membrane-bound Na<sup>+</sup>/K<sup>+</sup>-ATPase in human myocardium. Steroid-like compounds containing a core structure similar to cardiac glycosides are found in many Chinese medicines conventionally used for promoting blood circulation. Some of them are demonstrated to be Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitors and thus putatively responsible for their therapeutic effects via the same molecular mechanism triggered by cardiac glycosides. On the other hand, magnesium lithospermate B of danshen is also proposed to exert its cardiac therapeutic effect by effectively inhibiting Na<sup>+</sup>/K<sup>+</sup>-ATPase. Theoretical modeling suggests that the number of hydrogen bonds and the strength of hydrophobic interaction between the effective ingredients of various medicines and residues around the binding pocket of Na<sup>+</sup>/K<sup>+</sup>-ATPase are crucial for the inhibitory potency of these active ingredients. Ginsenosides, the active ingredients in ginseng and sanqi, substantially inhibit Na<sup>+</sup>/K<sup>+</sup>-ATPase when sugar moieties are attached only to the C-3 position of their steroid-like structure, equivalent to the sugar position in cardiac glycosides. Their inhibitory potency is abolished, however, when sugar moieties are linked to C-6 or C-20 position of the steroid nucleus; presumably, these sugar attachments lead to steric hindrance for the entrance of ginsenosides into the binding pocket of Na<sup>+</sup>/K<sup>+</sup>-ATPase. Neuroprotective effects of cardiac glycosides, several steroid-like compounds, and magnesium lithospermate B against ischemic stroke have been accordingly observed in a cortical brain slice-based assay model, and cumulative data support that effective inhibitors of Na<sup>+</sup>/K<sup>+</sup>-ATPase in the brain could be potential drugs for the treatment of ischemic stroke.

**Keywords:** cardiac glycoside; ginsenoside; magnesium lithospermate B; neuroprotection, Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitors; promoting blood circulation; steroid-like compound

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## Introduction

In an aging population cardiovascular diseases, such as congestive heart failure, have gradually become serious health challenges. Congestive heart failure, generally defined as incapability of the heart to supply sufficient blood flow to meet the body's needs, frequently leads to significantly adverse influences on physical and mental health, resulting in a markedly decreased quality of life and even death<sup>[1]</sup>. While cardiac glycosides, such as digoxin, digitoxin and ouabain, have been a cornerstone of the treatment of congestive heart failure for more than two centuries, severe side effects have been reported for these steroid-like compounds<sup>[2,3]</sup>. The narrow therapeutic index (the margin between effectiveness and toxicity) of cardiac glycosides further limits their clinical applications<sup>[4]</sup>. The limited benefit with certain side effects of

the existing remedies has prompted investigators to search for complementary and alternative therapies and drugs.

Many traditional Chinese medicines have long been used in the treatment of various cardiovascular diseases, such as coronary heart disease, heart stroke and myocardial infarction, and some of them are effective in promoting blood circulation, removing blood stasis and supplementing vital energy<sup>[5,6]</sup>. Although these medicines display considerable therapeutic effects with low toxicity, little is known about their primary active ingredients and detailed pharmaceutical mechanisms. Nevertheless, these traditional Chinese medicines, dating back 2000 years, have been regarded as valuable sources to screen potential drugs for the treatment of cardiovascular diseases.

With the rapid development of technology, numerous constituents in traditional Chinese medicines have been isolated and structurally determined<sup>[7]</sup>. Abundant steroid-like compounds, structurally similar to cardiac glycoside, have been found in many Chinese medicines used for promoting blood

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circulation<sup>[8, 9]</sup>. It is likely that these steroid-like compounds are responsible for the therapeutic effects of these medicines via the same molecular mechanism triggered by effective inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase. On the other hand, no appreciable contents of steroid-like compounds are found in danshen (*Salvia miltiorrhiza*), a well-known Chinese herb traditionally regarded as an effective medicine for promoting blood circulation<sup>[10]</sup>. Instead, magnesium lithospermate B (MLB), the major soluble ingredient in danshen, is assumed to be responsible for the therapeutic effect by inhibiting Na<sup>+</sup>/K<sup>+</sup>-ATPase in a manner comparable to that of cardiac glycosides.

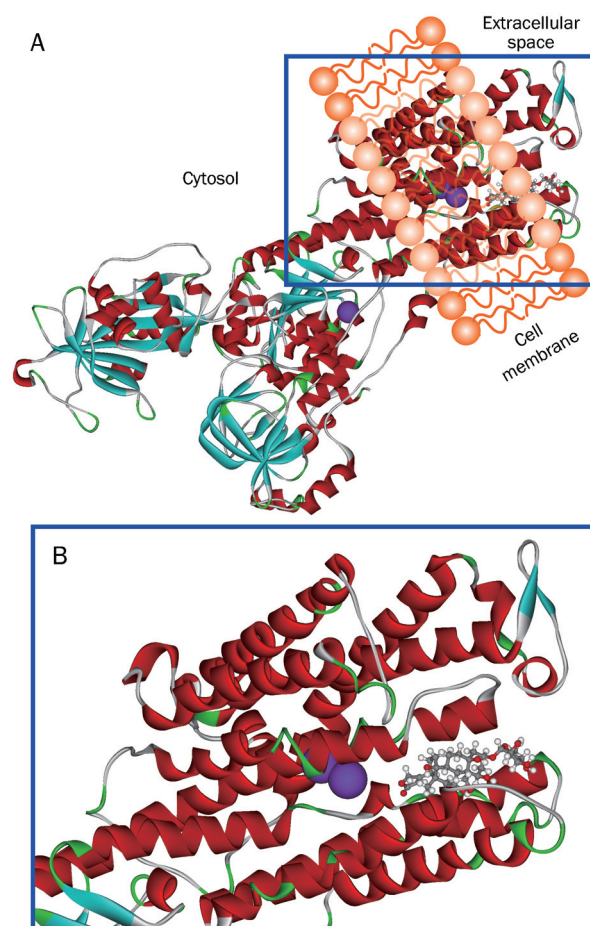
This review summarizes recent research on Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitory potency of some active ingredients identified in the Chinese medicines used for promoting blood circulation. Molecular modeling and docking of these compounds to Na<sup>+</sup>/K<sup>+</sup>-ATPase resolve at molecular level the difference in their inhibitory potency. Potential usage of these active ingredients as inhibitors of Na<sup>+</sup>/K<sup>+</sup>-ATPase in the brain for the treatment of ischemic stroke is also discussed.

### Structure and function of Na<sup>+</sup>/K<sup>+</sup>-ATPase

Na<sup>+</sup>/K<sup>+</sup>-ATPase, a P-type ATPase also known as sodium pump, is an active transport system of sodium and potassium ions that is highly conserved in all animal cells. It commonly consumes 20%–30% of the adenosine triphosphate (ATP) energy generated in animal cells at rest to actively transport three Na<sup>+</sup> out of and two K<sup>+</sup> into cells<sup>[11]</sup>. The x-ray crystal structure of Na<sup>+</sup>/K<sup>+</sup>-ATPase recently resolved<sup>[12–14]</sup> shows that it is composed of three subunits ( $\alpha$ ,  $\beta$  and  $\gamma$  subunits) with distinct properties<sup>[15–17]</sup>; and the  $\alpha$  catalytic subunit, a 112 kDa protein, contains sites important for ATP binding and phosphorylation as well as ion occlusion (Figure 1A) and an ouabain-binding site (Figure 1B), the primary binding site for many pharmacological agents, such as cardiac glycosides, that affect pump activity<sup>[18]</sup>.

Physiological functions of Na<sup>+</sup>/K<sup>+</sup>-ATPase have been deduced from its role as an ion pump. Specifically, the ability of Na<sup>+</sup>/K<sup>+</sup>-ATPase to establish and maintain ion gradients makes it essential for the generation and maintenance of electrical membrane potentials, which are necessary for neuronal excitability, transmission<sup>[19]</sup> and cardiac muscle contraction<sup>[20]</sup>. Na<sup>+</sup>/K<sup>+</sup>-ATPase also generates the Na<sup>+</sup> gradient that is critical for the reabsorption of sodium ion and water from the glomerular filtrate in the nephron<sup>[21]</sup> and absorption of fluid from the lungs and intestine<sup>[22]</sup>. It can also drive secondary active co-/countertransporters, which are coupled to the gradient of extracellular to intracellular Na<sup>+</sup> concentration, such as the sodium glucose cotransporter and the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger<sup>[23]</sup>. In addition, recent findings have suggested additional signaling modes of action of Na<sup>+</sup>/K<sup>+</sup>-ATPase<sup>[24–26]</sup>, implicating its regulation of several important cellular processes and highlighting potential therapeutic roles of its inhibitors (such as cardiac glycosides) in various diseases.

### Inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase by cardiac glycosides leading to a positive inotropic effect



**Figure 1.** (A) Crystal structure of ouabain binding to the extracellular pocket of shark rectal gland Na<sup>+</sup>/K<sup>+</sup>-ATPase (PDB code 3A3Y)  $\alpha$  subunit<sup>[17]</sup>. Amino acid residues of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$  subunit are shown in ribbon structure, and ouabain in scaled ball and stick. K<sup>+</sup> binding sites are shown in purple balls. (B) Enlarged diagram without the membrane bilayer shown in the blue box of (A).

Cardiac glycosides, such as ouabain and digoxin, are a diverse family of naturally derived compounds that bind to and inhibit Na<sup>+</sup>/K<sup>+</sup>-ATPase. While they show considerable structural diversity, all members of this family share a common steroidal framework, regarded as the pharmacophoric moiety responsible for the activity of these compounds<sup>[27]</sup>. Generally, this steroidal core is double-substituted with an unsaturated lactone ring at position 17 and a sugar portion at position 3; and this lactone moiety of cardiac glycosides is critical for their potent inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase. Although the sugar moiety does not greatly influence their biological activity, the addition of sugars to the steroidal core affects the pharmacodynamic and pharmacokinetic profile of each glycoside<sup>[28]</sup>.

Cardiac glycosides have been in clinical use for many years for the treatment of congestive heart failure and cardiac arrhythmia, and the mechanism of their positive inotropic effect is well characterized. According to the most widely accepted molecular mechanism responsible for the therapeutic effect of cardiac glycosides, they act through reversible inhi-

bition of  $\text{Na}^+/\text{K}^+$ -ATPase in the membrane of heart muscle cells<sup>[29, 30]</sup>. In human heart, inhibition of the  $\text{Na}^+/\text{K}^+$ -ATPase leads to the accumulation of intracellular sodium ion, which decreases the sodium gradient across the membranes of cardiac muscle cells. This reduced sodium gradient in turn limits the activity of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger in the cell membrane, which normally uses the sodium gradient for energy in the extrusion of calcium ion<sup>[31]</sup>. Each cardiac action potential is thus followed by elevated levels of residual intracellular calcium ion, and the net effect of which is to strengthen successive heart contractions<sup>[32]</sup>. In this way, inhibition of the  $\text{Na}^+/\text{K}^+$ -ATPase by cardiac glycosides produces beneficial effects in patients with congestive heart failure. However, severe side effects and narrow therapeutic index of cardiac glycosides have apparently limited their clinical applications<sup>[29]</sup>.

### Steroid-like compounds in Chinese medicines for promoting blood circulation and their inhibitory potency on $\text{Na}^+/\text{K}^+$ -ATPase

Many Chinese medicinal products traditionally used in the treatment of cardiovascular diseases seem to achieve their therapeutic effects via promotion of blood circulation. A number of steroid-like compounds, such as triterpenoids, steroids and saponins, have been found in Chinese medicinal products used for promoting blood circulation (Table 1), and regarded as the active ingredients responsible for their therapeutic effects<sup>[33-64]</sup>. In light of the structural similarity between the core structure of these steroid-like compounds and the steroidal framework of cardiac glycosides, we propose that some of these medicines may possess therapeutic effects via inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase by their steroid-like

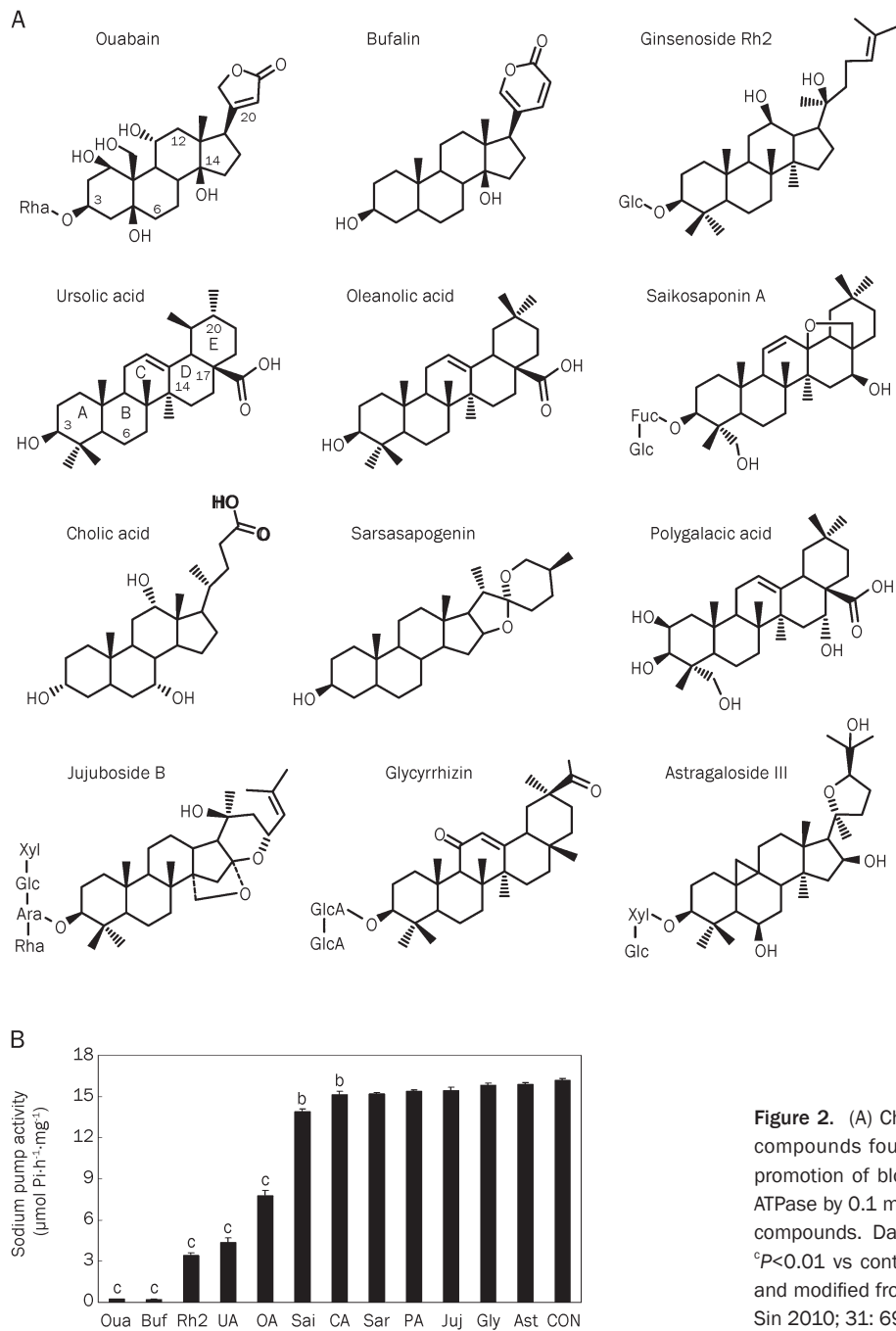
compounds<sup>[65]</sup>. Some of the steroid-like compounds listed in Table 1 are commercially available and thus subjected to  $\text{Na}^+/\text{K}^+$ -ATPase inhibition assay (Figure 2). The results show that all examined steroid-like compounds found in Chinese medicines for promoting blood circulation displayed more or less inhibitory potency on  $\text{Na}^+/\text{K}^+$ -ATPase. Among these steroid-like compounds, bufalin (structurally almost equivalent to ouabain) exhibits significantly higher inhibitory potency ( $\text{IC}_{50}$  value around 1  $\mu\text{mol/L}$ ) than the others while ginsenoside Rh2, ursolic acid and oleanolic acid are relatively moderate inhibitors of  $\text{Na}^+/\text{K}^+$ -ATPase with  $\text{IC}_{50}$  values around 50-100  $\mu\text{mol/L}$ . Based on the experimental observation of inhibitory potency of steroid-like compounds on  $\text{Na}^+/\text{K}^+$ -ATPase, the therapeutic effects of many cardiac Chinese medicines may be partly attributed to various steroid-like compounds that promote blood circulation via the same molecular mechanism triggered by cardiac glycosides, that is, accentuating the force of myocardial contraction by elevating calcium concentration via the inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase.

### Inhibition of $\text{Na}^+/\text{K}^+$ -ATPase by MLB, a non-steroid compound, leading to the therapeutic effect of danshen

Danshen, the dried roots of medicinal plant *Salvia miltiorrhiza*, is one of the most popular Chinese medicines widely used in many medicinal preparations and formulae taken by people in several Asian countries. Traditionally it is regarded as an effective herb for promoting blood circulation, removing blood stasis, relieving pain, stimulating menstrual discharge, and relaxing the mind. Therefore, danshen has also been extensively used in the treatment of coronary heart disease, myocardial infarction, heart failure, menstrual disorders, and

**Table 1.** Steroid-like compounds in Chinese medicines used for the promotion of blood circulation.

Compound	Chinese medicinal sources	References
Bufalin	The dried venom of <i>Bufo bufo gargarizans</i> Cantor or <i>B. bufo melanostictus</i> Schneider	33, 34
Ginsenoside Rh2	The dry root of <i>Panax ginseng</i> or <i>P. notoginseng</i> F H Chen	35-37
Ursolic acid	Whole plant of <i>Prunella vulgaris</i> L with dry flowers	38, 39
Oleanolic acid	Whole plant of <i>Prunella vulgaris</i> L with dry flowers	38, 39
Saikosaponin A	The dry root of <i>Bupleurum chinense</i> DC or <i>B. scorzonifolium</i> Willd	40, 41
Cholic acid	The dried bile of <i>Ursus arctos Linnaeus</i> or <i>Selenarctos thibetanus</i> G Cuvier	42, 43
Sarsasapogenin	The dry root of <i>Anemarrhena asphodeloides</i> Bge	44
Polygalacic acid	The dry root of <i>Platycodon grandiflorum</i> (Jacq) A DC	45
Jujuboside B	The mature seeds of <i>Ziziphus jujube</i> Mill var <i>spinosa</i> (Bunge) Hu ex H F Chou	46
Glycyrrhizin	The root of <i>Glycyrrhiza uralensis</i> Fisch - <i>G. inflata</i> Bat or <i>G. glabra</i> L.	47, 48
Astragaloside III	The dry root of <i>Astragalus membranaceus</i> (Fisch) Bge or var <i>mongholicus</i> (Bge) Hsiao	49
Betulinic acid	The dried root cortex of <i>Paeonia suffruticosa</i>	50
Inokosterone	The dry root of <i>Achyranthes bidentata</i> Blume	51
Dehydrocorydaline	The tuber of <i>Corydalis yanhusuo</i> W T Wang	52, 53
Rhynchophylline	The hook of <i>Uncaria rhynchophylla</i> (Miquel) Jacks	54, 55
Hirsutine	The hook of <i>Uncaria rhynchophylla</i> (Miquel) Jacks	56-58
Cucurbitacin D	The fruit of <i>Trichosanthes kirilowii</i> Maxim or <i>T. rosthornii</i> Harms	59, 60
$\beta$ -boswellic acid	The oleogum resin of <i>Boswellia carterii</i> Birdwood	61
Isolimonoidic acid	The fruit of <i>Citrus aurantium</i> L	62
$\beta$ -sitosterol	The rhizome of <i>Sparganium stoloniferum</i> Buch-Ham	63
Pachymic acid	The sclerotium of <i>Poria cocos</i> (Schw) Wolf	64



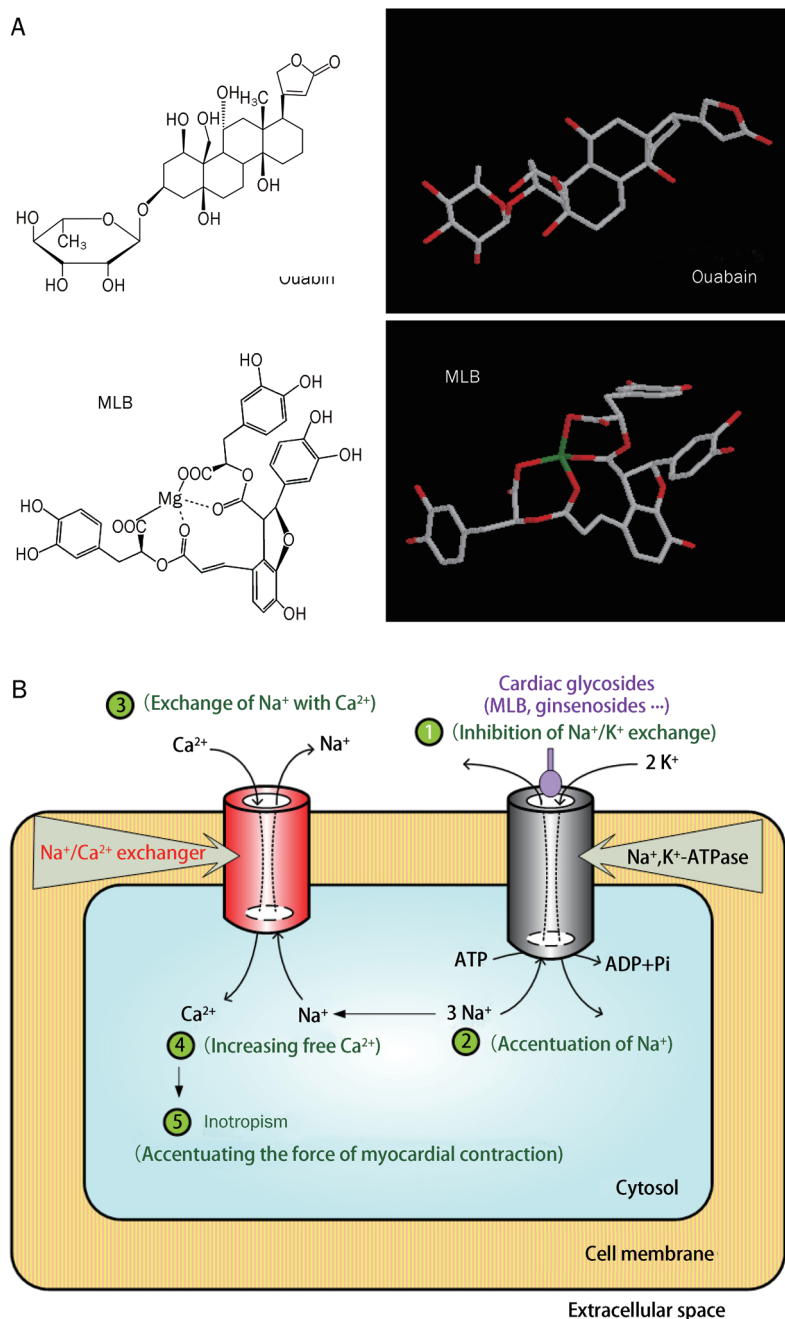
**Figure 2.** (A) Chemical structures of ouabain and 11 steroid-like compounds found in Chinese medicinal products used for the promotion of blood circulation. (B) Inhibition of porcine  $\text{Na}^+/\text{K}^+$ -ATPase by 0.1 mmol/L of ouabain and the selected 11 steroid-like compounds. Data represent mean  $\pm$  SEM of 5 replicates. <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs control group (CON; deionized water only). (Adopted and modified from Figures 1 and 2 of Chen *et al.* Acta Pharmacol Sin 2010; 31: 696–702).

other cerebrovascular diseases<sup>[66]</sup>. Ingredients in danshen are mainly divided into water-soluble and lipid-soluble compounds. Although some lipid-soluble constituents in this herb, such as tanshinones, have been conventionally considered the active ingredients<sup>[67, 68]</sup>, the major water-soluble component MLB, a derivative of caffeic acid tetramer, recently has also been demonstrated to possess several medicinal effects, such as vasodilating, antihypertensive, antioxidative, and free radical scavenging activities<sup>[69–73]</sup>. MLB possesses a relatively rigid structure due to the formation of salt bridges between  $\text{Mg}^{2+}$  and the four oxygen atoms of carboxyl groups from the

four caffeic acid fragments.

In spite of being a non-steroid compound, MLB possesses potent inhibition on  $\text{Na}^+/\text{K}^+$ -ATPase *in vitro*<sup>[74]</sup>. The molecular organization and configuration of MLB in the 3D structure is somewhat similar to ouabain, a cardiac glycoside with a rigid steroid backbone (Figure 3A), although they are totally different compounds with distinct molecular weights (584.65 for ouabain and 740.67 for MLB). Based on experimental observation and theoretical modeling, we propose that MLB acts as the active component responsible for the cardiac therapeutic effect of danshen by the same molecular mechanism triggered





**Figure 3.** (A) Chemical structures of ouabain and MLB. The 3D structures of ouabain and MLB (in dark background) were displayed using RasWin Molecular Graphics Windows Version 2.6. Gray, red, and green colors represent C, O, and  $\text{Mg}^{2+}$  atoms, respectively. (B) Proposed molecular mechanism responsible for the therapeutic effects of cardiac glycosides, ginsenosides, MLB, and other steroid-like compounds in cardiac cells. Step 1: Inhibiting the cellular exchange of  $\text{Na}^+$  and  $\text{K}^+$  by drugs binding to  $\text{Na}^+/\text{K}^+-\text{ATPase}$ . Step 2: Accumulation of  $\text{Na}^+$  in the intracellular space due to the inhibition of  $\text{Na}^+/\text{K}^+-\text{ATPase}$  activity. Step 3: Promotion of the cellular exchange of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  via the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger system. Step 4: Increasing the intracellular  $\text{Ca}^{2+}$  concentration owing to the activation of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger system. Step 5: The elevated intracellular  $\text{Ca}^{2+}$  concentration leads to an increased inotropism and accentuates the force of myocardial contraction. (Adopted from Figure 1 and the cover page of Tzen et al. Acta Pharmacol Sin 2007; 28: 609–15).

by cardiac glycosides, *ie*, inhibition of  $\text{Na}^+/\text{K}^+-\text{ATPase}$  (Figure 3B). In agreement with this proposal, the intracellular  $\text{Ca}^{2+}$  levels of SH-SY5Y neuroblastoma cells treated with MLB are substantially elevated in a manner similar to that observed in cells treated with ouabain<sup>[75]</sup>. The elevated  $\text{Ca}^{2+}$  levels seem to be supplied by both extracellular influx through the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger and intracellular release from endoplasmic reticulum.

Cell toxicity caused by cardiac glycosides at high concentrations has been noticed and blamed to their putative triggering of several signaling cascade responses that lead to cell death<sup>[76]</sup>. In contrast, MLB is generally regarded as an antioxidant without notable toxicity<sup>[74]</sup>. Similar results were found in our

studies: severe cell toxicity accompanied with dendritic shrink was observed in SH-SY5Y cells treated with ouabain, but not in those treated with MLB<sup>[75]</sup>. Therefore, we surmise that MLB has a great potential, with extensive clinical trials, to become a safer drug than cardiac glycosides.

#### Molecular modeling: the binding mode of potential inhibitors to $\text{Na}^+/\text{K}^+-\text{ATPase}$

Molecular modeling and docking of steroid-like compounds and MLB to the  $\alpha$  subunit of  $\text{Na}^+/\text{K}^+-\text{ATPase}$  revealed the observed difference in their  $\text{Na}^+/\text{K}^+-\text{ATPase}$  inhibition at molecular level. The drastic difference observed in the inhibitory potency of these active ingredients is ascribed mainly to

the number of hydrogen bonds (H-bonds) and partially to the strength of hydrophobic interaction between the compounds and residues around the deep cavity close to the two  $K^+$  binding sites of  $Na^+/K^+$ -ATPase (Figure 4). As expected from structural similarity, the interaction of bufalin with the binding pocket of  $Na^+/K^+$ -ATPase nearly matched to that of ouabain reported previously<sup>[16]</sup> with the unique lactone ring penetrating deeply into the cavity close to two  $K^+$  binding sites. For ouabain, three H-bonds are formed between the lactone ring and Ile328 and Ala330 of  $Na^+/K^+$ -ATPase, and two H-bonds between the hydroxyl group at C-14 and Thr804 of  $Na^+/K^+$ -ATPase. In contrast, one H-bond is formed between the lactone ring of bufalin and Ala330 of  $Na^+/K^+$ -ATPase, and two H-bonds between the hydroxyl group at C-14 of bufalin and Thr804 of  $Na^+/K^+$ -ATPase. Two H-bonds are formed between the hydroxyl groups at C-12 and C-20 of ginsenoside Rh2 and Asn129 and Thr804 of  $Na^+/K^+$ -ATPase, respectively. Strong hydrophobic interaction is found between the lactone ring of ouabain or bufalin and six hydrophobic residues (Ile327, Ile328, Val329, Ile787, Phe790, and Ile807) around the deep cavity of  $Na^+/K^+$ -ATPase. A hydrophobic interaction between the alkyl group of ginsenoside Rh2 and the same six hydrophobic residues of  $Na^+/K^+$ -ATPase is also found. Only one H-bond is formed between the carboxyl group of ursolic acid and Ile322 of  $Na^+/K^+$ -ATPase, and a moderate hydrophobic interaction is found between the ring E of ursolic acid and four hydrophobic residues (Ile327, Val329, Phe790, and Ile807) of  $Na^+/K^+$ -ATPase. On the whole, no H-bond is formed between those steroid-like compounds of weak inhibitory potency and the binding pocket of  $Na^+/K^+$ -ATPase<sup>[65]</sup>. However, hydrophobic interaction of similar strength is observed between the hydrophobic steroidal core of all 12 steroid-like compounds examined and 8 other hydrophobic residues (Leu132, Tyr315, Ile322, Phe323, Ile325, Phe793, Ile794, and Leu802 located in the upper portions of the figures) around the binding pocket of  $Na^+/K^+$ -ATPase.

Three H-bonds are formed between the hydroxyl group at C-4 position of MLB and Lys912 (forming two H-bonds) and Glu915 (forming one H-bond) of  $Na^+/K^+$ -ATPase, one H-bond between the carbonyl group at C-9 position of MLB and Thr804 of  $Na^+/K^+$ -ATPase, and one H-bond between the hydroxyl group at C-4 position of MLB and Leu110 of  $Na^+/K^+$ -ATPase. Similar to the hydrophobic steroidal core of ouabain, the four aromatic rings of MLB form strong hydrophobic interaction with hydrophobic residues (Leu132, Tyr315, Ile322, Phe323, Ile325, Phe793, Ile794, and Leu802) around the binding pocket of  $Na^+/K^+$ -ATPase.

### Effects of different sugar attachments to ginsenosides on $Na^+/K^+$ -ATPase inhibitory potency

Ginsenosides are triterpenoidal saponins that have a common four ring hydrophobic steroid-like structure with various sugar moieties attached mostly at the C-3, C-6, or C-20 position (Figure 4). To date, more than 80 ginsenosides have been isolated from over ten *Panax* taxa, and most of them are derived from four types of aglycones: protopanaxadiol, protopanaxa-

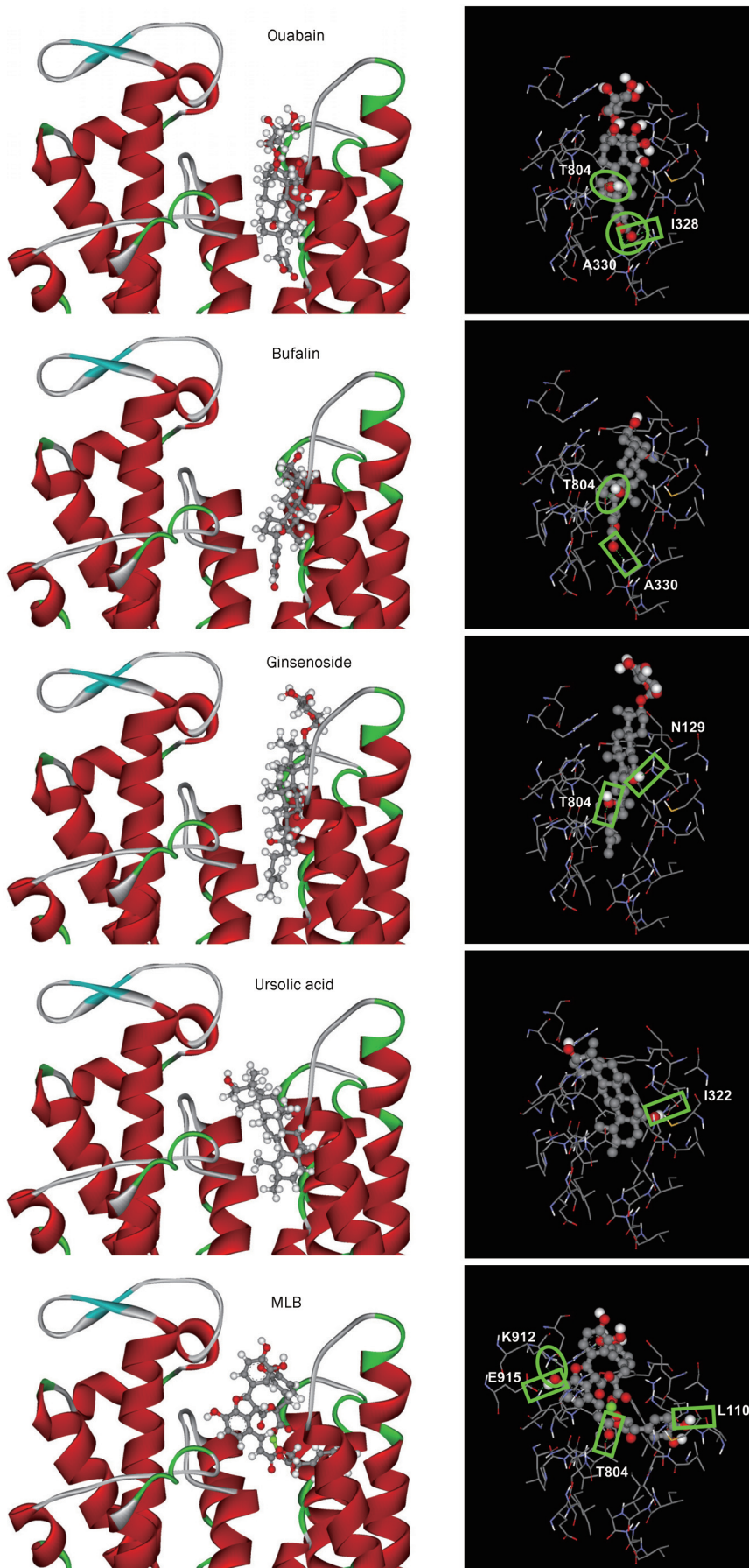
triol, oleanolic acid, and ocotillo<sup>[77]</sup>. Several biological activities, such as neuroprotective effects, antitumour activity, and cardiac therapeutic effects, have been documented for many ginsenosides<sup>[78, 79]</sup>. Ginsenosides have also been regarded as the active ingredients in many Chinese herbs, for instance, ginseng and sanqi (the roots of *Panax ginseng* and *Panax notoginseng*), two well-known traditional Chinese herbs commonly used for the treatment of coronary heart disease and cerebral vascular disease<sup>[80-82]</sup>.

Based on experimental observation and theoretical modeling, the therapeutic effects of ginseng and sanqi in promoting blood circulation should be at least partly attributed to the effective inhibition of  $Na^+/K^+$ -ATPase by ginsenosides<sup>[83]</sup>, such as ginsenoside Rh2. However, different sugar moieties in ginsenosides affect their inhibitory potency on  $Na^+/K^+$ -ATPase. In our study, ginsenosides with sugar moieties attached only to the C-3 position of the steroid-like structure, equivalent to the sugar position in cardiac glycosides, possess inhibitory potency on  $Na^+/K^+$ -ATPase. However, their inhibitory potency is significantly reduced or completely abolished when a monosaccharide was linked to the C-6 or C-20 position of the steroid-like structure; the inhibitory potency is lost completely when the monosaccharide is replaced by a disaccharide molecule at either positions. Molecular modeling and docking show that sugar attachment to the C-6 or C-20 position of the steroid-like structure apparently causes steric hindrance to the entrance of ginsenosides into the extracellular binding pocket of the  $Na^+/K^+$ -ATPase  $\alpha$  subunit, and thus greatly reduces or completely abolishes their inhibitory potency.

Paradoxically, most ginsenosides found in ginseng and sanqi do not seem to be competent inhibitors of  $Na^+/K^+$ -ATPase due to their sugar attachment to the C-6 or C-20 position of the steroid-like structure. Nevertheless, ginsenosides might act as prodrugs, as they tend to be metabolized to their active forms by intestinal bacterial deglycosylation after oral administration<sup>[84]</sup>. Generally, the metabolites, easily absorbed by the intestines due to an increase of hydrophobicity after deglycosylation, might display the same or different pharmacological actions in comparison with their parent compounds<sup>[85]</sup>. Therefore, the cardiac therapeutic effects of ginseng and sanqi could very possibly be attributed to the effective inhibition of  $Na^+/K^+$ -ATPase by the metabolized ginsenosides, with sugar moieties attached only to the C-3 position of the steroid-like structure.

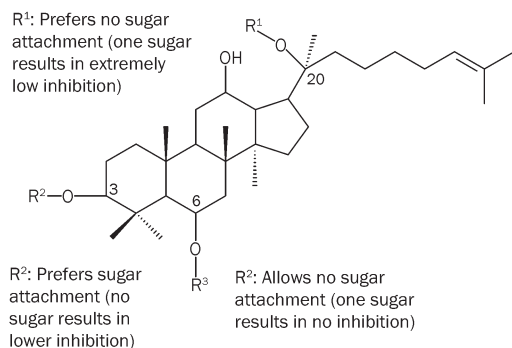
### Combinational usage of danshen and sanqi for the treatment of cardiovascular diseases

In contrast with western medicines, combinational usage of traditional Chinese medicines of similar or different therapeutic effects is a common practice. Currently, several commercial products for cardiovascular diseases comprise mainly danshen and sanqi in variable ratios, mostly with danshen as the major and sanqi the minor constituents<sup>[86]</sup>. The active ingredients in danshen and sanqi for cardiac therapeutic effects are MLB and ginsenosides, respectively. MLB, a polyphenolic compound, is metabolized within a few hours after consumption<sup>[87]</sup>. In



**Figure 4.** Detailed molecular interactions between the extracellular binding pocket of  $\text{Na}^+/\text{K}^+$ -ATPase and ouabain, bufalin, ginsenoside Rh2, ursolic acid, or MLB (Left panels) Modeling is displayed for ligand compounds, ouabain, bufalin, ginsenoside Rh2, ursolic acid, and MLB binding to the extracellular pocket of  $\text{Na}^+/\text{K}^+$ -ATPase  $\alpha$  subunit. Amino acid residues around the binding pocket of  $\text{Na}^+/\text{K}^+$ -ATPase are shown in ribbon structure, and ligand compounds in stick structure. (Right panels) Amino acid residues of  $\text{Na}^+/\text{K}^+$ -ATPase close to ligand compounds (ball-and-stick structure) are shown in line structure. Green box or oval represents one or two hydrogen bonds formed between  $\text{Na}^+/\text{K}^+$ -ATPase and ligand compounds. (Adopted and modified from Figure 6 of Chen et al. *Acta Pharmacol Sin* 2010; 31: 696–702 and Figure 5 of Chen et al. *Acta Pharmacol Sin* 2010; 31: 923–9).





**Figure 5.** A summarized diagram for the effects of sugar attachments in three different positions of the steroid nucleus of ginsenosides. Detailed 3D diagrams are shown in Figures 4, 5, and 6 of Chen et al. *Acta Pharmacol Sin* 2009; 30: 61–9.

contrast, it takes a couple of days to metabolize the steroid-like ginsenosides in human body<sup>[88, 89]</sup>. In light of our recent studies, we rationalize that the combinational usage of danshen and sanqi for the treatment of cardiovascular diseases takes the advantages of the non-toxic and yet strong Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitory potency of MLB to relieve the symptoms promptly and the moderate Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibitory potency of ginsenosides to provide a relatively long-term basal therapeutic effect.

### Inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase and its potential neuroprotection

In brain cells, approximately half of all energy is spent for the active exchange of cytosolic sodium for extracellular potassium, a process carried out by Na<sup>+</sup>/K<sup>+</sup>-ATPase for the maintenance of transmembrane ionic gradients for all mammalian cells<sup>[90–92]</sup>. As the brain's primary consumer of ATP, Na<sup>+</sup>/K<sup>+</sup>-ATPase is particularly vulnerable to ATP depletion commonly observed in ischemic stroke. This vulnerability suggests that pharmacological inhibition of the Na<sup>+</sup>/K<sup>+</sup>-ATPase in brain cells should further compromise ATP-depleted neurons. Indeed, there is accumulating evidence that inhibiting the brain Na<sup>+</sup>/K<sup>+</sup>-ATPase can actually provide neuroprotection in the context of ischemia<sup>[93]</sup>.

Cardiac glycosides, being potent inhibitors of Na<sup>+</sup>/K<sup>+</sup>-ATPase, have been demonstrated to provide neuroprotection against ischemic stroke in a cortical brain slice-based compound screening platform<sup>[94]</sup>. Moreover, it has been hypothesized that blockade of Na<sup>+</sup>/K<sup>+</sup>-ATPase may provide neuroprotective effect in ischemia-reperfusion through ATP conservation and modulating intracellular calcium levels just as the cardiac glycosides do in the heart contraction cycle<sup>[93]</sup>. Similar neuroprotective effects have also been documented for ginsenosides against ischemic stroke, and some of the results were observed using the same brain slice assay model<sup>[95–99]</sup>. Oleanolic acid, a steroid-like compound and moderate inhibitor of Na<sup>+</sup>/K<sup>+</sup>-ATPase, also displayed neuroprotective effect against focal cerebral ischemic injury<sup>[100]</sup>. Furthermore, the same phenomenon was observed when we examined the neu-

roprotective effect of MLB against ischemic stroke in a similar brain slice assay model<sup>[74]</sup>. Taken together, these experimental data support that inhibiting Na<sup>+</sup>/K<sup>+</sup>-ATPase may provide neuroprotection in the context of ischemia as well as other neurodegenerative conditions. It is more than likely that effective inhibitors of Na<sup>+</sup>/K<sup>+</sup>-ATPase in the brain are potential drugs for the treatment of ischemic stroke.

### Conclusions and perspectives

With a history of at least 2000 years, traditional Chinese medicines have long been regarded as precious resources for screening new drugs. Our recent studies have shown that steroid-like compounds in many Chinese medicines used for promoting blood circulation display more or less inhibitory potency on Na<sup>+</sup>/K<sup>+</sup>-ATPase, and may thus provide the therapeutic effects of corresponding medicines via the same molecular mechanism triggered by cardiac glycosides. Except bufalin in ChanSu, steroid-like compounds in traditional Chinese medicines used for promoting blood circulation are expected to exert less severe side effects than cardiac glycosides. Nevertheless, side effects have to be expected of a continuous consumption of these steroid-like compounds for a long period time. In contrast, MLB, the non-steroid active ingredient responsible for the cardiac therapeutic effect of danshen by effective inhibition of Na<sup>+</sup>/K<sup>+</sup>-ATPase, is generally regarded as a non-toxic antioxidant without apparent adverse effects. Therefore, we believe that MLB is of great potential to replace cardiac glycosides for the treatment of congestive heart failure, provided it undergoes necessary clinical trials. Moreover, searching from traditional Chinese medicines for additional antioxidant polyphenolic compounds possessing inhibitory potency on Na<sup>+</sup>/K<sup>+</sup>-ATPase may lead to the discovery of novel drugs for the treatment of cardiovascular diseases without side effects.

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