Active Compounds in Chinese Herbs and Medicinal Animal Products Which Promote Blood Circulation via Inhibition of Na^+, K^+-ATPase

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The therapeutic effect of cardiac glycosides for congestive heart failure lies in their reversible inhibition on Na^+, K^+-ATPase located in human myocardium. Several steroid-like compounds containing a core structure similar to cardiac glycosides have been found in many Chinese herbs and medicinal animal products conventionally used to promote blood circulation. They are putatively responsible for the therapeutic effect of those medicinal products via the same mechanism of inhibiting Na^+, K^+-ATPase. Inhibitory potency on Na^+, K^+-ATPase by ginsenosides, one of the identified steroid-like compounds, is significantly affected by sugar attachment that might cause steric hindrance of their binding to Na^+, K^+-ATPase. Ginsenosides with sugar moieties attached only to the C-3 position of the steroid-like structure, equivalent to the sugar position in cardiac glycosides, substantially inhibit Na^+, K^+-ATPase. However, their inhibitory potency is abolished when sugar moieties are linked to the C-6 or C-20 position of the steroid-like structure. In contrast, no appreciable contents of steroid-like compounds are found in danshen, a well-known Chinese herb traditionally regarded as an effective medicine promoting blood circulation. Instead, magnesium lithospermate B (MLB), the major soluble ingredient in danshen, is assumed to be responsible for the therapeutic effect by inhibiting Na^+, K^+-ATPase in a manner comparable to cardiac glycosides. Neuroprotective effects of cardiac glycosides, ginsenosides and MLB against ischemic stroke were accordingly observed in a cortical brain slice-based assay model. Whether the neuroprotection is also triggered by inhibition of Na^+, K^+-ATPase remains to be investigated. Molecular modeling suggests that cardiac glycosides, ginsenosides and MLB presumably bind to the same extracellular pocket of the Na^+, K^+-ATPase α subunit. *(Chang Gung Med J 2010;33:126-36)*

Key words: cardiac glycoside, ginsenoside, magnesium lithospermate B, Na^+, K^+-ATPase, promoting blood circulation

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Received: Apr. 28, 2009; Accepted: Sep. 8, 2009
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Na^+, K^+-ATPase and cardiac glycosides

Gradients of Na^+ and K^+ across the plasma membrane of animal cells are important for maintaining membrane potentials, cell volume, and active transport of other solutes.[1] Homeostasis of these two gradients is maintained by a specialized pump termed Na^+, K^+-ATPase that commonly consumes 20-30% of the adenosine triphosphate (ATP) energy generated in animal cells at rest to actively transport Na^+ out of and K^+ into cells. Na^+, K^+-ATPase belongs to the family of P-type cation pumps, and generally consists of a heterodimer of α- and β-subunits. Isoforms of α- and β-subunits encoded by individual genes have been found to be expressed in different tissues and cell types in mammals.[2,3] In the kidney outer medulla, the γ-subunit, belonging to the FXYD family, is often associated with the αβ complex as a third subunit and regulates the pumping activity in a tissue- and isoform specific manner.[4,5] Recently, the three dimensional structure of the pig renal Na^+, K^+-ATPase αβγ complex was resolved by X-ray crystallography.[6]

Cardiac glycosides, e.g., ouabain and digoxin, have been applied in the treatment of congestive heart failure for more than two centuries since William Withering published his famous monograph in 1785.[7] The therapeutic effect of cardiac glycosides lies in their reversible inhibition on the membrane-bound Na^+, K^+-ATPase located in human myocardium.[8,9] So far, the detailed binding site for cardiac glycosides on the α-subunit of Na^+, K^+-ATPase has not been properly defined. Based on molecular modeling and docking, two different locations for ouabain binding in the α-subunit of Na^+, K^+-ATPase have been proposed. Cerri et al. proposed that ouabain penetrated into and bound with transmembrane regions,[10] that is, both the extracellular loops of the α-subunit and the interprotodimeric cleft constituted by the transmembrane helices of both subunits. In contrast, Qiu et al. predicted that ouabain lay on the surface of the transmembrane ion channel without penetration,[11] i.e., ouabain was mainly located in the area surrounded by some extracellular loops of the α-subunit and a few amino acid residues of the transmembrane helices exposed to the extracellular space.

The molecular mechanism responsible for the therapeutic effect of cardiac glycosides through inhibition of Na^+, K^+-ATPase causes accumulation of sodium in cardiac cells which are enforced to promote the sodium-calcium exchange system in the cell membrane, thus leading to a higher level of intracellular and myocardial calcium concentration (Fig. 1).[7] The elevated intracellular calcium concentration results in increased inotropism, accentuating the force of myocardial contraction by increasing the velocity and extent of sarcomere shortening, thus translating into increased stroke work for a given filling volume of pressure. Severe side effects have been reported for cardiac glycosides, and safe administration of these drugs has been regarded as a difficult task because of their narrow safety margin.[7,8] In the past two decades, extensive effort has been made to develop novel cardiotonic agents such as new digitalis-like molecules through chemical synthesis and modification.[12-16] As these derivatives possess the same or similar steroid backbones, side effects are unlikely to be eliminated.

Steroid-like compounds in Chinese herbs and medicinal animal products which promote blood circulation

Many Chinese herbs and medicinal animal products have been conventionally used to promote blood circulation, and thus we speculate that some of these medicines may possess therapeutic effects via inhibition of Na^+, K^+-ATPase in a manner similar to cardiac glycosides. Reported steroid-like compounds include bufalin in Chan-Su (*Venenum Bufonis*) (Fig. 2),[17] ginsenosides in ginseng and sanqi (*Panax ginseng and Panax notoginseng*),[18] oleanolic acid and ursolic acid in selfheal (*Prunella vulgaris* L.),[19] saikosaponin A in Chaihu (*Bupleuri Radix*),[20] cholic acid in bear bile,[21] sarsasapogenin in longstamen onion bulb (*Allium macrostemon*),[22] polygalacic acid in yuanzhi (*Polygala tenuifolia*),[23] jujuboside B in suanzoren (*Ziziphus jujuba Mill. var. spinosa*),[24] glycyrrhizin in Glycyrrhiza radix (*Glycyrrhiza glabra* L.),[25] and astragaloside III in Huang-qi (*Astragalus membranaceus*).[26] In a preliminary in vitro assay, all these steroid-like compounds possessed more or less inhibitory activities on Na^+, K^+-ATPase (unpublished data), and thus were proposed to act as active ingredients which are at least partly responsible for promoting blood circulation in their corresponding medicinal sources.

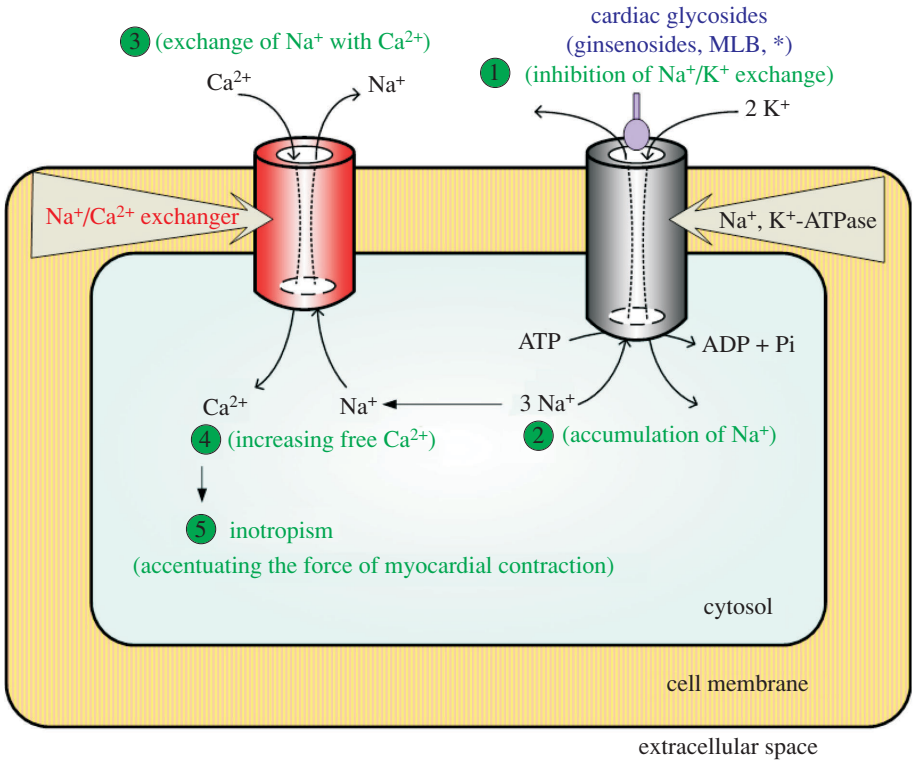

Fig. 1 Proposed molecular mechanism responsible for the therapeutic effects of cardiac glycosides, ginsenosides, MLB, and other steroid-like compounds in cardiac cells. Step 1: The cellular exchange of Na^+ and K^+ is inhibited by drug binding to Na^+, K^+-ATPase. Step 2: Na^+ accumulates in the intracellular space because of inhibition of Na^+, K^+-ATPase activity. Step 3: The cellular exchange of Na^+ and Ca^{2+} is promoted via the Na^+/Ca^{2+} exchanger system. Step 4: The intracellular Ca^{2+} concentration increases owing to activation of the Na^+/Ca^{2+} exchanger system. Step 5: The elevated intracellular Ca^{2+} concentration leads to an increased inotropism and accentuates the force of myocardial contraction. (Adopted and modified from the cover page for Tzen et al. Acta Pharmacol Sin 2007;28:609-15).

Inhibition of Na^+, K^+-ATPase by ginsenosides

Ginseng and sanqi (the roots of *Panax ginseng* and *Panax notoginseng*) are two well-known traditional Chinese medicinal herbs that have been used extensively in several Asian countries for thousands of years. Belonging to the same genus, ginseng and sanqi possess similar constituents including their unique active ingredients, ginsenosides.[27,28] Ginsenosides are triterpene saponins that have a common four-ring hydrophobic steroid-like structure with sugar moieties attached mostly at the C-3, C-6 and C-20 positions.[29] Several biological activities, such as neuroprotective effects, antitumour activity, and cardiac therapeutic effects, have been documented for many ginsenosides.[30-32] The different sugar moieties in ginsenosides are assumed to provide specificity for the diverse therapeutic effects of various ginsenosides. In spite of certain distinct remedial usages, comparable therapeutic effects, such as promotion of blood circulation, have been reported for ginseng and sanqi.[33]

Based on experimental observation and theoretical modeling, we propose that 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.[34] 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 activity (Fig. 3A). The inhibitory potency of ginsenosides on porcine Na^+, K^+-ATPase is evidently lower than that of ouabain: the IC_{50} of ouabain (0.45 μm) is approximately 120 times lower than that of

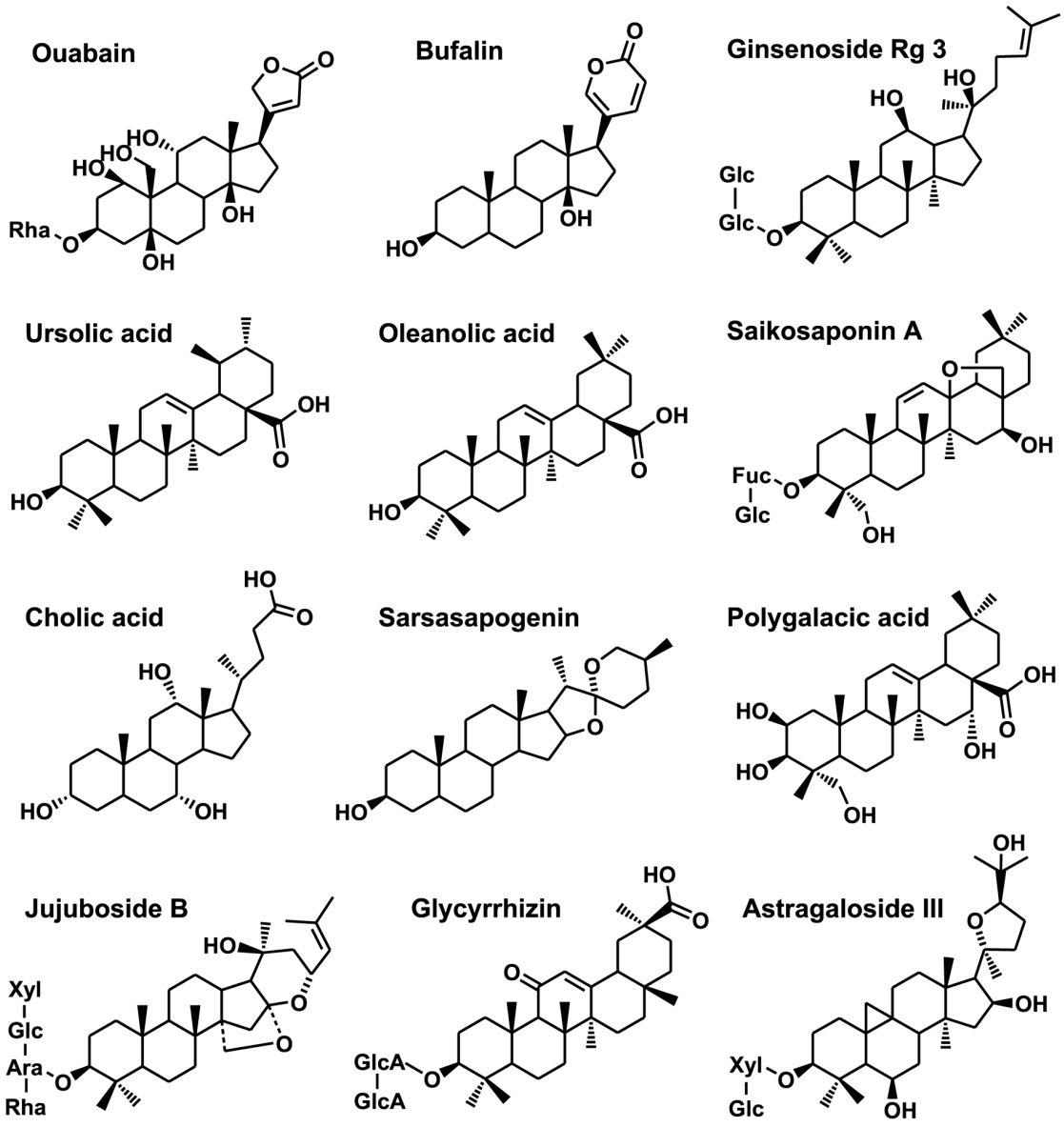

Fig. 2 Chemical structures of ouabain and 11 steroid-like compounds containing a core structure similar to cardiac glycosides from Chinese herbs and medicinal animal products.

ginsenoside Rh2 (55 μm), the strongest inhibitor of Na^+, K^+-ATPase among the ginsenosides examined in the study.[34]

Sugar attachment to the C-6 or C-20 position of the steroid-like structure apparently causes steric hindrance of the entrance of ginsenosides into the extracellular binding pocket of the Na^+, K^+-ATPase α subunit, and thus greatly reduces or completely abolishes their inhibitory potency (Fig. 3B). Paradoxically, most ginsenosides found in ginseng and sanqi do not seem to be competent inhibitors of Na^+, K^+-ATPase because of their sugar attachment to the C-6 or C-20 position. Nevertheless, ginsenosides may act as prodrugs as they tend to be metabolized to their active forms by intestinal bacterial deglycosylation after oral administration.[29] Commonly, the metabolites are

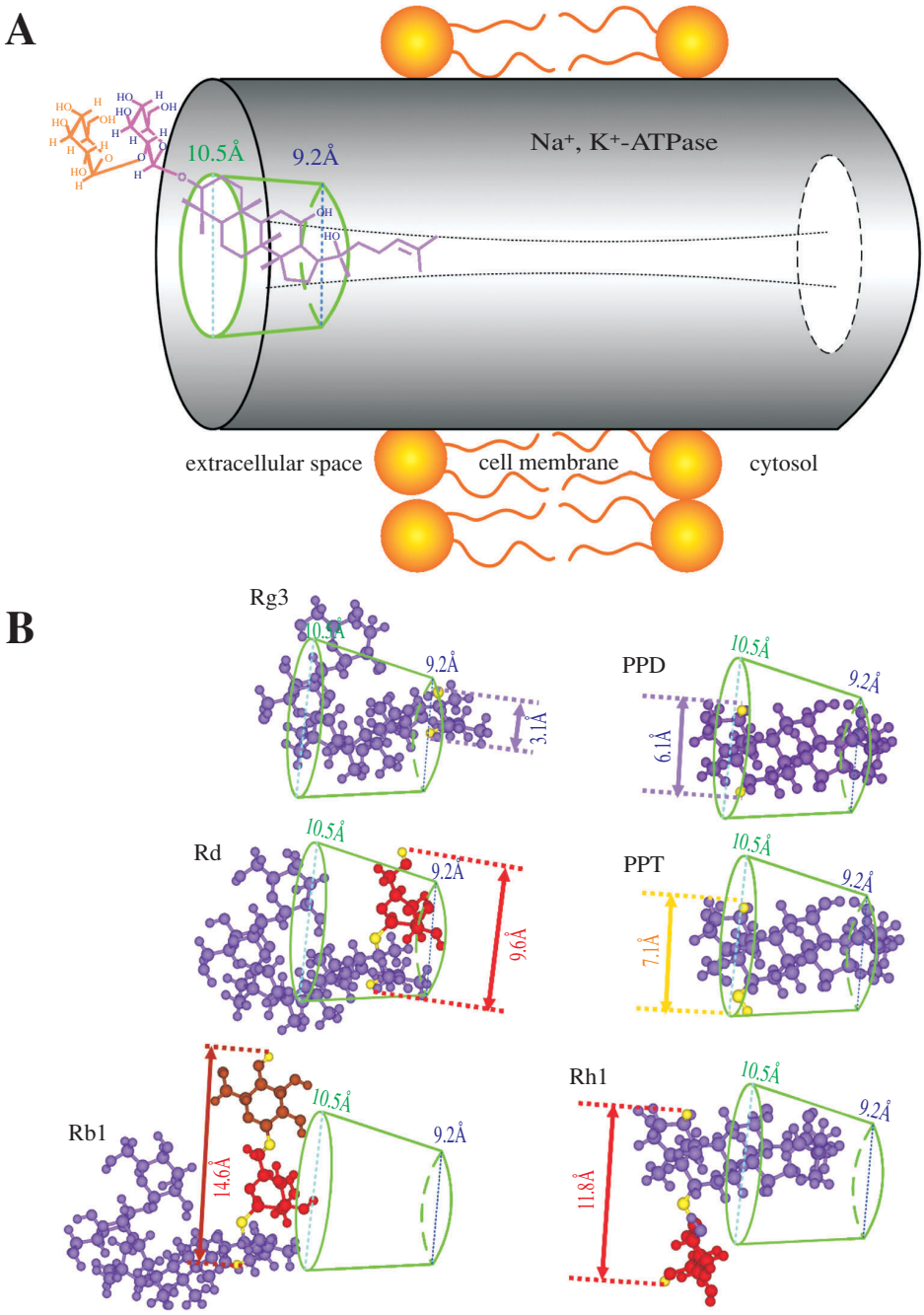

Fig. 3 (A) Modeling of ginsenoside Rg3 binding to the extracellular pocket of the Na^+, K^+-ATPase α subunit. The core steroid-like structure of ginsenoside Rg3 was trapped in a cave (shown in green with dimensional limitations of 10.5Å for the top and 9.2Å for the bottom) of the extracellular pocket of the Na^+, K^+-ATPase α subunit. (B) Molecular fitness of representative ginsenosides in the cave of the extracellular binding pocket of the Na^+, K^+-ATPase α subunit. The glucose molecules attached to the C-6 and C-20 positions of the steroid-like structure are shown in red and brown. Yellow balls indicate the extended dimensions of ginsenosides after hydroxylation (PPT) or glycosylation (Rh1, Rd, and Rb1) at the C-6 or C-20 positions. (Adopted and modified from Figures 4 and 6 of Chen et al. Acta Pharmacol Sin 2009;30:61-9)

easily absorbed by the intestines due to an increase of hydrophobicity after deglycosylation, and may display the same or different pharmacological actions in comparison with their parent compounds.[35,36]

Ginsenosides have also been demonstrated as pharmacologically active ingredients responsible for the effects of ginseng on the central and peripheral nervous systems.[30] It has been reported that ginsenosides have reversible and selective inhibitory effects on voltage-dependent ion channels (such as Ca^{2+}, K^+ and Na^+ channels) and ligand-gated ion channels (such as N-methyl-D-aspartate, some subtypes of nicotinic acetylcholine, and 5-hydroxytryptamine type 3 receptors), although little is known about the exact mechanisms. As the inhibition of Na^+, K^+-ATPase also leads to a fluctuation of Ca^{2+}, K^+ and Na^+ concentrations, it will be interesting to see if there is any cross-talk among these ion channels after inhibition by ginsenosides, which leads to the pharmacological actions of ginseng and sanqi.

Inhibition of Na^+, K^+-ATPase by magnesium lithospermate B

Danshen, the dried root of the medicinal plant *Salvia miltiorrhiza*, is one of the most popular Chinese herbal products used in medicine preparations and formulae in certain Asian countries. Traditionally regarded as an effective medicine for eliminating blood stasis, relieving pain, promoting blood flow, stimulating menstrual discharge, and relaxing the mind, danshen has been extensively used in the treatment of coronary heart disease, heart failure, myocardial infarction, other cerebrovascular diseases, and menstrual disorders.[37]

In contrast with those Chinese herbs and medicinal animal products in Fig. 2 that presumably promote blood circulation by their steroid-like compounds, danshen does not contain appreciable amounts of any steroid-like compounds. Instead, the water-soluble components of danshen have attracted growing attention on the basis of their reported medicinal potency, although some lipid-soluble constituents in this herb, such as tanshinones, have been conventionally considered the active ingredients.[38-40] Among the water-soluble components, magnesium lithospermate B (MLB), a derivative of caffeic acid tetramer, is the major soluble ingredient in danshen, and has been demonstrated to possess several medicinal effects, such as vasodilating, antihypertensive, antioxidative, and free radical scavenging activities.[41-46]

Ouabain is a cardiac glycoside with a rigid structure because of its steroid backbone. Similarly, MLB also possesses a relatively rigid structure because of the formation of salt bridges between Mg^{2+} and the four oxygen atoms of carboxyl groups from the four caffeic acid fragments (Fig. 4). The molecular organization and configuration of ouabain and MLB in the 3D structures are somewhat similar from a particular viewpoint (lower portions of their 3D structures), 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 may trigger the same molecular mechanism responsible for the therapeutic effect of cardiac glycosides via the reversible inhibition of Na^+, K^+-ATPase.[47]

Neuroprotection by cardiac glycosides, ginsenosides and MLB

Cardiac glycosides were recently demonstrated to provide neuroprotection against ischemic stroke in a cortical brain slice-based compound screening platform.[48] In this brain slice assay model, neuroprotective activity and delayed therapeutic potential were observed for neriifolin as well as other cardiac glycosides. The same phenomenon was observed when we examined the neuroprotective effect of MLB against ischemic stroke in a similar brain slice assay model (Fig. 5).[47] Furthermore, a protective effect against cerebral ischemia-reperfusion injury and attenuation of the infarct area in a middle cerebral artery occlusion animal model by a mixture of total salvianolic acids extracted from danshen have also been reported.[49] Similar neuroprotective effects have been reported for ginsenosides against ischemic stroke, and some of the results were observed using the same brain slice assay model.[50-54] It remains to be investigated whether cardiac glycosides, ginsenosides and MLB exert neuroprotection against ischemic stroke via the same mechanism triggered by the inhibition of Na^+, K^+-ATPase.

Modeling of ouabain, ginsenoside Rg3 and MLB binding to Na^+, K^+-ATPase

Molecular modeling and docking of ouabain,

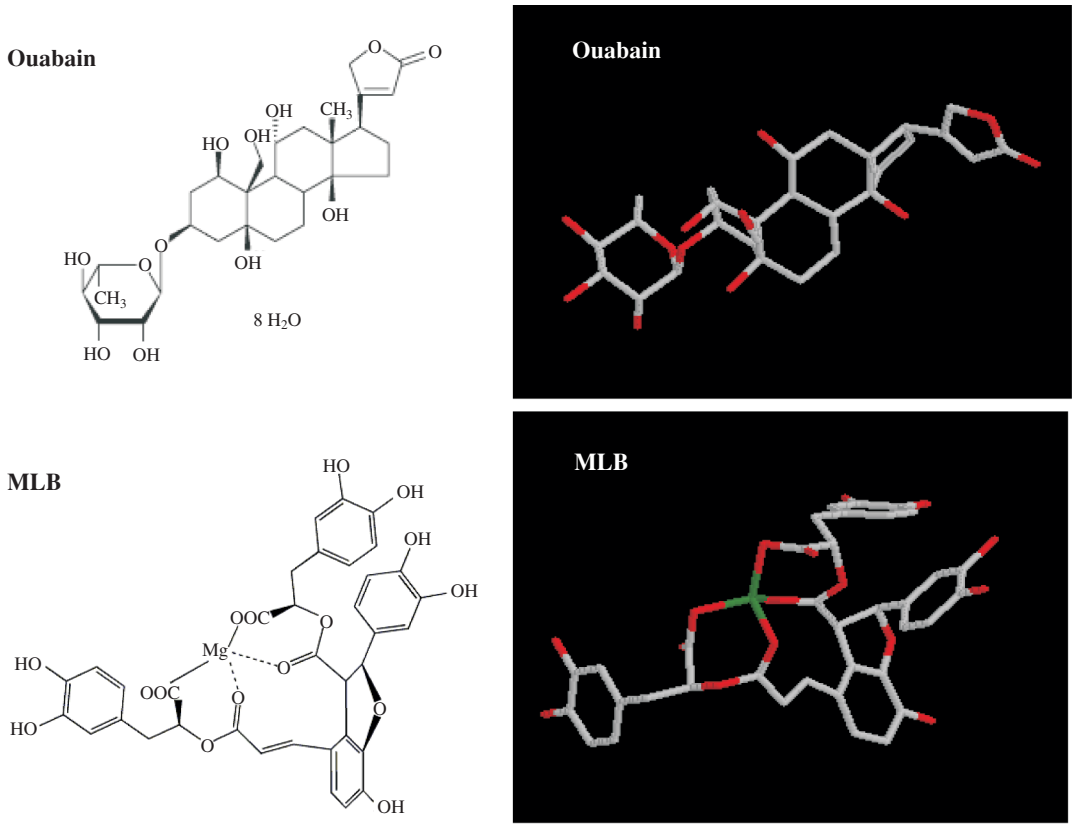

Fig. 4 Chemical structures of ouabain and MLB. The 3D structures of ouabain and MLB (dark background) are displayed using RasWin Molecular Graphics Windows Version 2.6. C, O, and Mg^{+2} atoms are shown in gray, red and green, respectively. (Adopted from Figure 1 of Tzen et al. Acta Pharmacol Sin 2007;28:609-15)

ginsenoside Rg3 and MLB to Na^+, K^+-ATPase suggest that these three inhibitors seem to bind to the same pocket located in the extracellular domain of the α subunit of Na^+, K^+-ATPase (Fig. 3 and 6). The core steroid-like structure of ouabain or ginsenoside Rg3 is trapped in a cave (shown in green in Fig. 3 with dimensional limitations of 10.5Å for the top and 9.2Å for the bottom) of the extracellular pocket. Detailed molecular interactions between the extracellular pocket of the Na^+, K^+-ATPase α subunit and the active compounds which promote blood circulation, including cardiac glycosides, ginsenosides, MLB, and other steroid-like compounds depicted in Fig. 2, are being investigated and will be compared after refinement.

Concluding remarks

Cardiac glycosides are drugs clinically used to relieve the symptoms of congestive heart failure.[55] Although these compounds unquestionably improve the condition of patients, safe administration of these drugs has been regarded as a difficult task because of their narrow safety margin and severe side effects. Comparably, side effects are probably similar with the utilization of ginsenosides and other steroid-like compounds shown in Fig. 2 which promote blood circulation, although the side effects are expected to be much less severe because of their relatively low affinity to Na^+, K^+-ATPase compared with cardiac glycosides.[34] In contrast to cardiac glycosides, MLB has been considered an antioxidant without significant adverse effects.[38] Therefore, we believe that MLB has great potential to replace cardiac glycosides in the treatment of congestive heart failure, provided it undergoes the necessary clinical trials. Of course, identification of the signal transduction path-

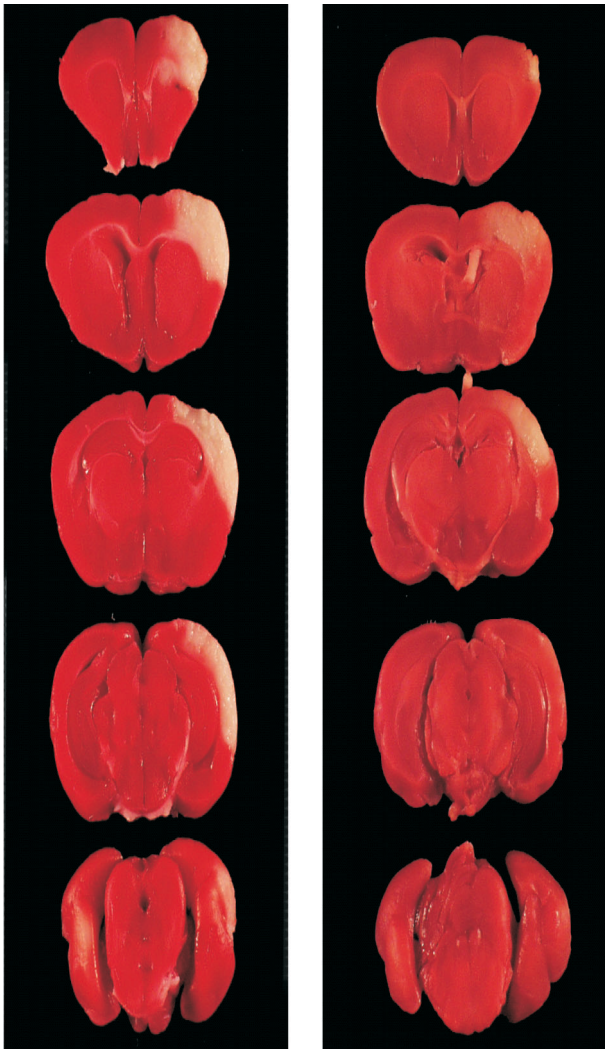

Fig. 5 Neuroprotective effect of MLB on infarct sizes of gerbil brains in cerebral ischemia. The infarct sizes were visualized by staining with a 2% solution of 2,3,5-triphenyltetrazolium chloride. (Adopted and modified from Figure 5 of Tzen et al. Acta Pharmacol Sin 2007;28:609-15)

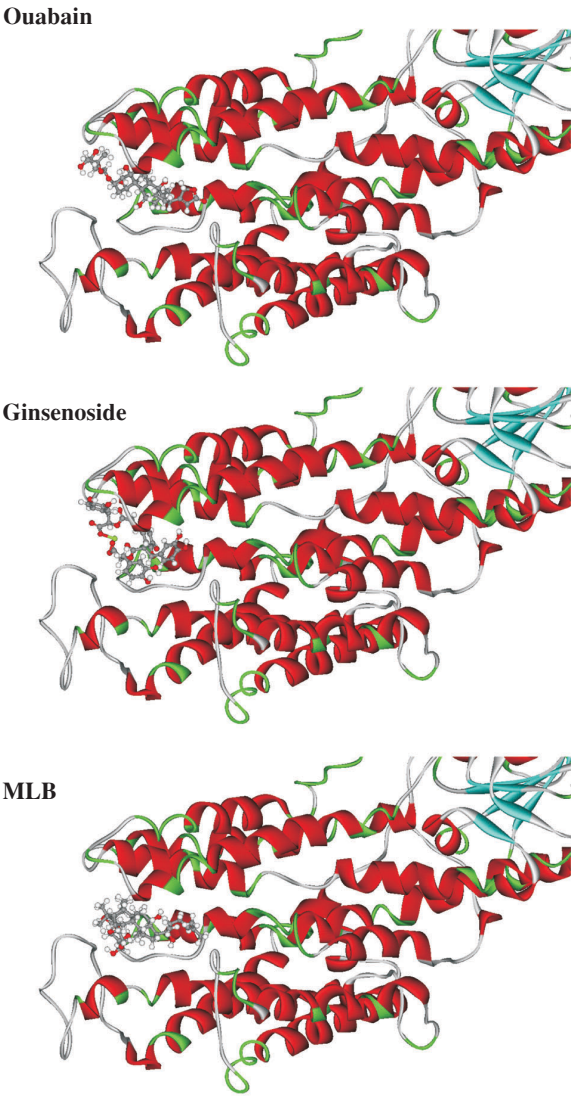

Fig. 6 Molecular modeling of the binding of ouabain, ginsenoside Rg3 and MLB to the extracellular pocket of the Na^+, K^+-ATPase α subunit. Relative locations of molecules are comparable to those shown in Fig. 3A. Ouabain, ginsenoside Rg3 and MLB are displayed in a ball-&-stick style.

way via the inhibition of Na^+, K^+-ATPase by MLB and assessment of other stimulatory effects in the brain of this potential drug are also interesting and challenging tasks.

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經由抑制鈉鉀幫浦達成行血功能的中藥材有效成分

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強心配醣體臨床上治療鬱血性心臟衰竭的分子機制是藉由抑制心肌細胞膜上的鈉鉀幫浦。許多用於活血化瘀的傳統中草藥材裏，如蟾酥、人參、三七、夏枯草、柴胡、熊膽、薤白、遠志、酸棗仁、甘草、黃耆等發現一些與強心配醣體相近的似固醇皂苷成分；推論這些似固醇皂苷成分，例如人參皂苷，應該也是藉由與強心配醣體一樣的分子機制來達成行血功能。因立體障礙之故，不同部位醣基化對人參皂苷抑制鈉鉀幫浦活性有明顯差異：僅在似固醇皂苷構造 C-3 位置醣基化的人參皂苷對於鈉鉀幫浦具有相當好的抑制性，而在似固醇皂苷構造 C-6 或 C-20 位置醣基化的人參皂苷則不具抑制鈉鉀幫浦活性。然而，中草藥材裏廣泛用於活血化瘀的丹參之主要成分中，並未發現似固醇皂苷成分。取而代之的是其水萃物中的主要成分丹參酚酸 B 鎂鹽，推論此成分亦是藉由抑制鈉鉀幫浦的分子機制來達成行血功能。模擬人類中風的腦缺血動物試驗中發現，強心配醣體、人參皂苷、丹參酚酸 B 鎂鹽均具有抗缺血、保護神經細胞的作用；此功效是否與抑制鈉鉀幫浦相關，仍有待後續研究證實。電腦 3D 影像模擬顯示，此三類藥用分子與鈉鉀幫浦胞外區間結合的位置應該是相同的。(長庚醫誌 2010;33:126-36)

關鍵詞：強心配醣體，人參皂苷，丹參酚酸 B 鎂鹽，鈉鉀幫浦，活血化瘀

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