Magnesium Lithospermate B Extracted from *Salvia Miltiorrhiza*, A Potential Substitute for Cardiac Glycosides

Tse-yu Chung[1], Feng-yin Li[2], Wen-Hua Chiou[2] and Jason T.C. Tzen[1,3,4,*]

[1]*Graduate Institute of Biotechnology and* [2]*Department of Chemistry, National Chung Hsing University, Taichung, 40227, Taiwan;* [3]*School of Chinese Medicine, China Medical University, Taichung, 40402, Taiwan;* [4]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei 11529, Taiwan*

Abstract: The therapeutic effect of cardiac glycosides lies in their reversible inhibition on the membrane-bound Na^+/K^+-ATPase in human myocardium. Unfortunately, the utilization of steroidal cardiac glycosides suffers some severe adverse side effects. Magnesium lithospermate B (MLB), a derivative of caffeic acid tetramer found in Danshen (*Salvia miltiorrhiza*), is suggested to provide a comparable cardiac therapeutic effect by effectively inhibiting Na^+/K^+-ATPase via the same molecular mechanism triggered by cardiac glycosides. Molecular modeling suggests that ouabain, a cardiac glycoside, and MLB presumably bind to the same extracellular pocket of the Na^+/K^+-ATPase. Moreover, MLB outperforms ouabain in cell toxicity, and thus has a great potential, with extensive clinical trials, to become a safe substitute for cardiac glycosides. Neuroprotective effects of cardiac glycosides and MLB against ischemic stroke have been observed, and cumulative data suggest that effective inhibitors of Na^+/K^+-ATPase in the brain may be potential drugs for the treatment of ischemic stroke. Taken together, recent studies on MLB seem to promote a paradigm shift of searching potential drugs for the treatment of cardiac diseases from steroid-like compounds to non-steroid ones.

Keywords: Cardiac glycoside, Danshen, magnesium lithospermate B, Na^+/K^+-ATPase, ouabain, *Salvia miltiorrhiza.*

1. INTRODUCTION

Improvement of the treatment of congestive heart failure, generally defined as incapability of the heart to supply sufficient blood flow to meet the body's needs, is a major medical challenge. Cardiac glycosides, such as ouabain and digoxin, are steroid-like compounds and have been used in the treatment of congestive heart failure for more than two centuries [1]. Their therapeutic effect mainly lies in reversible inhibition on the α-subunit of membrane-bound sodium-potassium pump (Na^+/K^+-ATPase), mainly but not exclusively, located in human myocardium [2, 3]. However, the narrow therapeutic index (the margin between effectiveness and toxicity) of cardiac glycosides apparently limits their clinical applications [4]. The limited benefit with certain side effects of the existing remedies has prompted investigators to search for complementary and alternative therapies and drugs.

The core structure of cardiac glycosides consists of a tetracyclic steroidal framework, which is considered the pharmacophoric moiety responsible for their inhibition on Na^+/K^+-ATPase [5]. Interestingly, a number of steroid-like compounds, such as triterpenoids, steroids and saponins, are also found in many Chinese medicinal products used for promoting blood circulation, and regarded as the active ingredients responsible for their therapeutic effects [6-8]. Although these compounds unquestionably improve the conditions of patients, their arrhythmogenic potential together with a low therapeutic index has been regarded as a serious problem [9, 10]. Efforts have been made to develop novel cardiotonic agents, such as new digitalis-like molecules through chemical synthesis and modification [11, 12]. Unfortunately, these digitalis-like molecules still possess the same or similar steroid backbone, and thus the drawbacks and side effects are unlikely to be eliminated.

Danshen, the dried roots of medicinal plant *Salvia miltiorrhiza*, is one of the most popular Chinese herbal products widely used in many medicine preparations and formulae taken by people in several Asian countries [13, 14]. It was the first traditional Chinese medicine subjected to phase 2 and 3 clinical trials in the USA in 1997. 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 either alone or in combination with other herbal ingredients in the treatment of coronary heart disease, heart stroke, myocardial infarction, menstrual disorder, and other cerebrovascular diseases [15-20].

Recently, magnesium lithospermate B (MLB) was suggested to be responsible for the cardiac therapeutic effect of Danshen by its effective inhibition on Na^+/K^+-ATPase via the same molecular mechanism triggered by cardiac glycosides [21-23]. This finding has an intriguing impact on the future molecular design of cardiotonic agents, especially inhibitors of Na^+/K^+-ATPase. Structurally MLB has no central steroid core, which is commonly shared among the current cardiotonic agents. This creates a new dimension for the structure-activity relationship in the inhibition of Na^+/K^+-ATPase to explore new molecular design rules of future cardiotonic agents. Furthermore, without the steroid core, MLB can provide a way to prevent the possible cardiac arrhythmia, which is the main side effect found in the current cardiotonic prescriptions. Last but not the least, as a derivative of caffeic acid tetramer, MLB can be synthesized through a single reactant species, which provides a great challenge in organic synthesis. In this review, we intend to provide the overview about the cardiac therapeutic effect of MLB through both in vitro study and molecular modeling.

2. 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 [24]. Homeostasis of these two gradients is maintained by a specialized pump termed Na^+/K^+-ATPase, a P-type ATPase also known as sodium pump. This specialized pump commonly consumes 20-30% of the adenosine triphosphate (ATP) energy generated in animal cells at rest to actively transport three Na^+ out of and two K^+ into cells. The x-ray crystal structure of Na^+/K^+-ATPase recently resolved shows that it is composed of three subunits (α, β and γ subunits); and the α catalytic subunit, a 112 kDa protein, contains sites important for ATP binding and phosphorylation as well as ion occlusion and an ouabain-binding site, the primary binding site for many pharmacological agents, such as cardiac glycosides, that affect pump activity [25-28].

*Address correspondence to this author at the Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan (R.O.C.) Tel.: 886-4-22840328 ext. 776; Fax: 886-4-22853527; E-mail: TCTZEN@dragon.nchu.edu.tw

1875-6298/12 $58.00+.00

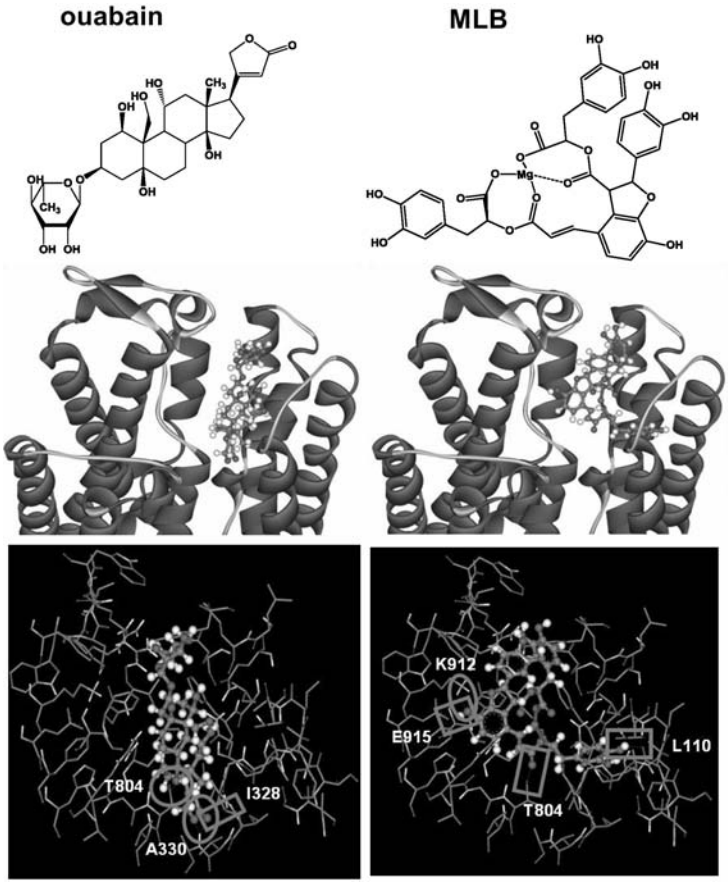


Fig. (1). (Upper panels) Chemical structures of ouabain and MLB. (Middle panels) Modeling of ouabain and MLB binding to the binding pocket of Na^+/K^+-ATPase. The amino acid residues around the binding pocket of Na^+/K^+-ATPase are shown in ribbon structure, and ouabain and MLB in scaled ball and stick. (Lower panels) The amino acid residues of Na^+/K^+-ATPase close to ouabain or MLB are shown in wireframe, and the structures of ouabain and MLB in scaled ball and stick. Box or oval represents one or two hydrogen bonds formed between Na^+/K^+-ATPase and ouabain or MLB. (Adopted and modified from Figure 5 of *Chen et al. Acta Pharmacol Sin 2010; 31: 923-29*)

Cardiac glycosides, such as ouabain and digoxin, are a diverse family of naturally derived compounds that bind to and inhibit Na^+/K^+-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 [29]. The steroid core of cardiac glycosides is double-substituted with an unsaturated lactone ring at position 17 and a sugar portion at position 3; and their lactone moiety is critical for their potent inhibition of Na^+/K^+-ATPase [30].

The therapeutic effect of cardiac glycosides for the treatment of congestive heart failure lies in their reversible inhibition on the membrane-bound Na^+/K^+-ATPase located in human myocardium [2]. Inhibition of the Na^+/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 Na^+/Ca^{2+} exchanger in the cell membrane, which normally uses the sodium gradient for energy in the extrusion of calcium ion [31]. Each cardiac action potential is thus followed by elevated levels of residual intracellular calcium ion. The elevated intracellular calcium concentration results in an 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 [32]. In this way, inhibition of the Na^+/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 [33].

3. MLB IN DANSHEN

Similar to cardiac glycosides, Danshen has been used for the treatment of congestive heart failure as well as other myocardial and cerebrovascular diseases. Ingredients in Danshen are mainly divided into lipid-soluble and water-soluble compounds. Before 1990, identification of active ingredients putatively responsible for biological activities of Danshen was mainly focused on its lipid-soluble constituents, such as diterpenoids and tanshinones [13]. In the past two decades, water-soluble components of Danshen, particularly caffeic acid derivatives, have attracted escalating attention on the basis of their reported medicinal potency [34]. So far, more than 20 caffeic acid derivatives have been isolated from Danshen and structurally determined through chemical and spectroscopic methods [14]. Among these water-soluble components, MLB, a derivative of caffeic acid tetramer, is present as 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 [35, 36].

4. COMPARISON OF CARDIAC GLYCOSIDES AND MLB

4.1. Structural Comparison between Ouabain and MLB

Similar to cardiac glycosides that possess a rigid structure due to their steroid backbone, MLB also possesses a relatively rigid structure due to the formation of salt bridges between Mg^{2+} and the four oxygen atoms of carboxyl groups originated from the four caffeic acid fragments (Fig. **1**, upper panels). The molecular organization and configuration of MLB and ouabain are somewhat com-

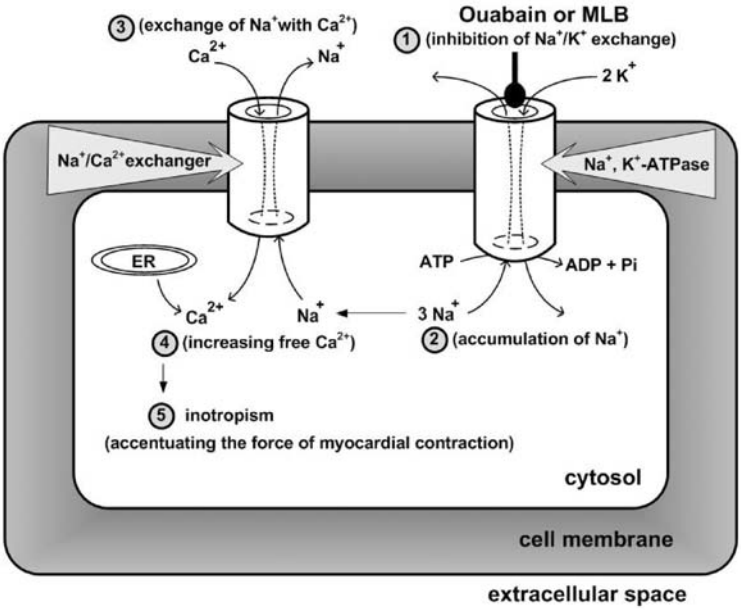


Fig. (2). Proposed molecular mechanism responsible for the therapeutic effects of ouabain, a cardiac glycoside, and MLB in cardiac cells. Step 1: Inhibiting the cellular exchange of Na^+ and K^+ by drug binding to Na^+/K^+-ATPase. Step 2: Accumulation of Na^+ in the intracellular space due to the inhibition of Na^+/K^+-ATPase activity. Step 3: Promotion of the cellular exchange of Na^+ and Ca^{2+} via the Na^+/Ca^{2+} exchanger system. Step 4: Increasing the intracellular Ca^{2+} concentration owing to the activation of the Na^+/Ca^{2+} exchanger system and intracellular release from endoplasmic reticulum (ER). 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*).

parable in their structures, although they are totally different compounds with distinct molecular weights (584.65 for ouabain and 740.67 for MLB, respectively). Structurally, ouabain is composed of three major parts, i.e., the central rigid core, the head part and the tail part. The central rigid core is composed of 4-ring hydrophobic steroid skeleton with the hydroxyl groups. The head part is formed by a five-member lactone ring. The tail part usually contains a carbohydrate moiety. MLB can also be divided into the three sections as found in ouabain. For example, the rigid structure around the salt bridges formed between Mg^{2+} and carboxyl groups as mentioned previously mimics partially the steroid structure of ouabain. One of the three o-hydroxyphenol groups in MLB resembles the lactone head group. The other two o-hydroxyphenol groups of MLB form the tail group, quite different from that of ouabain. With great difference in structure, the comparison between MLB and ouabain regarding to the inhibition potency toward Na^+/K^+-ATPase should be intriguing.

4.2. Inhibition of Na^+/K^+-ATPase by Ouabain and MLB

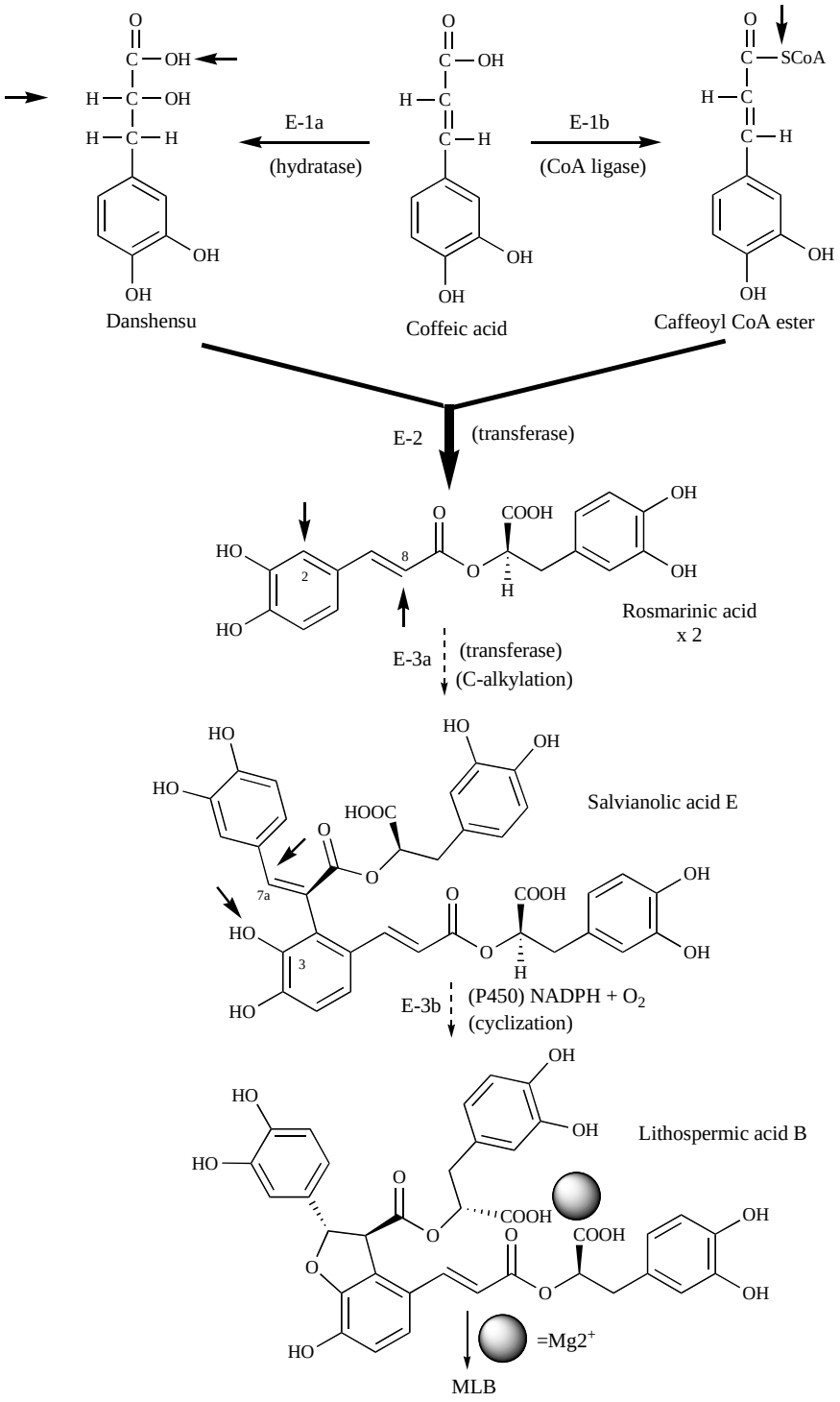
In spite of being a non-steroid compound, MLB possesses potent inhibition on Na^+/K^+-ATPase in vitro [21]. Under the same assay condition, inhibition effectiveness on Na^+/K^+-ATPase equivalent to that for ouabain was observed for MLB with approximately half dosage by weight. It is proposed that ouabain and MLB may lead to a similar therapeutic effect via the same mechanism, i.e., accentuating the force of myocardial contraction by elevating calcium concentration via the inhibition of Na^+/K^+-ATPase (Fig. **2**). In agreement with this proposal, the intracellular 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 [22]. The elevated Ca^{2+} levels seem to be supplied by both extracellular influx through the Na^+/Ca^{2+} exchanger and intracellular release from endoplasmic reticulum.

4.3. Cell Toxicity of Ouabain and MLB

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 [37]. In contrast, MLB, regarded as an antioxidant, possesses no appreciable toxicity [38]. Our studies showed that severe cell toxicity accompanied with dendritic shrink was observed in SH-SY5Y cells treated with ouabain, but not in those treated with MLB [23]. Therefore, we propose that MLB has a great potential, with extensive clinical trials, to become a safe substitute for cardiac glycosides.

4.4. Molecular Modeling of Ouabain and MLB Binding to Na^+/K^+-ATPase

The comparable inhibitory potency between ouabain and MLB on Na^+/K^+-ATPase can be explained with molecular modeling and their docking to the extracellular domain of Na^+/K^+-ATPase α subunit (Fig. **1**, middle and lower panels). The docking results show that MLB is localized in the ouabain binding pocket of Na^+/K^+-ATPase, and that equivalent interaction with the binding cavity of Na^+/K^+-ATPase is observed for ouabain and MLB by forming five intermolecular hydrogen bonds (H-bonds), respectively. Furthermore three H-bonds are formed between the lactone of ouabain and Ile328 (forming one H-bond) and Ala330 (forming two H-bonds) of Na^+/K^+-ATPase, and two H-bonds between the hydroxyl group at C-14 of ouabain and Thr804 of Na^+/K^+-ATPase. In contrast, 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. Evidently, the docking results support that MLB, being a potent inhibitor of Na^+/K^+-ATPase, acts as the active component responsible for the cardiac therapeutic effect of Danshen via the same physiological responses subsequently activated by effective inhibition of cardiac glycosides on Na^+/K^+-ATPase.



Scheme 1. Plausible biosynthetic pathway of MLB. This pathway involves only caffeic acid as the starting material and five enzymatic reactions, E-1a, E-1b, E-2, E-3a, and E-3b. The arrows indicate the functional groups where the next reactions take place. In the last step, MLB is formed by chelating Mg^{2+} to lethospermic acid B.

4.5. Neuroprotection of Cardiac Glycosides and MLB

As the primary consumer of ATP, Na^+/K^+-ATPase in the brain is particularly vulnerable to ATP depletion commonly observed in ischemic stroke. Indeed, accumulating evidence suggests that inhibiting the brain Na^+/K^+-ATPase can actually provide neuroprotection in the context of ischemia [39]. Cardiac glycosides, being potent inhibitors of Na^+/K^+-ATPase, have been demonstrated to provide neuroprotection against ischemic stroke in a cortical brain slice-based compound screening platform in a recent study [40]. Similarly, the same neuroprotective activity and delayed therapeutic potential of MLB were observed also in this brain slice assay model [21]. It remains to be investigated whether cardiac glycosides and MLB exert neuroprotection against ischemic stroke via the same mechanism triggered by the inhibition of Na^+/K^+-ATPase. Of course, identification of the subsequent pathway in signal transduction and assessment of other stimulatory effects of this drug in brain are also interesting and challenging tasks.

5. PLAUSIBLE BIOSYNTHETIC PATHWAY OF MLB

Structurally, MLB is a derivative of caffeic acid tetramer putatively formed by dimerization of rosmarinic acid, and can be synthesized through a single reactant species. On the basis of the biosynthetic pathway of rosmarinic acid [41], we proposed a plausible biosynthetic pathway of MLB including four steps of five enzymatic reactions by using caffeic acid, an intermediate compound in the lignin biosynthesis, as the starting material (Fig. **3**). The first step comprises two reactions: one is to generate danshensu by introducing H_2O into the double bond of caffeic acid (E-1a reaction), and the other is to generate caffeoyl CoA ester by forming a carbon-sulfur bond between the sulfur atom of coenzyme A and the carboxyl acid group of caffeic acid (E-1b reaction). The second step is to generate rosmarinic acid by combining danshensu and caffeoyl CoA ester via a S_N2 reaction (E2 reaction) in which the oxygen atom of the hydroxyl group of danshensu formed in the E-1a reaction attacks caffeoyl CoA ester and leads to the leave of coenzyme A. The third step comprises two reactions: one is to form salvianolic acid E by dimerization of two rosmarinic acid molecules linked together by C-alkylation (E-3a reaction), and the other is to form lithospermic acid B by intramolecular cyclization between C-3 hydroxyl group and C-7a in salvianolic acid E (E-3b reaction). The C-alkylation reaction is initialized by binding a pyrophosphate group to the C-8 of one rosmarinic acid, which is then attacked by the C2 of the other rosmarinic acid with the pyrophosphate group as the leaving group. The intramolecular cyclization reaction is possibly catalyzed by cytochrome P450 in the presence of NADPH and O_2. In the final step, chelation of Mg^{2+} to form salt bridges with the four oxygen atoms of lithospermic acid B leads to the formation of MLB, the major water-soluble compound in Danshen with four chiral carbons. However, the organic synthesis of MLB is still a great challenge due to its complicated structure and the four chiral carbon centers.

6. CONCLUSION

Cardiac glycosides are drugs clinically used to relieve the symptoms of congestive heart failure. Although these compounds unquestionably improve the condition of patients, safe administration of these drugs remains a difficult task because of their narrow safety margin and severe side effects. Recent studies suggest that MLB may provide an alternative solution to the above issue. In contrast with cardiac glycosides, MLB, lack of steroid-like structure and generally considered as an antioxidant without significant adverse effects, possesses the cardiac therapeutic effect with no apparent cell toxicity. It seems that MLB has great potential to be a substitute for cardiac glycosides in the treatment of congestive heart failure, provided it undergoes the necessary clinical trials. Of course, the assessment of other stimulatory effects in the brain of this potential drug is also an interesting and challenging task. Moreover, the proposed substitution of cardiac glycosides with MLB may promote a paradigm shift of searching potential drugs for the treatment of cardiac diseases from steroid-like compounds to non-steroid ones. It is expectable that other natural or synthetic non-steroid compounds will be reveled and used as substitutes for cardiac glycosides in the follow-up investigation.

ACKNOWLEDGEMENTS

Project supported by a grant to Jason TC Tzen from the National Science Council, Taiwan, (No 96-2752-B-005-008-PAE).

CONFLICT OF INTEREST

None declared.

DISCLOSURE

Part of information included in this chapter/article has been previously published in Acta Pharmacologica Sinica (2011) 32: 141–151; doi: 10.1038/aps.2010.197.

REFERENCES

[1] Fisch, C. William Withering: An account of the foxglove and some of its medical uses 1785-1985. *J. Am. Coll. Cardiol.*, **1985**, *5*(5 Suppl A), 1A-2A.
[2] Li-Saw-Hee, F.L.; Lip, G.Y. Digoxin revisited. *QJM*, **1998**, *91*(4), 259-264.
[3] Kjeldsen, K.; Nørgaard A.; Gheorghiade, M. Myocardial Na,K-ATPase: the molecular basis for the hemodynamic effect of digoxin therapy in congestive heart failure. *Cardiovasc. Res.*, **2002**, *55*(4), 710-713.
[4] Prassas, I.; Diamandis, E.P. Novel therapeutic applications of cardiac glycosides. *Nat. Rev. Drug Discov.*, **2008**, *7*(11), 926-935.
[5] Schönfeld, W.; Menke, K.H.; Schönfeld, R.; Repke, K.R. 5 Beta,14 beta-androstane-3 beta,14-diol binds to the digitalis receptor site on Na/K-ATPase. *J. Enzyme. Inhib.*, **1987**, *2*(1), 37-45.
[6] Zhou, J. Bioactive glycosides from Chinese medicines. *Mem. Inst. Oswaldo Cruz*, **1991**, *86*, 231-234.
[7] Chen, R.J.; Chung, T.Y.; Li, F.Y.; Lin, N.H.; Tzen, J.T. Effect of sugar positions in ginsenosides and their inhibitory potency on Na^+/K^+-ATPase activity. *Acta Pharmacol. Sin.*, **2009**, *30*(1), 61-69.
[8] Chen, R.J.; Chung, T.Y.; Li, F.Y.; Yang, W.H.; Jinn, T.R.; Tzen, J.T. Steroid-like compounds in Chinese medicines promote blood circulation via inhibition of Na^+/K^+ -ATPase. *Acta Pharmacol. Sin.*, **2010**, *31*(6), 696-702.
[9] Repke, K.R.H. *In: Drug Discovery Today*, Elsevier, Amsterdam, **1997**, Vol. *2*, pp.110-160.
[10] Bayram, M.; De Luca, L.; Massie, M.B.; Gheorghiade, M. Reassessment of dobutamine, dopamine, and milrinone in the management of acute heart failure syndromes. *Am. J. Cardiol.*, **2005**, *96*(6A), 47G-58G.
[11] Micheletti, R.; Mattera, G.G.; Rocchetti, M.; Schiavone, A.; Loi, M.F.; Zaza, A.; Gagnol, R.J.; De Munari, S.; Melloni, P.; Carminati, P.; Bianchi, G.; Ferrari, P. Pharmacological profile of the novel inotropic agent (E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride (PST2744). *J. Pharmacol. Exp. Ther.*, **2002**, *303*(2), 592-600.
[12] Micheletti, R.; Palazzo, F.; Barassi, P.; Giacalone, G.; Ferrandi, M.; Schiavone, A.; Moro, B.; Parodi, O.; Ferrari, P.; Bianchi, G. Istaroxime, a stimulator of sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a activity, as a novel therapeutic approach to heart failure. *Am. J. Cardiol.*, **2007**, *99*(2A), 24A-32A.
[13] Ji, X.Y.; Tan, B.K.; Zhu, Y.Z. *Salvia miltiorrhiza* and ischemic diseases. *Acta Pharmacol. Sin.*, **2000**, *21*(12), 1089-1094.
[14] Lu, Y.; Foo, L.Y. Polyphenolics of *Salvia*--a review. *Phytochemistry*, **2002**, *59*(2), 117-140.
[15] Yokozawa, T.; Lee, T.W.; Oura, H.; Nonaka, G.; Nishioka, I. Effect of magnesium lithospermate B in rats with sodium-induced hypertension and renal failure. *Nephron*, **1992**, *60*(4), 460-465.
[16] Fung, K.P.; Wu, J.; Zeng, L.H.; Wong, H.N.; Lee, C.M.; Hon, P.M.; Chang, H.M.; Wu, T.W. Lithospermic acid B as an antioxidant-based protector of cultured ventricular myocytes and aortic endothelial cells of rabbits. *Life Sci.*, **1993**, *53*(12), 189-193.
[17] Yokozawa, T.; Chung, H.Y.; Dong, E.; Oura, H. Confirmation that magnesium lithospermate B has a hydroxyl radical-scavenging action. *Exp. Toxicol. Pathol.*, **1995**, *47*(5), 341-344.
[18] Kasimu, R.; Tanaka, K.; Tezuka, Y.; Gong, Z.N.; Li, J.X.; Basnet, P.; Namba, T.; Kadota, S. Comparative study of seventeen *Salvia* plants: aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. *Chem. Pharm. Bull. (Tokyo)*, **1998**, *46*(3), 500-504.
[19] Wu, X.J.; Wang, Y.P.; Wang, W.; Sun, W.K.; Xu, Y.M.; Xuan, L.J. Free radical scavenging and inhibition of lipid peroxidation by magnesium lithospermate B. *Acta Pharmacol. Sin.*, **2000**, *21*(9), 855-858.
[20] O, K.; Lynn, E.G.; Vazhappilly, R.; Au-Yeung, K.K.; Zhu, D.Y.; Siow, Y.L. Magnesium tanshinoate B (MTB) inhibits low density lipoprotein oxidation. *Life Sci.*, **2001**, *68*(8), 903-912.
[21] Tzen, J.T.; Jinn, T.R.; Chen, Y.C.; Li, F.Y.; Cheng, F.C.; Shi, L.S.; She, H.; Chen, B.C.; Hsieh, V.; Tu, M.L. Magnesium lithospermate B possesses inhibitory activity on Na^+,K^+-ATPase and neuroprotective effects against ischemic stroke. *Acta Pharmacol. Sin.*, **2007**, *28*(5), 609-615.
[22] Chen, Y.C.; Jinn, T.R.; Chung, T.Y.; Li, F.Y.; Fan, R.J.; Tzen, J.T. Magnesium lithospermate B extracted from *Salvia miltiorrhiza* elevates intracellular Ca(2+) level in SH-SY5Y cells. *Acta Pharmacol. Sin.*, **2010**, *31*(8), 923-929.
[23] Chen, R.J.; Jinn, T.R.; Chen, Y.C.; Chung, T.Y.; Yang, W.H.; Tzen, J.T. Active ingredients in many Chinese medicines promoting blood circulation are Na^+/K^+-ATPase inhibitors. *Acta Pharmacol. Sin.*, **2011**, *32*(2), 141-151.
[24] Skou, J.C.; Esmann, M. The Na,K-ATPase. *J. Bioenerg. Biomembr.*, **1992**, *24*(3), 249-261.
[25] Rose, A.M.; Valdes, R., Jr. Understanding the sodium pump and its relevance to disease. *Clin. Chem.*, **1994**, *40*(9), 1674-1685.

[26] Morth, J.P.; Pedersen, B.P.; Toustrup-Jensen, M.S.; Sørensen, T.L.; Petersen, J.; Andersen, J.P.; Vilsen, B.; Nissen, P. Crystal structure of the sodium-potassium pump. *Nature*, **2007**, *450*(7172), 1043-1049.

[27] Ogawa, H.; Shinoda, T.; Cornelius, F.; Toyoshima, C. Crystal structure of the sodium-potassium pump (Na^+,K^+-ATPase) with bound potassium and ouabain. *Proc. Natl. Acad. Sci. U. S. A.*, **2009**, *106*(33), 13742-13747.

[28] Shinoda, T.; Ogawa, H.; Cornelius, F.; Toyoshima, C. Crystal structure of the sodium-potassium pump at 2.4 Å resolution. *Nature*, **2009**, *459*(7245), 446-450.

[29] Schönfeld, W.; Weiland, J.; Lindig, C.; Masnyk, M.; Kabat, M.M.; Kurek, A.; Wicha, J.; Repke, K.R. The lead structure in cardiac glycosides is 5 beta, 14 beta-androstane-3 beta 14-diol. *Naunyn Schmiedebergs Arch. Pharmacol.*, **1985**, *329*(4), 414-426.

[30] Melero, C.P.; Medarde, M.; A., S.F. A Short Review on Cardiotonic Steroids and Their Aminoguanidine Analogues. *Molecules*, **2000**, *5*, 51-81.

[31] Blaustein, M.P. The interrelationship between sodium and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmacol.*, **1974**, *70*, 33-82.

[32] Ferrandi, M.; Barassi, P.; Molinari, I.; Torielli, L.; Tripodi, G.; Minotti, E.; Bianchi, G.; Ferrari, P. Ouabain antagonists as antihypertensive agents. *Curr. Pharm. Des.*, **2005**, *11*(25), 3301-3305.

[33] Lin, S.C.; Way, E.L. A high affinity Ca^{2+}-ATPase in enriched nerve-ending plasma membranes. *Brain Res.*, **1982**, *235*(2), 387-392.

[34] Jiang, R.W.; Lau, K.M.; Hon, P.M.; Mak, T.C.; Woo, K.S.; Fung, K.P. Chemistry and biological activities of caffeic acid derivatives from *Salvia miltiorrhiza*. *Curr. Med. Chem.*, **2005**, *12*(2), 237-246.

[35] Tanaka, T.; Morimoto, S.; Nonaka, G.; Nishioka, I.; Yokozawa, T.; Chung, H.Y.; Oura, H. Magnesium and ammonium-potassium lithospermates B, the active principles having a uremia-preventive effect from *Salvia miltiorrhiza*. *Chem. Pharm. Bull.*, **1989**, *37*, 340-344.

[36] Shigematsu, T.; Tajima, S.; Nishikawa, T.; Murad, S.; Pinnell, S.R.; Nishioka, I. Inhibition of collagen hydroxylation by lithospermic acid magnesium salt, a novel compound isolated from *Salviae miltiorrhizae* Radix. *Biochim. Biophys. Acta*, **1994**, *1200*(1), 79-83.

[37] Kulikov, A.; Eva, A.; Kirch, U.; Boldyrev, A.; Scheiner-Bobis, G. Ouabain activates signaling pathways associated with cell death in human neuroblastoma. *Biochim. Biophys. Acta*, **2007**, *1768*(7), 1691-1702.

[38] Tzen, J.T.; Chen, R.J.; Chung, T.Y.; Chen, Y.C.; Lin, N.H. Active compounds in Chinese herbs and medicinal animal products which promote blood circulation via inhibition of Na^+, K^+-ATPase. *Chang Gung Med. J.*, **2010**, *33*(2), 126-136.

[39] Annunziato, L. *New strategies in stroke intervention*, Humana Press, New York, **2009**.

[40] Wang, J.K.; Portbury, S.; Thomas, M.B.; Barney, S.; Ricca, D.J.; Morris, D.L.; Warner, D.S.; Lo, D.C. Cardiac glycosides provide neuroprotection against ischemic stroke: discovery by a brain slice-based compound screening platform. *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*(27), 10461-10466.

[41] Petersen, M.; Simmonds, M.S. Rosmarinic acid. *Phytochemistry*, **2003**, *62*(2), 121-125.

Received: November 29, 2010 Revised: May 13, 2011 Accepted: June 30, 2011