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Danshen diversity defeating dementia ☆

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ABSTRACT

Salvia miltiorrhiza (danshen) is widely used for the clinical treatment of cerebral ischemia and cardiovascular diseases. Its diverse molecular makeup of simple and poly hydroxycinnamic acids and diterpenoid quinones are also associated with its beneficial health effects such as improved cognitive deficits in mice, protection of neuronal cells, prevention of amyloid fibril formation and preformed amyloid fibril disaggregation related to Alzheimer's disease. Whilst the in vitro studies have therapeutic promise, the anti-dementia effect/impact of danshen however depends on its absorbed constituents and pharmacokinetic properties. Both the water and lipid danshen fractions have been shown to have low oral bioavailability and at physiological pH, the polyphenolic carboxylate anions are not brain permeable. To tap into the many neuroprotective and other biological benefits of danshen, the key challenge resides in developing danshen nanopharmaceuticals, semi-synthetic pro-drug forms of its constituents to improve its biocompatability, that is, absorption, circulation in bloodstream and optimization of BBB permeability.

Over the last few decades, aging diseases such as dementia and Alzheimer's disease (AD) have evolved into a global epidemic in an aging population. Physical and mental exercise and dietary regimes can counteract the development of aging[1] and dementia. AD is characterized by depositions of amyloid proteins (Aβ) and cholinergic neurotransmission deficits in the brain. Current therapeutic intervention for AD is primarily based on the inhibition of brain acetylcholinesterase (AChE) to improve the brain acetylcholine levels. A noninvasive early detection protocol for the presence of Aβ would aid in the diagnosis of dementia onset prior to extensive neuronal damage and provide a therapeutic window to combat the disease.[2] The complementation of such strategies with safe preventive drugs or tailored food supplements will be needed to combat the epidemic of cognitive decline, a hallmark of aging.

Danshen analysis: A considerable body of information has accumulated on the therapeutic potential of Chinese herbs that are associated with improved cognition, enhancing the fight against dementia diseases.[3] *Salvia miltiorrhiza* known as danshen, one of the most popular Chinese traditional herbs is used to improve blood circulation[4] in the treatment of cardiovascular disorders, cerebrovascular diseases. The specific clinical uses of danshen in China relate to angina pectoris, hyperlipidemia and ischemic stroke. In this review, the emerging research surrounding the anti-dementia/AD effects and mechanisms of danshen that have been investigated/proposed in the last 10 years as illustrated in Scheme 1 is presented.

Analysis of the chemical constituents of danshen revealed two dominant classes of secondary metabolites. A family of lipid soluble, hydrophobic diterpenoids known as tanshinones and water soluble, hydrophilic, polyphenolic combination of compounds consisting mainly of caffeic acid monomers, dimers, trimers or tetramers in the form of Salvianolic acids. The tanshinones and polyphenolic acids are unique to the *Salvia* genus[5] and the eight major constituents found in danshen were isolated using microwave assisted extraction procedures.[6] A synopsis of the amounts isolated, chemical structures, oral bioavailability and neuroprotection studies are presented in Table 1.

Danshen bioavailability: For clinical applications for the treatment of cerebrovascular, cardiovascular, cognitive diseases, it is necessary to determine how much danshen is required daily to provide protection, and this depends on the bioavailability of its constituents. Upon oral administration of danshen decoctions, bioavailability generally designates simply the quantity or fraction of the ingested dose that is absorbed, its metabolism and excretion. Animal studies have provided data on the uptake, absorption, metabolism and excretion of danshen constituents. Chromatographic, spectrometric and spectroscopic analytical methods have been utilized for the identification of the in vivo metabolites and pharmacokinetic properties in the urine of miniature pigs after oral administration of danshen decoctions.[44]

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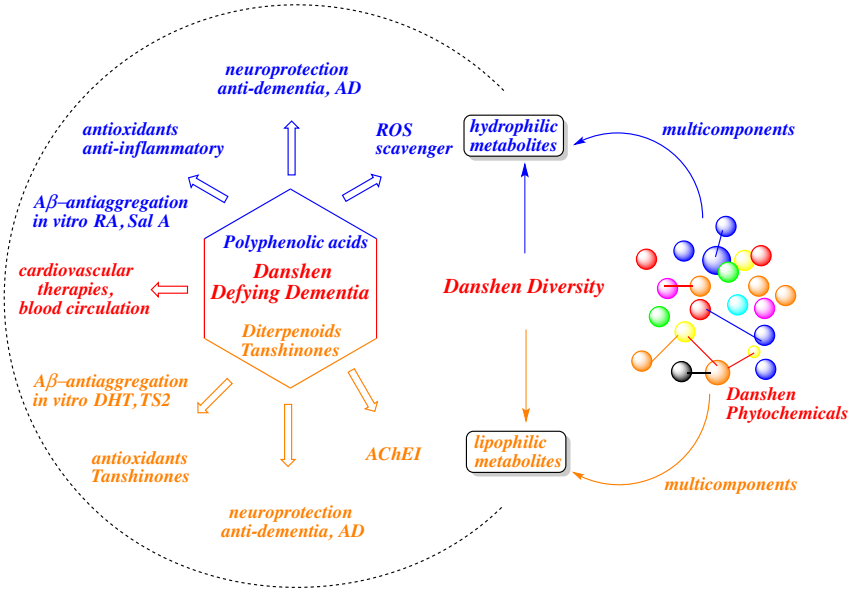


Scheme 1. Overview of the diverse therapeutic applications of danshen constituents.

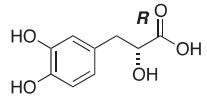
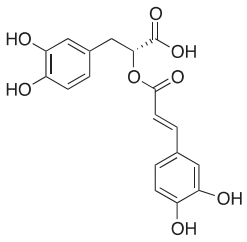
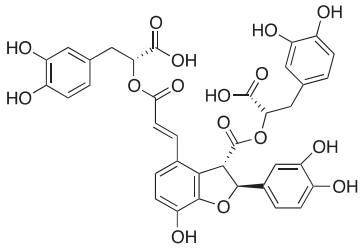
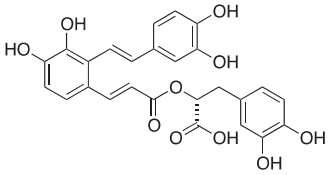
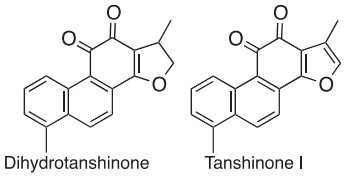
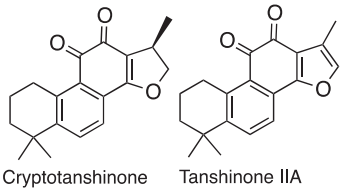
Of the fifty compounds that constituted the decoction, danshen bioavailability related to the uptake/absorption/metabolism/circulation/excretion of ten phenolic substances present in the decoction shown is Scheme 2. These active phenolic compounds were found to undergo metabolic transformations in the colon and liver including hydrolytic reactions, glucuronidation, sulfation, methylation, hydrogenation, decarboxylation and glycine conjugation before being excreted in the urine. The analysis also revealed that 87 % of the urine metabolite fraction is attributed to protocatechuic aldehyde, caffeic acid and danshensu, suggesting that these are the most significant bioactives and that intestinal metabolism significantly increased their concentration/circulation and therefore their potential bioactivity. This is further supported by analysis data that indicated that colonic polyphenol metabolism led to a fourfold increase in the mono-phenolic fraction as shown in Scheme 3 below.

Danshen metabolites: The metabolism of dietary plant mono-phenolic hydroxycinnamic acids[45] in both rats and human subjects undergo metabolic transformations in the gastrointestinal tract, intestinal mucosa, intestinal microflora, liver, and kidneys. It is known that these modifications include dehydroxylation, demethylation, hydrogenation, *O*-methylation, sulfation, glucuronisation, GSH conjugation, and/or glycination. The relative rates of urinary excretion of caffeic and ferulic acid and their metabolites range[46–48] from 5.9 to 27%. In rats fed 2.8 mmol of caffeic acid (505 mg), 11% of the ingested dose was excreted[49] in the urine. Studies with mice indicate that caffeic acid in the brain has neuroprotective actions.[50] For instance 12.4 ± 1.8 mg/100 g of caffeic acid was detected in the brain of mice with a diet containing 2% caffeic acid for 4 weeks.[51] Furthermore, caffeic acid protected the PC12 cells against Aβ-induced cell death through the attenuation of intracellular calcium influx and reduction of τ phosphorylation by the decrease to GSK-3β activation.[52] However studies on male Sprague–Dawley rats that ingested 140 × 10^6 dpm of [3-^{14}C] trans-caffeic acid, found that over the ensuing 72 h period, there was little or no accumulation of radioactivity in body tissues, including the brain.[53] A large number of human and rat urine metabolites have been reported for caffeic and ferulic acids including aromatic hydroxy acids[45] following their ingestion. In this context it is noteworthy that in vitro studies[54] have shown that simple dihydroxybenzoic acid isomers exhibit different abilities to dissociate preformed biotinyl-Aβ (1–42) oligomers. Whilst it is known that the rapid and extensive metabolism of free hydroxycinnamic acids results in low plasma concentrations and their rapid elimination from circulation, a complete analysis of the concentration of caffeic acid administered, prodrug forms, its conjugates and metabolite end products in plasma and brain is therefore needed to determine the nature, bioavailability/accessibility of caffeic acid related compounds/metabolites and their brain permeability to evaluate and measure the extent and significance of their contribution to the improvement of spatial learning, memory and resistance to cognitive diseases.

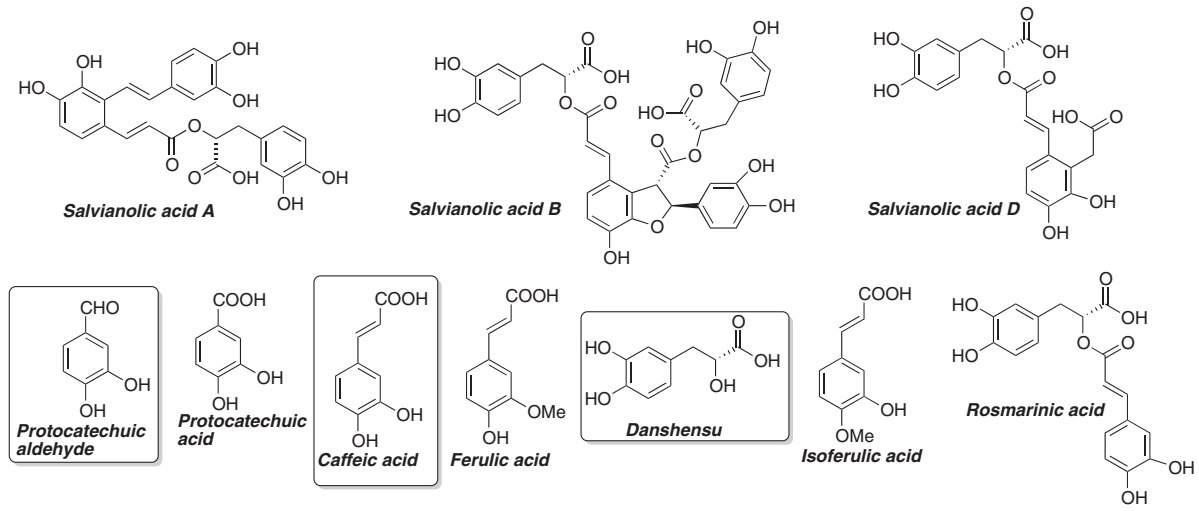
Rosmarinic acid: The evaluation of a standardized extract from the leaves of sage *Salvia officinalis* specifically indicated that its main ingredient, rosmarinic acid[11] as being able to reduce/protect cultured rat pheochromocytoma [PC12] cells from multiple β-amyloid peptide induced neurotoxicity insults including reactive oxygen species generation, lipid peroxidation, DNA decomposition, caspase-3-activation, tau protein hyperphosphorylation and inhibition of phosphorylated p38 mitogen protein kinase activation. In a mouse model, rosmarinic acid at a dose of 0.25 mg/kg protected against the impairment of memory by β-amyloid peptide through scavenging of the peroxynitrite ($ONOO^-$) anion, leading to the implication that daily consumption of culinary herbs containing rosmarinic acid may chemo-protect against dementia.[14] The utilization of nuclear magnetic resonance [NMR] spectrometer techniques revealed the binding of rosmarinic acid, a major component of the butanol extract of *Salvia sclareoides*, to Aβ oligomers,[15] inhibited both Aβ oligomerization and deposition.[55] Also the effect of rosmarinic acid, methyl caffeate, and methyl cinnamate on Aβ peptide aggregation was evaluated with the thioflavin T assay which supported the NMR results and confirming that these natural compounds, methyl caffeate, methyl cinnamate, and rosmarinic acid present in many herbs could have multiple neuroprotective/therapeutic effects against AD. The oral bioavailability of RA in *Isodi Rubescentis* extract by LC–MS-MS in rat plasma[56] was 13.96 (+/–) 3%. The multicomponent nature of herbal teas effectively enhanced rosmarinic acid absorption[10] by intestinal Caco-2 cells to 43 %.

Polyphenolic acid bioavailability: The pig urinary metabolite[44] data showed that salvianolic acids are rapidly metabolized, consistent with the reported[12] bioavalability studies that indicate salvianolic acids have very limited uptake and absorption. The bioavailability[12] values of RA, Sal B and Sal A (derived from

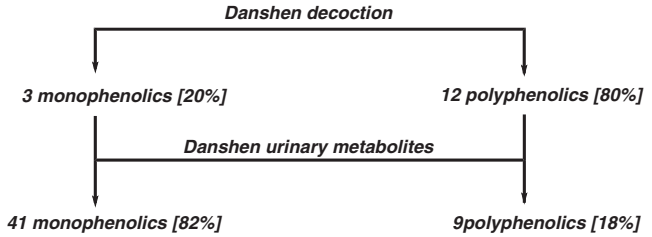
Table 1
Bioactivity profiles of the major danshen constituents

Danshen constituent analysis in mg/g, chemical structure	Oral bioavailability, in animal studies	Neuroprotective effects
Danshensu [DSS] **0.24–0.35** 	Detected[7] brain/blood ratio is 0.25, Pgp inhibition enhanced brain uptake.[8]	Danshensu-cysteine compound cytoprotective against H_2O_2-induced cell death.[9]
Rosmarinic acid, [RA] **2.4–3.5** 	RA absorption from herbal teas[10] (in presence of flavonoids), by intestinal Caco-2 cells increased to 43%. Calcd abs bioavailability RA, Sal B and Sal A, 5.29%, 3.05% and 2.50%, respectively.[12] Carboxylate anionic compounds do not penetrate BBB.[13]	Neuroprotection of PC12 cells from Aβ-induced toxicity[11] chemo-protection due to peroxynitrite scavenging,[14] RA-Aβ binding inhibited Aβ oligomerization/deposition.[15]
Salvianolic acid B [Sal B] **56–75** 	Oral bioavailability[16,17] in freely moving rats was 2.3%, addition of borneol[12] improved bioavailability, and pharmacokinetics; compounds with carboxylic acid group do not readily cross BBB.[13]	Protection against Aβ cytotoxicity,[18] inhibition/disaggregation Aβ fibrils, antioxidant protection[19] against $Aβ_{25–35}$, attenuates cholinergic and Aβ dysfunctions and counters brain injury[20,21] potential treatment for vascular dementia,[22,23] treatment for neurodegenerative disease.[24]
Salvianolic acid A [Sal A] **0.29–0.37** 	Biovailability in beagle dog[25] 1.47–1.84%; chemical instability[26] at pH >7 subtracts from bioavailability. Polyphenolic acids with a free carboxylic group have a very low effective brain penetration.[27,13]	Inhibition of granulocyte adherence.[28] Prevents oxidative stress, platelet aggregation, ischemia, and hepatocirrhosis.[28] Matrix Metalloproteinase inhibitor.[29] Inhibition MKP-3 expression.[30] Sal A inhibited Aβ42 self-mediated aggregation & disaggregated Aβ42 aging fibrils.[31]
Dihydro-tanshinone [DHT] **0.10–0.24** Tanshinone I [TI], 0.37–1.1 	Low oral bioavailability.[32] The co-presence of other tanshinones and danxiongfang enhances CT bioavailability by decreasing the efflux transport of CT by P-glycoprotein.[33]	T1 better than TIIA for: inhibition amyloid aggregation of amyloid-β peptide, disaggregation amyloid fibrils, and protection cultured cells.[34] T1 improves memory impairments,[35] via cellular kinase signaling.
Cryptotanshinone, [CT] **0.24–0.88** Tanshinone IIA, [TIIA] **0.91–2.6** 	When CT was dosed at 100 mg/kg the oral and intra peritoneal bioavailability[32] in rats was estimated as 2.1% and 10.6%, respectively. A low molecular weight TIIA-chitosan [1:9] solid dispersion[36] resulted in complete dissolution, accelerated absorption rate and 30% improved oral bioavailability.	Animal studies with CT attenuated Aβ deposition.[37] CT acting as AChEI results in memory improvement,[38] causes APP processing to non-Aβ,[39] by translocation of ADAM10 and PKC-α. TIIA neuroprotection against cerebral ischemia via inhibition of macrophage migration inhibitory factor,[40] protection from hypoxia-induced mitochondrial apoptosis,[41] disaggregates Aβ fibrils, anti-hypoxia activity via Akt/Skp2/p27 pathway,[42] protection from Aβ via calpain, p35/Cdk5 pathway.[43]

the AUC) of rats that received Sal acid extract orally in comparison to receiving the extract intravenously, were calculated to be 5.29%, 3.05% and 2.50%, respectively. Previously, the absolute bioavailability of Sal B had been calculated to be 3.90% or 5.0% in the rat with oral administration of Sal B in pure form,[57,58] 1.07% in the beagle dog with oral administration of Sal acid extract,[59] and 5.6% in rabbit.[60] The low bioavailability of Sal acids is the result of inadequate absorption from the intestine and the first pass



Scheme 2. Ten phenolic compounds absorbed from danshen decoction.



Scheme 3. Comparison of the phenolic composition of danshen decoction and pig urinary metabolites.

elimination of Sal acids in the liver and intestine.[16,57,61] Relative to the oral administration of the Sal acid extract, the absolute bioavailability values of RA, Sal B and Sal A were improved by 21.61%, 33.77% and 17.90%, respectively, after oral administration of Sal acid extract plus the co-addition of borneol,[12] suggesting that borneol enhanced the intestinal absorption, and limited the metabolism of SAs. This was consistent also with the finding of the poor oral bioavailability of Sal A in beagle dogs[25] calculated to range from 1.47% to 1.84% reflecting its low oral absorption and quick clearance.

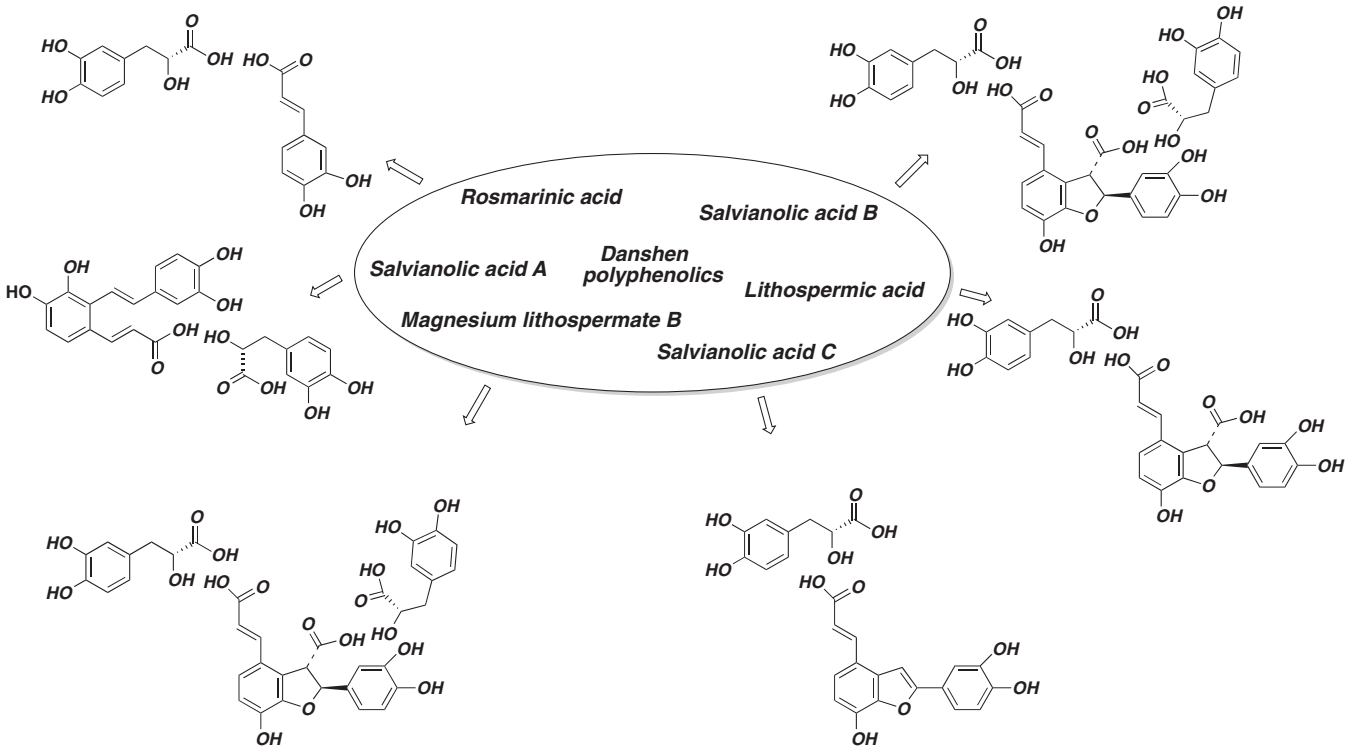
Salvianolic acid A anti-amyloid activity: In vitro studies of Sal A[62] found multiple modulation of Aβ induced toxicity whereby:

- It inhibited Aβ42 self-mediated aggregation and disaggregated Aβ42 aging fibrils.
- Significantly decreased Cu, Fe or Zn ion induced Aβ aggregation, thus suggesting that Sal A inhibits Aβ aggregation through and beyond its metal chelating activity.
- Sal A reduced the production of oxidative stress in SH-SY5Y cell lines.
- Sal A is a neuroprotective agent against Aβ42-induced toxicity in a dose-dependent manner.
- Sal A at 50 and 200 μM attenuated Aβ-induced paralysis in C. elegans strain CL4176 through decreasing the levels of total Aβ ($p = 0.005$ and 0.008 μM, respectively).
- Molecular dynamic simulations demonstrated that Sal A inhibits Aβ self-aggregation through binding to the C-terminus and therefore stabilizing the helical conformations.

Polyphenolic acid metabolism: As outlined in Scheme 3 and as illustrated in Scheme 4 danshen polyphenolics may be hydrolyzed to monophenolics according to the pig urine metabolite profile. After passing through the stomach, the unabsorbed salvianolic acids are unstable above pH 7 complicating their metabolic profile analysis.[26] The analysis of Sal B in stress free rats revealed that the oral bioavailability of Sal B in free ranging rats was calculated[17] as 2.3%. This supports the findings that polyphenolic compounds undergo extensive metabolism, are modified and transformed by colonic micro-flora, and thereby catabolized into simple phenolic acid derivatives that can readily be absorbed from the large intestine.[63] These small phenolic acid products may account for some of the biological activity associated with polyphenolic phytochemicals of danshen whereby the ester bond hydrolysis in rosmarinic acid, salvianolic acids A and B would substantially increase the concentration of danshensu. The brain to blood distribution ratio of danshensu has been calculated[7] as 0.25 ± 0.04 and it has been suggested that the good BBB permeability of danshensu is most likely due to its low molecular weight and its low 5% protein-binding rate. Recently it was demonstrated[8] that the combination of verapamil a P-gp inhibitor with danshensu increased the brain concentration of danshensu. A danshensu-cystine[9] conjugate inhibited apoptosis via upregulation of heme oxygenase-1 expression in SH-SY5Y cells. Further studies are required to investigate the neuroprotective properties of the hydrolysis products of the constituents in danshen.

Salvianolic acid B bioactivities: Sal B was found[18] to inhibit Aβ fibril aggregation with IC_{50} 1.54–5.37 μM as well as destabilize preformed Aβ fibril IC_{50} 5–5.19 μM in a dose- and time-dependent manner and proved to be more effective than ferulic acid but less active than curcumin in the inhibition of $Aβ_{1-40}$ aggregation. Sal B (10 mg/kg, p.o.) inhibited[64] GABAergic neurotransmitter system; Sal B showed anti-inflammatory benefits,[20] suppressed the expression of pro-inflammatory cytokines TNF-α and IL-1β, and enhanced the expression of anti-inflammatory cytokines IL-10 and TGF-β1. Danshensu and salvianolic acid B could protect[21] PC-12 cells by blocking Aβ(25–35)-induced Ca^{2+}-intake, lactate dehydrogenase release, cell viability decrease and apoptosis, TI and DHTI inhibited acetylcholinesterase in vitro.

Sal A inhibited granulocyte adherence[28] by decreasing the expression of intercellular cell adhesion molecule-1 in brain micro-vascular endothelial cells in the treatment of ischemic stroke.



Scheme 4. The hydrolysis of danshen polyphenolics in the colon increases circulating danshensu concentration.

A comparative study of the effects of Sal B and Ginkgo biloba extract EGb 761 on $A\beta_{25-35}$ fibril formation and cytotoxicity to PC12 cells revealed that both Sal B and EGb 761 inhibited the formation of amyloid fibrils, protected PC12 cells from $A\beta_{25-35}$ induced cytotoxicity, and also decreased ROS accumulation caused by $A\beta_{25-35}$. Significantly, Sal B was much more efficient than EGb 761 in inhibiting Aβ aggregation and in protecting PC12 cells from Aβ-induced cytotoxicity.[65] The anti-inflammatory properties of Sal B on interferon-gamma-induced JAK-STAT1activation, suggests a molecular mechanism for potential therapeutic application for vascular disorders.[66]

The redox transformations of the polyphenol EGCG to quinones leading to covalent modifications of proteins[67] and remodeling of amyloid fibrils[68] has been described. The three catechol rings in Sal B can similarly be oxidized in the cellular environment to electrophilic quinones providing potent anti-Aβ neuroprotective effects via Schiff base binding to Aβ peptide lysine amine groups. This would account for some of the potent anti-oxidative properties of Sal B and the other catechol containing polyphenolic constituents of danshen. The water-soluble danshen decoction is therefore potentially a potent pro-electrophilic mixture of compounds that may become redox-responsive and oxidized to quinones by the cellular oxidative-stress environment, resulting in amyloid protein remodeling and abating Aβ-induced cytotoxicity.

Sal B was found to promote neuronal stem progenitor cells (NSPCs) proliferation in vitro and in vivo.[24] The Sal B delayed post-ischemic treatment (7 days after ischemic stroke) with 25 mg/kg improved cognitive impairment after stroke in rats. Whilst studies showed that Sal B promoted the adult hippocampus neurogenesis and improved the cognitive functions in cerebral ischemia rats, evidence that Sal B permeates the blood–brain barrier to act on NSPCs is required. However the exact mechanism(s) by which Sal B acts on adult neurogenesis remain unclear. Additional mechanistic research to confirm NSPCs proliferation by Sal B on contributing to the cognitive improvement is required. The data clearly demonstrated that Sal B was capable of promoting proliferation of NSPCs and improving the learning and memory ability of cerebral ischemic rats. It was concluded that the Sal B promoted NSPCs self-renewal and neurogenesis were at least in part attributed to the PI3 K/Akt signaling pathway. These findings suggest that Sal B or its more lipophilic metabolites, could act as potential drugs for the treatment of brain injury or neurodegenerative disease.

BBB permeation: Useful reported guidelines[69] for the physicochemical properties of a molecule that are consistent with the potential for brain uptake include:

- CNS penetration decreased as MW increased.
- Compounds with MW <300 had brain/blood ratios of 2.2 compared to 0.1 for compounds with MW >700.
- CNS penetration was dependent on ionization state descending in the order basic > neutral > zwitterionic > acidic molecules.
- CNS penetration increases with *c*log*P*, but this correlation is weaker than that for MW.

From the analysis of the physicochemical properties and the chemical structural profiles of CNS and non-CNS oral drugs,[70] and the above molecular guidelines for brain penetration, this suggests that the hydrophilic danshen polyphenolics with: high polar surface area, negative log*D* values, high MW, with free carboxylic acid group(s) (acidic pKa values ranging from 2 to 4) ionization state affecting membrane permeability adversely in the case of negatively charged species[71] are unable to enter the brain by trans-cellular passive diffusion[72] through the lipid membranes that compose the BBB.

BBB permeation of polyphenolics: Nature may have created useful therapeutic agents against AD. Resveratrol in red wine, curcumin in turmeric spice curry, epidemiological evidence has shown an inverse correlation between wine and curry consumption and decreased AD risk. In cell culture experiments polyphenol compounds inhibit the formation and promote the dissociation of Aβ-fibrils by selective and reversible binding.[73–75] The link

between polyphenols and their effects on AD is controversial. Some contend that to date there have not emerged any definitive human intervention studies that have substantiated in vitro claims concerning the neuroprotective effect of polyphenols.[76] The key question is the degree of polyphenol brain penetration. Polyphenolics cannot be readily CNS tailor-made/redesigned, and so a prodrug, semi-synthetic property modification and/or a pharmaceutical formulation strategy may improve herbal CNS uptake.

Prodrug, formulations, and brain uptake: For example the masking of the carboxylic acid group as the 1,3-diacetyl glyceride ester prodrug of ketoprofen [2-(3-benzoylphenyl) propanoic acid] Scheme 5 resulted in the 50-fold improved brain uptake.[77,78] This suggests that carboxylate-prodrug modifications of the danshen hydrophilic constituents would increase their lipophilicity and higher brain concentrations, however this benefit also increases the polyphenol molecular weight. The increased utilization of nanoparticle, lipid and biopolymer combination formulation has resulted in improved pharmacokinetic properties and increased oral bioavailability and plasma concentration of bioactive substances. After the oral administration of a salvianolic acid B-phospholipid complex[79] the peak plasma concentration (C_{max}) of salvianolic acid B-phospholipid complex nanoparticles was 3.4 μg/ml much higher than that of salvianolic acid B at 0.9 μg/ml.

Bioavailability of tanshinones: Over forty lipophilic constituents collectively known as tanshinones and recognized as abietane diterpenes with four ring structures including the 15,16-dihydrotanshinone I (DH-TI) tanshinone I (TI), cryptotanshinone (CT), tanshinone IIA (TIIA), shown in Table 1 have been characterized and isolated. The most representative species that produce this type of diterpenoids is *S. miltiorrhiza*.[5] Furthermore the abietanes (including rearranged abietanes) form the largest group of components of Salvia plants and are ordered into nineteen subgroups.[5]

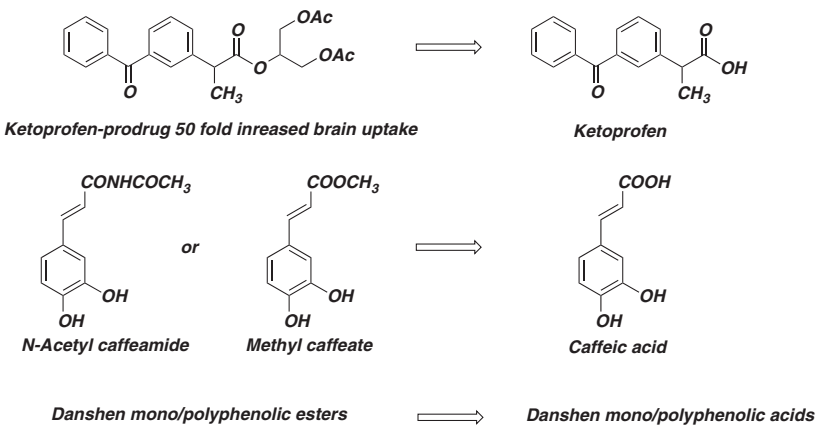
The recently collated pharmacokinetic data[32] for DH-TI, TI, CT, TIIA, indicated that:

- Despite their lipophilic nature, tanshinones exhibit poor bioavailability[80] with p.o. around 2.1% and ip 10.6% in rats, attributed to their low water solubility, poor membrane permeability, and the actions of the P-glycoprotein efflux pump.
- Intravenous administration was the most effective method of tanshinone uptake, pharmacokinetic interactions[81] can eventuate between the hydrophobic tanshinones and Sal B of hydrophilic extracts of danshen resulting in increased plasma levels of both phases.
- The occurrence of metabolic transformations[82] whereby in vivo CT is dehydrogenated to TIIA, is prevalent in pigs and rats.
- The high diversity of compounds in danshen extracts can lead to higher absorption[83,37] and bioavailability[81] of some of its constituents, increasing the competition for the same intake transporters, while promoting the inhibition of efflux transporters.
- Mixtures of diterpenoid tanshinones and danxingfang, improved the absorption of CT, probably by effectively decreasing the efflux transport of CT by P-glycoprotein.[33]

A promising approach to enhance the solubility/dissolution and bioavailability of TIIA has been reported.[36] A 5-fold increase in dissolution of TIIA base by a solid dispersion (SD) system with low-molecular-weight chitosan (TIIA/LMC 1:9 ratio) was recorded. The improved dissolution of the SD was mainly attributed to the high dispersion of the drug as microcrystalline, nano-crystalline or amorphous state in the carrier. Compared with TIIA powders, the oral bioavailability of the physical mixture increased about 30% and this may be due to the absorption-promoting activity of chitosan.

Bioactivities of tanshinones: The diterpenoids DHT and CT are both mixed non-competitive inhibitors for hAChE and uncompetitive inhibitors for hBChE. In hAChE, and from molecular docking studies DHT and CT were found to adopt different orientations inside the active-site gorge.[84,85] While both compounds interact with the ACh enzyme mainly through hydrophobic interactions, DT was predicted to form extra hydrogen bonds with Tyr337 and Gly120. This binding mode would explain the difference in their inhibition potencies towards hAChE. On the other hand, CT and DT are bound at a similar position in hBChE that allows them to interact with the product analogues, suggesting that they inhibit the enzyme through blocking the dissociation of reaction products. Further investigation on the interactions/molecular docking of these inhibitors to hAChE and hBChE may provide insight for designing of a new class of AChE inhibitor. Tanshinones have been identified as potent human carboxylesterase (CE) inhibitors[86] and have been found to modulate the metabolism/efficacy of the anticancer prodrug irinotecan (CPT-11) induced cytotoxicity by inhibiting human CEs. Remedies containing tanshinones should be avoided when individuals are taking esterified agents to avoid potential drug–drug interactions.

As previously stated[32] many studies have demonstrated the anti-proliferation and pro-apoptosis activities of tanshinones [T1, TIIA, CT, DHT1] on various cancer cells, involving multiple targets were all performed in cell culture models. It is difficult to estimate how likely these targets and mechanisms can be translated into in vivo applications because most of the reported studies did not have in vivo data to support the pharmacodynamic targets.



Scheme 5. Ketoprofen prodrug and potential danshen phenolic prodrugs.

Aβ-inhibition of tanshinones: The examination[34] of the in vitro inhibitory activity of TS1 and TSIIA, on the aggregation and toxicity of Aβ1–42 using atomic force microscopy, thioflavin-T (ThT) fluorescence assays[31] cell viability assays, and molecular dynamics simulations suggested that:

- The (ThT) fluorescence assays showed that both TS1 and TSIIA exhibit different albeit general inhibitory abilities to prevent unseeded amyloid fibril formation.
- TS1 showed better inhibitory potency than TSIIA to disaggregate preformed amyloid fibrils.
- Small amounts of tanshinones enabled the protection of cultured SH-SY5Y cells against Aβ-induced cell toxicity.
- Comparative molecular dynamic simulation results reveal a general tanshinone binding mode to prevent Aβ peptide association, showing that both TS1 and TSIIA preferentially bind to a hydrophobic β-sheet groove formed by the C-terminal residues of Isoleucine31-Methionine35 and Methionine35-Valine39 and several aromatic residues.
- The differences in binding distribution, residues, sites, population, and affinity between TS1-Aβ and TSIIA-Aβ systems also interpret different inhibitory effects on Aβ aggregation as observed by in vitro experiments.
- Tanshinones, particularly TS1 compound, offer promising lead compounds with dual protective role in anti-inflammation and antiaggregation for further development of Aβ inhibitors to prevent and disaggregate amyloid formation.

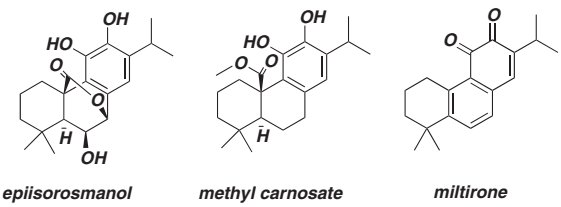
Neuroprotection of tanshinones: DHTI, TI, TIIA, CT, have all been reported to attenuate scopolamine induced learning and memory impairments[38] on the passive avoidance task.[87] It has been proposed[88] that TIIA protects rat brain from pristine ischemic damage in the cerebral cortex that might be correlated with induced nuclear translocation of transducers of regulated cAMP response element binding protein (CREB) activity (TORCs). TORC1 upregulated expression of CREB and brain derived neurotrophic factor. Also the task learning ability of scopolamine-treated rats evaluated by the acquisition protocol of the Morris water maze was significantly reversed by CT (5 mg/kg) and the CT-fed rats were able to develop a spatial searching strategy comparable to that of the control animals. T1 in rats improved learning, memory, and ameliorates memory impairment in mice via signal-regulated kinase signaling pathway.[35] The antioxidative activity of TIIA protected cultured cortical neurons against $A\beta_{25-35}$-induced neurotoxicity[89] and is an effective neuroprotective agent via PI3 K/Akt activation and GSK3b phosphorylation.[90] It provided protection against neuropathological changes induced by Aβ (1–40) injection into the hippocampus.[91] Danshen increased the expressions of urokinase PA, cyclin D1, E and ERK, JNK, and P38 MAP kinases via the FGF-2 signaling pathway in a dose-dependent manner, indicating that danshen and tanshinone IIA may enhance neuron regeneration,[92] however optimum dose requirements were not determined.

TIIA protected the brain from ischemic injury by suppressing the oxidative stress and the radical-mediated inflammatory insult.[93] The effects of CT, on amyloid precursor protein (APP) processing in rat cortical neuronal cells overexpressing Swedish mutant human APP695 was to decrease[94] Aβ generation in concentration-dependent manner. Also the alpha-secretase (sAβPPα) activity was increased toward the non-amyloidogenic product pathway. Further studies[39] suggested that CT-induced sAβPPα secretion is regulated by a PKC-α and the ADAM10 cascade in neuroblastoma cells and may be involved in the lowering of Aβ production.

Compound screening for BBB permeation: To provide a guide for the BBB penetration of natural and synthetic compounds, the parallel artificial membrane permeation assay (PAMPA) for the prediction of blood–brain barrier penetration (PAMPA-BBB) was developed.[27,13] This innovative system models the rate of trans-cellular passive diffusion of drugs across the BBB by measuring the effective permeability (Pe, cm/s) using a porcine brain artificial lipid membrane extract as the assay membrane, impregnated on a solid filter support. When applied to the constituents of *Salvia officinalis* the screening/analysis characterized epiisorosmanol, and methyl carnosate, (Scheme 6) two phenolic diterpenes as BBB permeable compounds supporting evidence for the beneficial effects of sage extracts for treatment of memory disorders.[95,96] These compounds also have structural similarity with miltirone a diterpene *ortho*-quinone found in danshen, reported for its antioxidative,[97] anxiolytic effect,[98,99] positive modulation on GABA (A) receptor[100] and anti-proliferative activities in multidrug-resistant cancer cells.[101] Importantly the PAMPA study exemplifies the possible brain penetration of carboxylate ester compounds as highly relevant for the modification/increasing the brain penetration of danshen hydrophilic and lipophilic compounds. The PAMPA-BBB assay should find increased application for screening/predicting passive blood–brain barrier penetration of herbal constituents.

Tanshinones, activities, interactions: Recent research has s uggested and provided evidence[43] that the neuroprotective properties of TIIA against the neurotoxicity of Aβ (25–35) had increased the viability of neurons and decreased expression of phosphorylated tau in neurons induced by Aβ (25–35). TIIA maintained the normal expression of p35 on peripheral membranes, and reduced p25 expression in the cytoplasm. Tan IIA also inhibited the translocation of Cdk5 from the nucleus into the cytoplasm of primary neurons induced by Aβ (25–35). These data suggested that tan IIA possessed neuroprotective action and the protection may involve calpain and the p35/Cdk5 pathway. The pharmacokinetics[102] of CT and TIIA in rats after administration of the tanshinones extract were significantly affected by the coexisting tanshinones. Indicating that the herb–drug interactions occurring between coexisting tanshinones and CT or TIIA affected their absorption, transformation and metabolism.

Herbal-nanoformulation therapeutics: Chemical modifications and novel formulations had been made to address the poor oral bioavailability of tanshinones.[32] Plasma levels of tanshinones in the nM to sub-μM ranges were commonly observed with ip and oral administration. Danshen mechanistic and therapeutic relevance should be interpreted whenever possible with relevant pharmacokinetic data. For clinical applications, Aβ inhibitors should resist premature enzymatic degradation, target specific tissues, cross the blood brain barrier (BBB), and facilitate nucleus uptake, while not inducing inflammation, toxicity, and other adverse immune responses. The facile brain penetration/concentration of curcumin-solid lipid nanoparticles[103] suggests that nano-pharmaceuticals, nano-composites, nano-structured lipid materials is a rapidly emerging pharmaceutical science that may provide ways of improving oral bioavailability and brain penetration of herbal-nano-formulations leading to more efficient therapies. To facilitate the preparation of a fully blood–brain permeable danshen, care and caution is required. The use of unconventional formulations alters the pharmacokinetics and bioavailability, leading to changes in experimental outcomes. Also



Scheme 6. *Salvia officinalis* brain penetrating phenolic diterpenoids, miltirone.

administration of artificially high and non-physiological levels of compounds triggers the saturation of drug elimination mechanisms.

There is growing evidence that compounds broken down in the colon form a key part of the 'in vivo bioavailability equation' of natural products and related compounds that can occur in danshen, botanical medicines, and also in fruit and vegetables products and extracts. Furthermore, the colon-derived phenolic acids appear to have in vitro anti-inflammatory activity, and to protect human nerve cells against oxidative damage. There appears to be a natural synergistic effect whereby the multiple components of danshen work together to provide benefits including enhanced bioavailability.

In vitro research suggests that both tanshinones and polyphenolics in danshen are the active constituents responsible for the beneficial effects of this herb in AD treatment.

Tanshinones, the main lipophilic components extracted from Chinese herb danshen, can inhibit Aβ aggregation, disaggregate Aβ fibers, and reduce Aβ-induced cell toxicity in vitro. However therapeutic potency and efficacy issues are unresolved. The mono- and polyphenolic danshen compounds have multiple specific and non-specific Aβ binding interactions; and could be very promising therapeutic inhibitors with both antiaggregation and antioxidant activities to protect neurons from Aβ damage.

AD is a multifactorial disease involving a wide range of molecular mechanisms/networks whereby proteins, enzymes, receptors, cell signaling, becomes dysfunctional, making poly-diagnosis and treatment challenging.[104] It is unclear which factors are essential for the pathogenesis of AD. In the longitudinal assessment of AD, Aβ deposition increases slowly from cognitive normality to moderate[105] severity of dementia. Extensive Aβ deposition precedes cognitive impairment, and is associated with the ApoE genotype and a higher risk of cognitive decline over 1–2 years. However, cognitive decline is only weakly related to change in Aβ burden,[106,107] underlining that the multi-pathological nature of dementia and AD involves an umbrella of factors, some of which have a more direct effect on symptom progression than Aβ deposition/toxicity.

Metabolism, efficacy of danshen anti dementia constituents: Chinese herbs show promise in the treatment[108] of AD. The unique mixture of caffeic acid monomers, dimers, trimers, tetramers, with various quinone diterpenes in danshen represent the multiple molecular/biological properties of danshen as outlined in Scheme 1 that actively target to eradicate most of the root causes of dementia-AD onset and may lead the future direction of new drug development. The poor bioavailability of danshen and all other natural products including fruit products, the cognitive benefits of which are attributed to their polyphenol content means that further research is necessary to develop the most appropriate/effective form of their intake and treatment. A better understanding of how the major danshen constituents impact metabolic phenolic profiles and their bioavailability is critical to development of herbal products designed to deliver specific anti dementia health benefits. The potential/possible need to initiate the herbal/natural products as neuroprotection therapy long before the onset of dementia symptoms suggests that lifestyle changes need to be addressed through education platforms. All these issues need to be incorporated into the design of future human trials of herbal and natural products to defeat dementia. We may never achieve complete eradication of cognitive decline, but such a pursuit serves as a constant source of inspiration to discover and develop new preventative therapies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.042. These data include MOL files and InChiKeys of the most important compounds described in this article.

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