Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Using Molecular Docking Analysis to Discovery *Dregea sinensis* Hemsl. Potential Mechanism of Anticancer, Antidepression, and Immunoregulation

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Submitted: 28-08-2016 Revised: 08-11-2016 Published: 19-07-2017

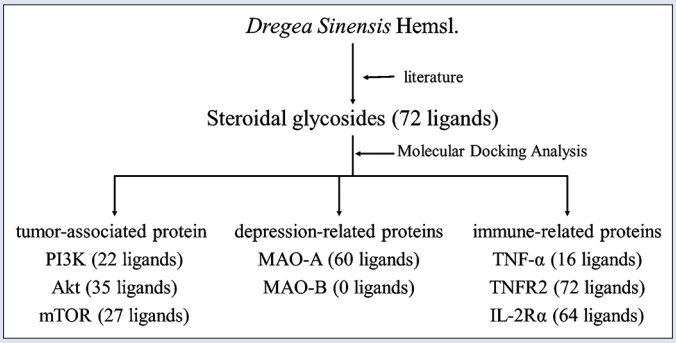
ABSTRACT

Background: *Dregea sinensis* Hemsl. plant of the genus *Dregea volubilis* (Asclepiadaceae), plays a vital role in anticancer, antidepression, and immunoregulation. Steroidal glycosides are the main constituents of this herb, which were significant biological active ingredients. **Objective:** The objective of this study is to recognize the mechanism of anticancer, antidepression, and immunoregulation of *D. sinensis* Hemsl. **Materials and Methods:** Seventy-two steroidal glycosides of *D. sinensis* Hemsl. were evaluated on the docking behavior of tumor-associated proteins (PI3K, Akt, mTOR), depression-related proteins (MAO-A, MAO-B) and immune-related proteins (tumor necrosis factor-α [TNF-α], tumor necrosis factor receptor 2 [TNFR2], interleukin-2Rα [IL-2Rα]) using Discovery Studio version 3.1 (Accelrys, San Diego, USA). **Results:** The molecular docking analysis revealed that mostly steroidal glycosides of *D. sinensis* Hemsl. exhibited powerful interaction with the depression-related protein (MAO-A) and the immune-related proteins (TNFR2, IL-2Rα). Some ligands exhibited high binding energy for the tumor-associated proteins (PI3K, Akt, mTOR) and the immune-related protein (TNF-α), but MAO-B showed none interaction with the ligands. **Conclusion:** This study has paved better understanding of steroidal glycosides from *D. sinensis* Hemsl. as potential constituents to the prevention of associated cancer, depression and disorders of immunoregulation.

Key words: Anticancer, antidepression, *D. sinensis* Hemsl., immunoregulation, steroidal glycosides

SUMMARY

- The ligand database was consist of 72 steroidal glycosides from *Dregea sinensis* Hemsl
- Steroidal glycosides had the potential to dock with the tumor-associated proteins (PI3K, Akt, mTOR)
- Steroidal glycosides were bounded with MAO-A rather than MAO-B, accorded with the inhibitor selectivity of MAOs, can be considered as potent candidate inhibitors of MAO-A
- 72 ligands got high interaction with TNFR2 and IL-2Rα, regard the steroidal glycoside as powerful candidate inhibitors of TNFR2 and IL-2Rα.



Abbreviations used: PI3K: Phosphatidyl inositol 3-kinase; Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; MAO-A: Monoamine oxidase A; MAO-B: Monoamine oxidase B; TNF-α: Tumor necrosis factor α; TNFR2: Tumor necrosis factor receptor 2; IL-2Rα: The alpha subunit (CD25) of the interleukin-2 receptor; DS: Discovery Studio; PDB: Protein Database Bank; 3D: three-dimensional.

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DOI: 10.4103/pm.pm_384_16

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INTRODUCTION

D. sinensis Hemsl. plant of the genus *Dregea volubilis* (Asclepiadaceae), widely distributed in the southwest of China and grown at an altitude of 500–3000 m of mountain jungles or bushes. As a common medicinal material, it is extensively used in Dai ethnic minorities with therapeutic effects including detoxification, blood-activating, defervesce, detumescence, and acesodyne.[1] In the system of Dai medicine, *D. sinensis* Hemsl. plays a vital role in enhancing human immunity. With the intensive researches on Dai medicine, various studies have been focused on this plant species.[2] At present, more than 100 compounds have been obtained from *D. sinensis* Hemsl. Steroidal glycosides are the main constituents in these obtained compounds, which are the significant biological active ingredients.[3-14] Since 1920's, steroidal glycosides compounds have been found from several plant species. These compounds mainly distributed in Asclepiadaceae,[15] yam,[16] Gentianaceae,[17] and solanaceae[18] species. Among the plant species, Asclepiadaceae has the highest content of steroidal glycosides. Many plants of Asclepiadaceae have already proved to be of significant value in treating cough, tumor, rheumatoid arthritis, asthma, etc.[19-22] However, the pharmacological mechanism of *D. sinensis* Hemsl. has not been clarified clearly. In this paper, the chemical composition database of D. *sinensis* Hemsl. was builded up, and then molecular docking was carried

Cite this article as: Liu X, Shi Y, Deng Y, Dai R. Using molecular docking analysis to discovery *Dregea sinensis* Hemsl. potential mechanism of anticancer, antidepression, and immunoregulation. Phcog Mag 2017;13:358-62.

out with the tumor-associated proteins, depression-related proteins, and immune-related proteins, respectively. Finally, the action mechanism of *D. sinensis* Hemsl. was explored at the level of protein molecules.

MATERIALS AND METHODS

Ligand preparation

Based on the published literature,[3-14] the database of *Dregea sinensis* Hemsl. including 72 steroidal glycosides were prepared by ChemBio Office software. Classified by the structural characteristic, steroidal glycosides can be divided into seven categories: (A) C5-C6 single bond, C17 hydroxylation; (B) C5-C6 double bond, C17 hydroxylation; (C) C5-C6 double bond, C17 hydroxylation, C20 carbonylation; (D) C5-C6 double bond, C17 non-hydroxylation, C20 carbonylation; (E) C5-C6 single bond, C17 hydroxylation, C20 carbonylation; (F) C5-C6 single bond, C17 hydroxylation, C20 carbonylation; and (G) the others. The type of A- F shown in the Table 1 below.

Target protein identification and preparation

The initial three-dimensional (3D) geometric coordinates of the X-ray crystal structure of the protein was downloaded from the Protein Database Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). The 3D structures of tumor-associated proteins: Phosphatidylinositol 3-kinase (PI3K, PDB ID: 1E8Y); protein kinase B (Akt, PDB ID: 4GV1); mammalian target of rapamycin (mTOR, PDB ID: 4JSP). The 3D structures of depression-related proteins: monoamine oxidase A (MAO-A, PDB ID: 2Z5Y); monoamine oxidase B (MAO-B, PDB ID: 4CRT). The 3D structures of immune-related proteins: Tumor necrosis factor-α (TNF-α, PDB ID: 2AZ5); tumor necrosis factor receptor 2 (TNFR2, PDB ID: 3ALQ); and the alpha subunit (CD25) of the interleukin-2 receptor (IL-2Rα, PDB ID: 2ERJ).

Docking studies

The molecular docking calculations were performed using the LibDock protocol under the protein-ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA), which the ligand would be structurally rearranged in response to the receptor. Docking was carried out as described elsewhere,[23] which hinted the target compounds as inhibitors of proteins.

RESULTS AND DISCUSSION

PI3Ks are enzymes which catalyze the phosphorylation of one or more inositol phospholipids in the 3-position of the inositol ring.[24] Akt is a serine/threonine protein kinase. After the pH-regulatory domain of Akt binds to PI3K, Akt is activated and translocated from the cytoplasm to the membrane, and consequently, mediates the activation of multiple downstream genes.[25] The mTOR is an important regulatory factor of cell growth and proliferation.[26] Many researches indicated that the PI3K/Akt/mTOR signaling pathway plays a crucial role in tumorigenesis and tumor progression. If this pathway disorders, it can induce a series of diseases, including cancer, neurological disease, and autoimmune diseases.[27-29] 72 constituents of *D. sinensis* Hemsl. were evaluated on the docking behavior of PI3K, Akt, and mTOR, respectively. The docking studies calculations as in Table 2. 22 ligands exhibited interaction with the PI3K. most of them were type A, B, C, F, G. In contrast, type D and E were hard to dock with the PI3K. C-44 had the highest LibDock score (116.41) with that of PI3K, D-47, C-43, B-36, and A-3 also had high interaction energy. Akt possessed 35 docking ligands, and D-52 had the highest LibDock score (171.88). A-type, B-type, and D-type compounds had the high LibDock score with Akt. As for the docking studies calculations with mTOR, 27 steroidal glycosides had interaction with this protein. A-29 got the highest score (159.14) with that of mTOR. The result showed that A-type, C-type, and D-type also got high LibDock score with this protein. PI3K, Akt and mTOR all had high LibDock score with A-3 (113.29, 133.63, 115.76), B-36 (109.13, 128.83, 153.20), C-43 (109.53, 113.79, 121.34), and G-60 (99.59, 125.20, 122.10). This result provided a direction for the next anticancer drug research and development, to a certain extent, the study explained anticancer mechanism of *D. sinensis* Hemsl.

Table 1: the steroidal glycosides Structural Characteristic of Dregea sinensis Hemsl

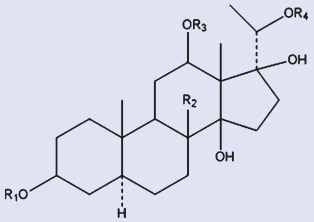
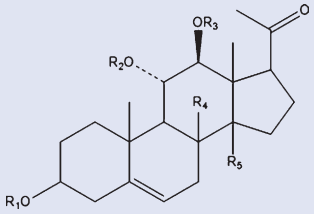
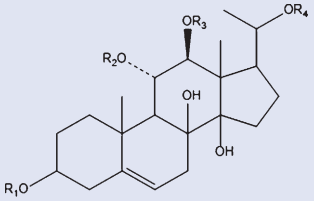
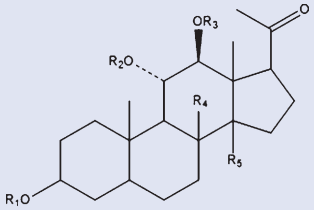
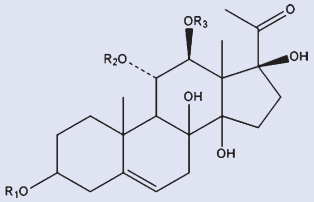
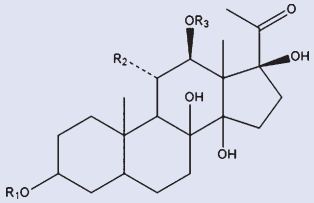
Type	Structure	Amount	Type	Structure	Amount
A		33	B		10
C		4	D		14
E		2	F		4
G	–	5			

Table 2: The libdockscore analysis of 72 ligands with that of PI3K, Akt, mTOR, MAO-A, MAO-B, TNFR2, TNF-α and IL-2Rα using Discovery Studio

LIGAND	PI3K	Akt	mTOR	MAO-A	MAO-B	TNFR2	TNF-α	IL-2Rα
A-1	92.26	112.45	102.43	115.72		87.21		89.62
A-2				116.63		84.18		111.04
A-3	113.29	133.63	115.76	114.75		101.86		109.84
A-4						37.93		
A-5	87.09	122.38	106.26	90.74		108.04		97.65
A-6		122.60		115.34		95.17		108.32
A-7				90.93		82.67	72.27	81.79
A-8						112.12		104.03
A-9		94.95		107.27		107.38		127.70
A-10				131.74		113.96		106.58
A-11				91.14		90.43		132.42
A-12				78.38		137.04		140.51
A-13	52.69	126.70		158.10		147.50		140.85
A-14				69.92		68.78		
A-15		98.49	90.09	110.66		90.68		91.69
A-16			109.42	95.92		79.79	77.44	76.22
A-17	58.53					69.74		79.11
A-18		85.22	86.18	86.38		90.90		79.14
A-19	86.90		87.04	124.48		90.63		84.87
A-20						39.06		
A-21	81.05					84.62		87.94
A-22		70.90		176.10		171.73		139.52
A-23				98.02		95.63		
A-24				121.77		96.88		
A-25				159.18		126.31		
A-26				117.81		163.71		100.54
A-27				44.30		107.85		
A-28		129.91		113.73		130.37		118.15
A-29		129.05	159.14	134.44		156.99		127.43
A-30						56.60		41.82
A-31						130.81		
A-32				106.12		101.15		82.65
B-33	65.65	108.44	84.95	96.45		76.73		81.74
B-34				199.36		155.59		131.43
B-35		130.78		124.37		111.06		108.09
B-36	109.13	128.83	153.20	141.09		134.08	130.89	144.16
B-37	84.66		142.08	156.95		147.24		151.63
B-38	108.25	93.04		164.71		134.71	134.49	146.39
B-39				157.74		153.96		143.02
B-40	104.10			159.81		123.67	121.58	142.53
B-41				125.81		179.70		149.55
B-42				127.25		122.93		125.67
C-43	109.53	113.79	121.34	159.74		147.36		142.70
C-44	116.41	106.78		145.50		139.98	75.33	150.10
C-45	72.68		129.24	169.57		143.57	121.92	134.49
C-46		94.04	130.86	164.52		143.55		131.86
D-47	114.54	157.05		128.55		115.36		115.67
D-48		156.31	157.72	167.57		162.36	123.78	144.10
D-49			108.81	175.27		163.80	125.80	133.38
D-50			108.81	175.27		163.80	125.80	133.38
D-51				137.66		153.89		161.04
D-52		171.88	141.59	155.49		147.86	69.52	152.77
G-53	98.09	68.77	87.05	80.01		76.86		84.59
G-54	79.33	57.12	93.36	92.18		89.08		90.54
G-55	98.09	98.64	82.41	62.21		83.40		92.88
A-56			52.94			72.10		78.91
F-57	79.33	105.42		96.75		91.25		87.20
F-58		89.84				77.27		83.71
G-59	81.57	111.57	110.38	123.23		92.75		94.80
G-60	99.59	125.20	122.10	118.22		94.34	80.92	94.66
F-61				161.76		129.92	102.82	138.55
F-62		103.60		161.10		151.74		129.60
D-63						106.11		63.74
D-64		151.61	105.36	169.42		127.20	118.06	157.97
D-65		94.64		139.60		128.69	119.42	161.10

Contd...

Table 2: Contd...

LIGAND	PI3K	Akt	mTOR	MAO-A	MAO-B	TNFR2	TNF-α	IL-2Rα
D-66				179.41		125.83		116.63
D-67						114.38		113.25
D-68		83.57		157.49		130.77		114.41
D-69		112.74	105.45	128.52		98.33		103.88
D-70		142.92	99.13	149.16		122.44	100.75	130.52
E-71				120.50		137.24		127.32
E-72		133.38				77.37		109.95

Monoamine oxidases (MAOs) localized to the outer mitochondrial membrane in various cells catalyzed amine to produce hydrogen peroxide by oxidative deamination in the brain and peripheral nerve tissues.[30] There exist two forms of MAOs: MAO-A and MAO-B. Two forms of MAOs have been identified by substrate and inhibitor selectivity.[31,32] They have different effects in neurotransmitter metabolism and biological behavior. As for the docking studies and binding free energy calculations with MAO-A and MAO-B [Table 2]. Sixty ligands exhibited interaction with MAO-A, including all the B-type and all the C-type ligands, but there were 8 A-type steroidal glycosides of 12 failed-ligands. Most of the docking ligands got high LibDock score, especially B-34 (199.36) was the highest. Meanwhile, all the ligands were failed to dock with MAO-B. The result showed steroidal glycosides of *D. sinensis* Hemsl. had a significant difference in interaction with MAOs and conform to the inhibitor selectivity. Steroidal glycosides can be considered as potent inhibitors of MAO-A.

TNF-α is a pleiotropic cytokine involved in immunity, inflammation, cell proliferation, differentiation, and apoptosis,[33] mainly secreted from activated macrophages. Both TNF receptors TNFR1 and TNFR2 are transmembrane proteins,[34] with high similarity in their extracellular regions although they differ widely in their intracellular domains.[35] All the ligands showed interaction with TNFR2, compared with 16 docking ligands to TNF-α [Table 2]. Then almost A-type ligands failed docking with the TNF-α. A-22 (171.73) had the highest score with that of TNFR2, and B-38 (134.49) to TNF-α. Finally, the docking studies calculations with that of IL-2Rα, in which 64 ligands exhibited interaction with IL-2Rα and the failed-ligands were all A-type. IL-2Rα got the highest LibDock score with D-64 (161.10). Molecular docking analysis of steroidal glycosides and immune-related proteins (TNFR2, TNF-α, and IL-2Rα) indicated that steroidal glycosides had interaction with these proteins, especially TNFR2 and IL-2Rα. As a powerful evidence to illuminate *D. sinensis* Hemsl. owning the function of immunoregulation.

CONCLUSION

In the present study, it was found that steroidal glycosides of *D. sinensis* Hemsl. had the potential to dock with the tumor-associated proteins (PI3K, Akt, mTOR). These compounds accorded with the inhibitor selectivity of MAOs, just were bound with MAO-A rather than MAO-B, can be considered as a potent candidate inhibitors of MAO-A. 72 ligands got high interaction with TNFR2 and IL-2Rα, regard the steroidal glycoside as powerful candidate inhibitors of TNFR2 and IL-2Rα. However, the ligands were weakly bound with TNF-α. Hence, it is strongly suggested that the results had paved better understanding of steroidal glycosides of *D. sinensis* Hemsl. as potential PI3K, Akt, mTOR, MAO-A, TNFR2, and IL-2Rα inhibitors in relation to the prevention of associated cancer, depression, and disorders of immunoregulation.

Financial support and sponsorship

This project was supported by the National Major Scientific Instruments and Equipment Development Project (Grant No. 2013YQ03059514).

Conflicts of interest

There are no conflicts of interest.

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