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Interaction of *Carthamus tinctorius* lignan arctigenin with the binding site of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase☆

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ABSTRACT

Mediterranean *Carthamus tinctorius* (Safflower) is used for treatment of inflammatory conditions and neuropsychiatric disorders. Recently *C. tinctorius* lignans arctigenin and trachelogenin but not matairesinol were described to interfere with the activity of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) in peripheral blood mononuclear cells *in vitro*. We examined a potential direct influence of compounds on IDO enzyme activity applying computational calculations based on 3D geometry of the compounds. The interaction pattern analysis and force field-based minimization was performed within LigandScout 3.03, the docking simulation with MOE 2011.10 using the X-ray crystal structure of IDO. Results confirm the possibility of an intense interaction of arctigenin and trachelogenin with the binding site of the enzyme, while matairesinol had no such effect.

1. Introduction

The herbaceous *Asteraceae* plant *Carthamus tinctorius* (Safflower) has been used in traditional Chinese medicine to promote circulation and menstruation [1] and to treat neuropsychological disorders such as major depression [2]. In the Mediterranean area, *C. tinctorius* extracts play a role in the treatment of cancer and are known for antihelmintic, antiseptic, diuretic and febrifugal properties [3]. Most of the clinical conditions mentioned are associated with immune activation and inflammation which is characterized by specific biochemical alterations. Among them alterations of tryptophan metabolism appear to be most prominent. Tryptophan breakdown due to enzyme indoleamine 2,3-dioxygenase (IDO) is involved in several physiological and pathophysiological conditions. IDO is an iso-enzyme of tryptophan 2,3-dioxygenase (TDO), and both enzymes catalyze the first and rate limiting step of tryptophan catabolism namely the conversion of tryptophan to *N*-formyl kynurenine. Whereas TDO is mainly located in the liver, IDO is more widely distributed in the organism [4]. During immune activation and inflammation IDO is mainly up-regulated by Th1-type cytokine interferon-γ (IFN-γ), and this is detectable locally and systemically by an increased tryptophan breakdown rate as is indicated by an increased kynurenine to tryptophan ratio (Kyn/Trp) [5], whereas in healthy conditions, the expression and activity of IDO is low. In animal model systems and *in vitro*, increased tryptophan breakdown was found to lead to immune tolerance via the induction of regulatory T-cells (Treg) and dendritic cells [6,7].

Tryptophan is also the substrate of the serotonin (5-hydroxytryptamine, 5-HT) pathway and its breakdown leads to reduced synthesis of serotonin which is related to depression, as observed, e.g., in cancer patients during treatment with IFNs [5,8]. Additionally, the accumulation of tryptophan catabolites, such as 3-hydroxykynurenine, kynurenic acid and quinolinic acid are believed to play a role in the pathogenesis of the AIDS-dementia complex, Huntington's disease, Alzheimer's disease, complex partial seizures, depression, anorexia and schizophrenia [9]. Thus, in neurological disorders, IDO inhibitors are supposed to reduce the production of the neurotoxic tryptophan metabolites [10].

Based on the observations made in patients and the ethnopharmacological use of Safflower, the three major lignans trachelogenin, arctigenin, and matairesinol (Fig. 1) were isolated from *C. tinctorius* extracts and pharmacologically investigated for their ability to interfere with activation of IDO in freshly isolated peripheral blood mononuclear cells (PBMC) [11]. Arctigenin and also trachelogenin

Abbreviations: GBVI/WSA, generalized-born volume integral/weighted surface area; 5-HT, 5-hydroxytryptamine, serotonin; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ; 1-MT, D-1-methyl tryptophan; Kyn/Trp, kynurenine to tryptophan ratio; MMFF94, Merck Molecular Force Field 94; PBMC, peripheral blood mononuclear cells; TDO, tryptophan 2,3-dioxygenase; Treg, regulatory T-cells.

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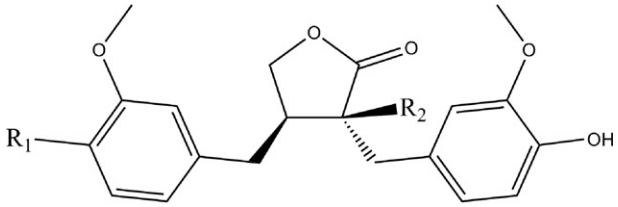

Fig. 1. Chemical structures of compounds trachelogenin (1), arctigenin (2) and matairesinol (3).

exerted a significant and dose-dependent effect to suppress tryptophan breakdown in the PBMC model, whereas matairesinol was only weakly effective [12]. Because of the relatively strong effect of compounds on tryptophan breakdown, we became interested to simulate possible direct interaction of the lignans with IDO, so a docking simulation was performed.

2. Methods

During the docking simulation the previously energetically minimized molecules were placed within the binding pocket of a protein, generating a set of energetically favorable poses. These poses are then ranked according to a score that the docking program assigns to each pose, estimating the binding free energy. The best ranked pose of each molecule was then further optimized and a three dimensional representation of its interaction pattern, a pharmacophore, was calculated to analyze the structure–activity relationship.

The possible direct interaction of the three lignans with IDO was simulated, 3D geometries of trachelogenin, arctigenin, and matairesinol were calculated with Discovery Studio [13] and energetically minimized with Omega 2.2.1. [14]. The docking simulation was performed with the software package MOE 2011.10 [15] using the X-ray crystal structure of IDO (Protein Data Bank entry 2d0t) [16]. A pharmacophore of the metal binding feature was generated within LigandScout 3.03 [17] based on the ligand (4-phenyl-imidazole) co-crystallized in the crystal structure and used as a constraint in the docking simulation. This means that the docked ligand must form a coordinated interaction with the heme iron ion from the IDO active site. For the docking, the A chain of 2d0t, which was crystallized as a dimer, was used. The original ligand 4-phenyl-imidazole, the two co-crystallized detergent molecules of 2-[*N*-cyclohexylamino]ethane sulfonic acid, and water were removed. The binding site of the original ligand was chosen for the docking. The program defines the open space in the binding pocket with so called alpha spheres that represent available atom positions. Placement was done by the triangle matcher algorithm using standard settings. The triangle matcher algorithm generates poses by aligning triplets of ligand atoms with triplets of alpha spheres to find possible binding orientations of the ligand within the binding site.

Rescoring was performed with London [18] and GBVI/WSA (Generalized-Born Volume Integral/Weighted Surface area) [19] scoring functions to rank the resulting poses of each molecule. The London scoring function estimates the free energy of a pose by forming a sum of energy term (considering ligand flexibility, H-bonds and desolvation) and geometric imperfections. The GBVI/WSA estimates the electrostatic solvation using a weighted solvent-accessible surface area. For structure activity analysis the best ranked docking pose of each molecule was energetically minimized within LigandScout using the Merck Molecular Force Field 94 (MMFF94) force field for optimization of the intermolecular interactions. 3D Protein–ligand interaction patterns (pharmacophores) were automatically generated using default settings. The final structure–activity relationship investigation was based on these pharmacophores and not on the absolute docking scores, due to their well known limited quantitative reliability, especially in small datasets [20].

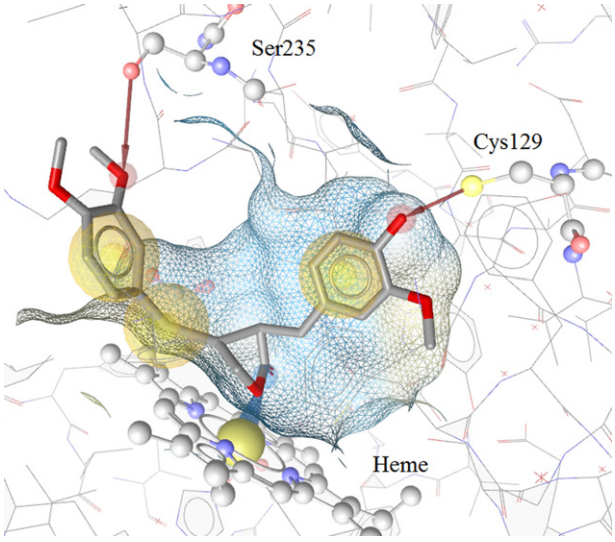

Fig. 2. Arctigenin fitted into the active site of IDO. Yellow spheres signify hydrophobic interactions between the molecule and the binding pocket. The blue cone stands for a metal bond between the lactone ring and the iron of the heme group. The red arrows mark hydrogen bond acceptors between Cys129 and a phenolic hydroxyl group and Ser235 and an aromatic methoxy group.

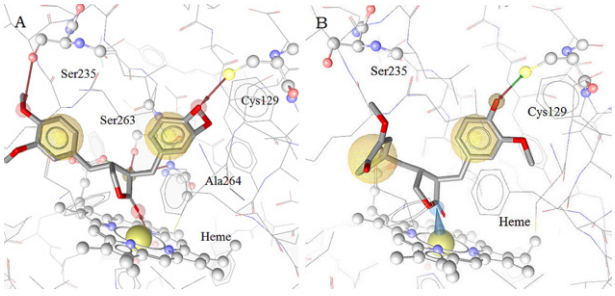

Fig. 3. (A) Docking pose of trachelogenin inside the IDO active site. Yellow spheres show hydrophobic parts of the molecule. Red arrows mark hydrogen bond acceptors, the green arrow marks a hydrogen bond donor. The hydrogen bonds to Ser263 and Ala264 stabilize a position farther removed from the iron compared to arctigenin. (B) The missing methoxy group in matairesinol leads to a loss of stabilization, because the hydrogen bond to Ser235 is not formed.

3. Results and discussion

In order to suggest a binding mode for the three ligands, the compounds were docked and energetically minimized into the active site of human IDO [16] (Figs. 2 and 3). For arctigenin the lactone ring was placed very close to the catalytic iron, forming a metal binding feature that explains the strong activity. This pose was stabilized by two hydrogen bonds to Ser235 and Cys129 on each side of the molecule. Trachelogenin was placed in a similar pose, but due to hydrogen bonds between the lactone hydroxyl group and Ser263/Ala264, the lactone was disposed from its ideal interaction geometry with the iron, possibly explaining the loss in activity (Fig. 3A). Compound matairesinol did not form the hydrogen bond with Ser235 and was less stabilized within the pocket (Fig. 3B).

The computational calculations confirmed the possibility of an intense interaction of two of out of the three lignin compounds with the binding site of the enzyme, and this was true for arctigenin and

trachelogenin only while matairesinol had no such effect. So even in the case when the structural differences between the three compounds appeared to be small at first glance, the interaction of them with the IDO binding site declined drastically. Thereby it is of particular interest to note that the dose-dependent suppressive effects which were observed in the *in vitro* test system of human PBMC were quite similar to the results of the calculations: also in PBMC arctigenin had the strongest influence on IDO activity followed by trachelogenin whereas matairesinol had no relevant effect. The calculated 50% inhibitory concentration (IC_{50} value) was 26.5 μmol/L for arctigenin followed by trachelogenin ($IC_{50} = 57.4$ μmol/L). Matairesinol showed only weak suppressive effects, decreasing KYN/TRP significantly only at a 193 μmol/L concentration. Moreover, IC_{50} concentration of arctigenin was only approximately 2.5-fold higher than that of the classical IDO inhibitor D-1-methyl tryptophan (1-MT) with and IC_{50} of 9.3 μM [12].

Molecular modeling calculation results showed that arctigenin and to a lesser extent also trachelogenin strongly interact with the IDO binding site, whereas matairesinol was predicted to be less stabilized within the binding pocket. Especially a hydrogen bond between the ligand and Ser235 was predicted as crucial for activity. Any effect of arctigenin and possibly also of trachelogenin to directly inhibit IDO activity would have several therapeutic consequences for antidepressant and immunomodulatory treatment strategies. It might explain treatment effects observed with *C. tinctorius* extracts in traditional medicine. However, further studies are needed to clarify this point and the possible application of arctigenin as an IDO inhibitor with additional immunomodulatory properties needs to be further tested in appropriate animal model systems.

Several natural products such as curcumin or reservatrol have been tested for IDO inhibitory activity [21,22]. However, most of published work employed assays based on cellular models like the human peripheral blood mononuclear cells (PBMC) assay [11,12,21,22], which was used also in this study and can detect a general effectiveness on IFN-γ-induced biochemical pathways like IDO but no direct influence of compounds on IDO is demonstrable. In most cases the suppression of tryptophan breakdown can be referred to an effect of compounds on IDO expression when the production of pro-inflammatory cytokines like IFN-γ becomes diminished. Therefore in most studies also parallel suppression of neopterin production is demonstrable. From a structural perspective the lactone moiety can been observed in some other IDO inhibitory compounds, like warfarin, coumarin and garvin B [23]. The lignan scaffold itself was (to our knowledge and recent literature search) not known to act on IDO previously.

The inhibitory effect of compounds on IDO cannot imply a similar influence on tryptophan 2,3-dioxygenase (TDO), because structural homology between TDO and IDO is rather limited, Uniprot identity 12.357%, (http://www.uniprot.org/uniprot/?query=reviewed%3Ayes), ClustalW alignment score −14 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). While both the enzymes act via an activated heme, the binding pocket anatomy is very different [24,25]. We assume it is unlikely that compounds like arctigenin or trachelogenin would also bind to TDO.

References

[1] He H., Cui L., exec. editors. Pharmacopoeia of PRC, vol. 1, English edition. Beijing: China Medical Science and Technology Press; 2010, p. 89–90.

[2] Zhao, G., Gai, Y., Chu, W.J., Qin, G.W. and Guo, L.H. (2009) A novel compound N(1),N(5)-(Z)-N(10)-(E)-tri-p-coumaroylspermidine isolated from *Carthamus tinctorius* L. and acting by serotonin transporter inhibition. J. Eur. Coll. Neuropsychopharmacol 19, 749–758.

[3] von Bruchhausen, F. (2007) In: W. Blaschek, S. Ebel, E. Hackenthal, U. Holzgrabe, K. Keller, J. Reichling, V. Schulz (Eds.), Hagers Enzyklopädie der Arzneistoffe und Drogen (4) 6th ed., vol. 3. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, pp. 880–883.

[4] Chen, Y. and Guillemin, G.J. (2009) Kynurenine pathway metabolites in humans: disease and healthy states. Int. J. Tryptophan Res. 2, 1–19.

[5] Schroecksnadel, K., Wirleitner, B., Winkler, C. and Fuchs, D. (2006) Monitoring tryptophan metabolism in chronic immune activation. Clin. Chim. Acta 364, 82–90.

[6] Chen, W., Liang, X., Peterson, A.J., Munn, D.H. and Blazar, B.R. (2008) The indoleamine 23-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. J. Immunol. 181, 5396–5404.

[7] Soliman, H., Mediavilla-Varela, M. and Antonia, S. (2010) Indoleamine 2,3-dioxygenase: is it an immune supressor? Cancer J. 16, 354–359.

[8] Byrne, G.I., Lehmann, L.K., Kirschbaum, J.G., Borden, E.C., Lee, C.M. and Brown, R.R. (1986) Induction of tryptophan degradation in vitro and in vivo: a gamma-interferon-stimulated activity. J. Interferon Res. 6, 389–396.

[9] Stone, T.W. (2001) Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. Prog. Neurobiol 64, 185–218.

[10] Takikawa, O. (2005) Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism. Biochem Biophys Res Commun 338, 12–19.

[11] Jenny, M., Klieber, M., Zaknun, D., Schroecksnadel, S., Kurz, K., Ledochowski, M. et al. (2011) In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. Inflamm. Res. 60, 127–135.

[12] Kuehnl, S., Schroecksnadel, S., Temml, V., Gostner, J.M., Schennach, H., Schuster, D. et al. (2013) Immune modulating effects of lig-nans from *Carthamus tinctorius*. Phytomedicine 20, 1190–1195.

[13] Accelrys Software Inc., Discovery studio modeling environment, Release 3.5, San Diego: Accelrys Software Inc.; 2012, http://accelrys.com/products/discovery-studio/.

[14] Hawkins, P.C., Skillman, G.A., Warren, G.L., Ellingson, B.A. and Stahl, M.T. (2010) Conformer generation with OMEGA: algorithm and validation using high quality structures from the protein databank and Cambridge structural database. J. Chem. Inf. Model. 50, 572–584.

[15] Molecular Operating Environment (MOE), 2012.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7; 2012.

[16] Sugimoto, H., Oda, S., Otsuki, T., Hino, T., Yoshida, T. and Shiro, Y. (2006) Crystal structure of human indoleamine 2,3-dioxygenase: catalytic mechanism of O_2 incorporation by a heme-containing dioxygenase. Proc. Natl. Acad. Sci. USA 103, 2611–2616.

[17] Wolber, G. and Langer, T. (2005) LigandScout: 3-d pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J. Chem. Inf. Model. 45, 160–169.

[18] Naïm, M., Bhat, S., Rankin, K.N., Dennis, S., Chowdhury, S.F., Siddiqi, I. et al. (2007) Solvated interaction energy (SIE) for scoring protein-ligand binding affinities. 1. Exploring the parameter space. J. Chem. Inf. Model. 47, 122–133.

[19] Corbeil, C.R., Williams, C.I. and Labute, P. (2012) Variability in docking success rates due to dataset preparation. J. Comput. Aided Mol. Des. 26, 775–786.

[20] Damm-Ganamet, K.L., Smith, R.D., Dunbar, J.B., Stuckey, J.A. and Carlson, H.A. (2013) CSAR Benchmark Exercise 2011–2012: evaluation of results from docking and relative ranking of blinded congeneric Series. J. Chem. Inf. Model. http://dx.doi.org/10.1021/ci400025f.

[21] Wirleitner, B., Schroecksnadel, K., Winkler, C., Schennach, H. and Fuchs, D. (2005) Resveratrol suppresses interferon-gamma-induced biochemical pathways in human peripheral blood mononuclear cells in vitro. Immunol. Lett. 100, 159–163.

[22] Maier, E., Kurz, K., Jenny, M., Schennach, H., Ueberall, F. and Fuchs, D. (2010) Food preservatives sodium benzoate and propionic acid and colorant curcumin suppress Th1-type immune response in vitro. Food Chem. Toxicol. 48, 1950–1956.

[23] Vottero, E., Balgi, A., Woods, K., Tugendreich, S., Melese, T., Andersen, R.J. et al. (2006) Inhibitors of human indoleamine 2,3-dioxygenase identified with a target-based screen in yeast. Biotechnol. J. 1, 282–288.

[24] Forouhar, F., Anderson, J.L., Mowat, C.G., Vorobiev, S.M., Hussain, A., Abashidze, M. et al. (2007) Molecular insights into substrate recognition and catalysis by tryptophan 2,3-dioxygenase. Proc. Natl. Acad. Sci. USA 104, 473–478.

[25] Sugimoto, H., Oda, S., Otsuki, T., Hino, T., Yoshida, T. and Shiro, Y. (2006) Crystal structure of human indoleamine 2,3-dioxygenase: catalytic mechanism of O_2 incorporation by a heme-containing dioxygenase. Proc. Natl. Acad. Sci. USA 103, 2611–2616.