International Journal of Biomedical and Pharmaceutical Sciences ©2013 Global Science Books

Docking Studies of Aldose Reductase Inhibitory Activity of Commercially Available Flavonoids

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ABSTRACT

Molecular docking is a frequently used tool in computer-aided structure-based rational drug design. Flavonoids are a group of natural products which exhibits various biological and pharmacological activities. The primary objective of this study was to investigate the aldose reductase inhibitory activity of flavonoids using *in silico* docking studies. In this perspective, flavonoids like aromadedrin, eriodictyol, homoeriodictyol, isorhamnetin, okanin, pachypodol, peonidin, robinetin, tangeritin were selected. Epalrestat, a known aldose reductase inhibitor was used as the standard. *In silico* docking studies were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. The interacting residues within the complex model and their contact types were identified. In the docking studies, three important parameters like binding energy, inhibition constant and intermolecular energy were determined. The results showed that all the selected flavonoids showed binding energy ranging between -9.20 kcal/mol to -8.02 kcal/mol when compared with that of the standard (-8.73 kcal/mol). Inhibition constant (181.13 nM to 1.32 μM) and intermolecular energy (-10.99 kcal/mol to -9.81 kcal/mol) of the flavonoids also coincide with the binding energy. All the selected flavonoids contributed aldose reductase inhibitory activity because of its structural properties. These molecular docking analyses could lead to the further development of potent aldose reductase inhibitors for the treatment of diabetes. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes.

Keywords: binding energy, diabetes, inhibition constant, intermolecular energy

INTRODUCTION

Diabetes has become a leading killer disease in recent years. According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%. The reasons behind this projected increase in prevalence rate are due to authorization, westernization and their associated lifestyle changes, increase in life expectancy at birth, obesity, physical inactivity, and possibly a genetic predisposition (Li *et al.* 2011).

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Type 1 diabetes is caused by a lack of *β*-pancreatic cells insulin secretion (Li *et al.* 2011). Type 2 diabetes is associated with obesity and is characterized by an early phase progressive insulin resistance, with ensuing reduction in the ability of pancreatic hormone to promote peripheral glucose disposal and to decreases hepatic glucose output (Lamba *et al.* 2011; Vianna *et al.* 2011).

Aldose reductase (ALR2; EC: 1.1.1.21) belongs to aldo-keto reductases super family. It is the first rate limiting enzyme in polyol pathway and reduces glucose to sorbitol by utilizing NADPH as a cofactor. sorbitol dehydrogenase is the enzyme responsible for the conversion of Sorbitol into fructose (Hwang *et al.* 2005; Ravindranath *et al.* 2009). However, in the presence of high glucose, the activity of this pathway is increased and could represent up to 30% of total glucose consumption (Yadav *et al.* 2009). Abnormal activation of the polyol pathway during diabetes leads to accumulation of osmotically active sorbitol leading to osmotic as well as oxidative stress, resulting in tissue injury (Dong *et al.* 2005; Saraswat *et al.* 2008). Evidence for the involvement of ALR2 in diabetic neuropathy, retinopathy, nephropathy and cataract emerged from several independent studies (Guzman and Guerrero 2005). Thus inhibiting ALR2 activity appears to be an effective means to prevent the diabetic complications.

Flavonoids and their related compounds are low molecular weight substances, which are a group of natural products which exhibits various biological and pharmacological activities like antibacterial, antiviral, antioxidant, anti inflammatory, anti allergic, hepatoprotective, antithrombotic, antiviral and antimutagenic effects and inhibition of several enzymes (Cushnie and Lamb 2011; Liu *et al.* 2007; Gonzalez *et al.* 2011; Nishumi *et al.* 2011).

Drug design is an important tool in the field of medicinal chemistry where new compounds are synthesized by molecular or chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effect (Cavasotto and Abagyan 2004). A huge breakthrough in the process of drug design was the development of *in silico* method to predict about the therapeutic efficacy of the molecule (Khokra *et al.* 2011; Saleshier *et al.* 2011).

Molecular docking is a frequently used tool in computer-aided structure-based rational drug design. It evaluates how small molecules called ligands (flavonoids) and the target macromolecule (ALR2 enzyme) fit together (Seeliger and de Grootligand 2010; Norgan *et al.* 2011). Auto Dock Tools (ADT) is a program package of automated docking tools and designed to predict how small molecules bind to a target protein of known 3D-structure. Besides generating binding energies in these docking studies, the position of the ligand in the enzyme binding site can be visualized (Zhang *et al.* 2008; Cosconati *et al.* 2010). It can be useful for developing potential drug candidates and also for the understanding the binding nature.

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Schames *et al.* 2004). Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid

Received: 20 January, 2012. Accepted: 22 March, 2012.

Original Research Paper

genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy (Cosconati *et al*. 2010).

There is a need to develop new and potent ALR2 inhibitors. The main objective of the present work is to study the *in silico* ALR2 inhibitory activity of commercially available flavonoids.

MATERIALS AND METHODS

Softwares required

Python 2.7 - language was downloaded from www.python.com, Cygwin (a data storage) c:¥program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Discovery studio visualizer 2.5.5 was downloaded from www.accelerys.com, Molecular orbital package (MOPAC), Chemsketch was downloaded from www.acdlabs.com. Online smiles translation was carried out using cactus.nci.nih.gov/translate/.

Docking methodology

Lamarckian genetic algorithm (LGA) for ligand conformational searching is used for the docking, which is a hybrid of genetic algorithm and local search algorithm. This algorithm first builds a population of individuals, each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a user-specified proportion of the individuals. The individuals with the low resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian genetic algorithm because every new generation of individuals is allowed to inherit the local search adaptations of their parents (Madeswaran *et al.* 2011).

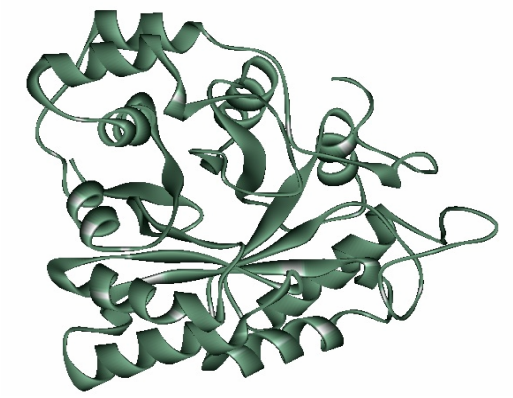

Fig. 1 Refined aldose reductase enzyme (3EL3).

An extended PDB format, termed as PDBQT file was used for coordinate files which includes atomic partial charges. AutoDock Tools was used for generating PDBQT files from traditional PDB files (Umamaheswari *et al.* 2012). Crystal structure of ALR2 enzyme was downloaded from the Brookhaeven protein data bank and the enzyme was refined using various steps with the help of Accelrys studio viewer. The refined enzyme structure was further utilized in the docking study (**Fig. 1**).

The flavonoid ligands like aromadedrin, eriodictyol, homo-

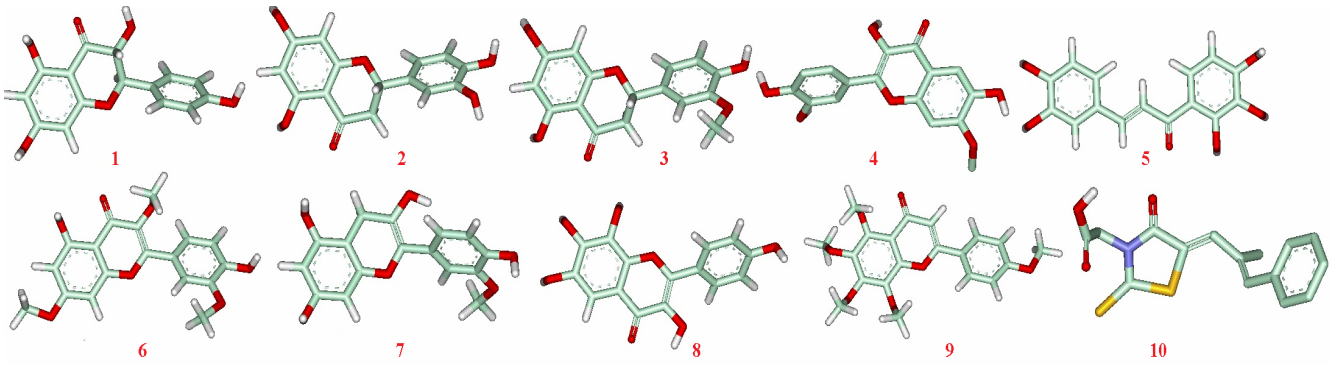

Fig. 2 The optimized ligand molecules (**1** aromadedrin, **2** eriodictyol, **3** homoeriodictyol, **4** isorhamnetin, **5** okanin, **6** pachypodol, **7** peonidin, **8** robinetin, **9** tangeritin and **10** epalrestat).

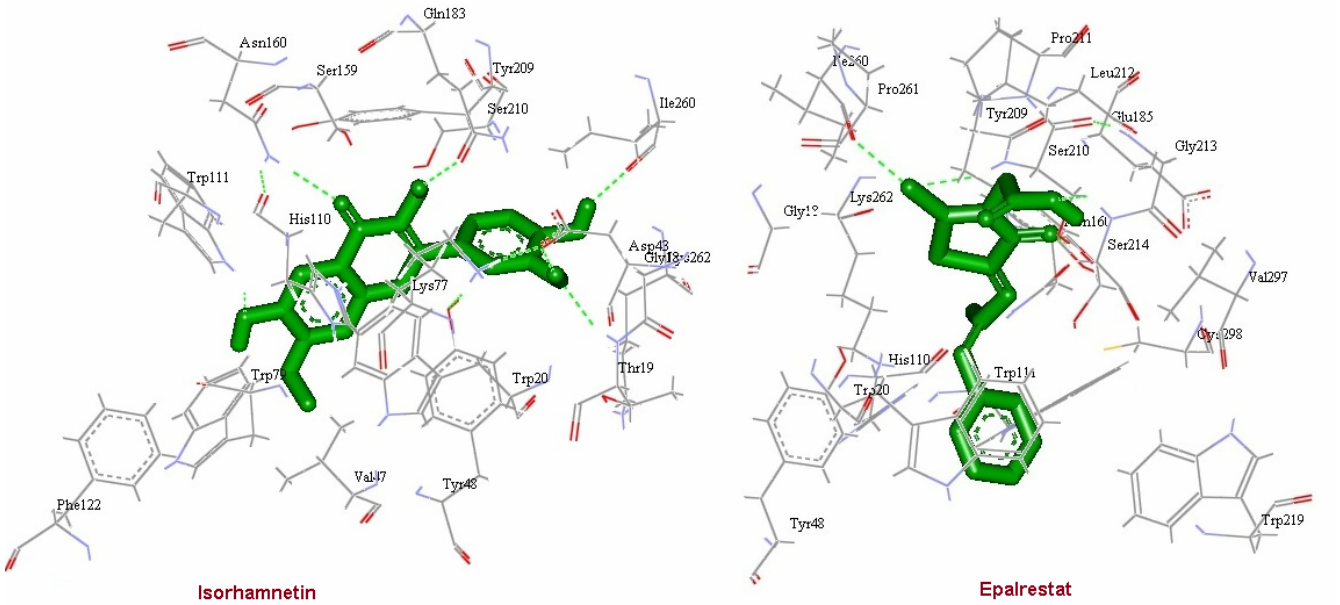

Fig. 3 Docked pose of aldose reductase enzyme with the ligands isorhamnetin and epalrestat.

eriodictyol, isorhamnetin, okanin, pachypodol, peonidin, robinetin, tangeritin and epalrestat were built using Chemsketch (**Fig. 2**) and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies. The optimized ligand molecules were docked into refined ALR2 model using "LigandFit" in the AutoDock 4.2 (Umamaheswari *et al.* 2011).

The preparation of the target protein 1EL3 (unbound target) with the AutoDock Tools software involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the macromolecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of the program. Three-dimensional affinity grids of size 277 × 277 × 277 Å with 0.6 Å spacing were centered on the geometric center of the target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein. Additionally, an electrostatic map and a desolvation map were also calculated (Umamaheswari *et al.* 2011).

Rapid energy evaluation was achieved by precalculating atomic affinity potentials for each atom in the ligand molecule. In the AutoGrid procedure, the target enzyme was embedded on a three dimensional grid point (Madeswaran *et al.* 2011). The energy of interaction of each atom in the ligand was encountered.

The selected important docking parameters for the LGA as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06.

AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates (Umamaheswari *et al.* 2011). AutoDock Tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and its energy and other parameters like intermolecular energy and inhibition constant. For each ligand, ten best poses were generated and scored using AutoDock 4.2 scoring functions (Madeswaran *et al.* 2011).

RESULTS AND DISCUSSION

Docking analysis

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against aldose reductase enzyme. In this study 9 different flavonoids were selected for the *in silico* docking studies. Lead optimization of the selected compounds was done by computation of drug-likeness properties. The druglikeness scores of the compounds were evaluated with the help of Lipinski's rule. The docking studies were performed by the use of AutoDock4.2. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity.

Docking is finding the binding geometry of two interacting molecules with known structures. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.

The best scoring ligands were identified by best docked scores obtained in comparitive docking studies of ligands with ALR2 enzyme. Identified ligands can be explored further to generate more effective and potential drug molecules through ligand based drug designing approaches. These docking studies also provide indepth understanding of the interaction at their binding sites of ligand groups and receptor sites.

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses (Madeswaran *et al.* 2011). This ranking of the compounds were based on their binding energy with the enzyme. If the binding energy of the compound is less, then the particular compound has more active in nature. In **Fig. 3**, docked pose of ALR2 enzyme with the ligands isorhamnetin and epalrestat clearly demonstrated the binding positions of the ligand with the enzyme. Binding energy of the individual compounds were calculated by using the following formula:

Binding energy = A+B+C-D

where A denotes final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol), B denotes final total internal energy (kcal/mol), C denotes torsional free energy (kcal/mol), D denotes unbound system's energy (kcal/mol).

The binding sites of the epalrestat was found to be GLY 18, TRP 20, LYS 21, TYR 48, HIS 110, TRP 111, TYR 209, SER 210, SER 214, ILE 260. The potential binding sites of the isorhamnetin was found that, GLY 18, THR 19, TRP 20, TYR 48, HIS 110, TRP 111, ASN 160, GLN 183, TYR 209, SER 210, ILE 260. This proves that the effective binding sites are present in the selected flavonoid isorhamnetin when compared with the standard epalrestat. It proves that the ability of inhibiting the ALR2 enzyme by the selected flavonoid.

Most of the flavonoids have anti-inflammatory properties. Therefore the consumption of flavonoids could be appropriate in medical conditions involving inflammation. Flavonoids are the excellent antioxidants when compared to other compounds. Extracts from onion and different flavonoids activate the cellular antioxidant system (Yadav *et al.* 2009). Analysis of the receptor/ligand complex models generated after successful docking of the flavonoids was based on the parameters such as hydrogen bond interactions, π – π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site (Azam *et al.* 2011).

As a general rule, in most of the potent anti inflammatory compounds, both hydrogen bond and π – π hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity. Analysis of the receptor/ligand complex models generated after successful docking of the flavonoids was based on the parameters such as binding energy, hydrogen bond interactions, π – π interactions, orientation of the docked compound within the active site, and RMSD of active site residues (Zhang *et al.* 2008).

As shown in **Table 1**, flavonoids showed binding energy ranging between -9.20 kcal/mol to -8.02 kcal/mol. All the selected flavonoids had showed better and consistent binding energy when compared to standard epalrestat (-8.73 kcal/mol). This proves that flavonoids consist of potential ALR2 inhibitory binding sites similar to that of the standard.

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. As shown in **Table 2**, flavonoids showed inhibition constant ranging from 181.13 nM to 1.32 µM. All the selected compounds had lesser inhibition constant when compared to the standard (397.18 nM). Inhibition constant is directly proportional to binding energy. Thus, the ALR2 inhibitory activity of the flavonoids were compared with the epalrestat.

As shown in **Table 3**, flavonoids showed intermolecular energy ranging between -10.99 kcal/mol to -9.81 kcal/mol which was lesser when compared to the standard (-10.52 kcal/mol). Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of all the selected compounds with a simultaneous decrease in the binding energy. This result further proved the ALR2 inhibitory activity of all the selected flavonoids.

Based on the docking studies, the ALR2 inhibitory activity of the selected compounds was found to be decreased in the order of isorhamnetin, okanin, eriodictyol, epalrestat, peonidin, homoeriodictyol, tangeritin, pachypodol, aroma-

Table 1 Binding energies of the compounds by the docking studies.

Compounds	Binding energies of the compounds based on their rank (kcal/mol)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aromadedrin	-8.09	-7.96	-7.92	-7.88	-7.91	-7.77	-7.64	-7.48	-7.47	-6.46
Eriodictyol	-8.86	-8.85	-8.65	-7.76	-8.16	-7.63	-7.39	-7.36	-7.35	-7.17
Homoeriodictyol	-8.40	-8.29	-8.13	-7.65	-7.63	-7.59	-7.52	-7.50	-7.32	-7.42
Isorhamnetin	-9.20	-9.12	-9.03	-8.87	-8.60	-8.56	-8.48	-8.38	-8.37	-7.59
Okanin	-9.11	-8.82	-8.69	-8.69	-8.67	-7.97	-7.51	-6.93	-6.92	-7.25
Pachypodol	-8.20	-8.19	-7.86	-7.74	-7.53	-7.53	-7.92	-7.81	-7.23	-7.12
Peonidin	-8.64	-8.64	-8.37	-7.93	-8.31	-8.27	-7.79	-7.77	-7.06	-6.71
Robinetin	-8.02	-7.99	-7.99	-7.98	-7.95	-7.95	-7.95	-6.61	-6.51	-6.22
Tangeritin	-8.23	-7.82	-7.68	-7.67	-7.27	-7.72	-7.67	-7.63	-7.24	-7.07
Epalrestat	-8.73	-8.65	-8.63	-8.12	-7.86	-7.85	-7.60	-7.23	-7.69	-7.07

Table 2 Inhibition constant of the compounds by the docking studies.

Compounds	Inhibition Constant of the compounds based on their rank (mM, * µM)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aromadedrin	1.17*	1.47*	1.57*	1.67*	1.59*	2.02*	2.52*	3.31*	3.36*	18.27*
Eriodictyol	320.02	325.20	460.33	2.04*	1.04*	2.54*	3.82*	4.03*	4.10*	5.57*
Homoeriodictyol	692.02	833.12	1.10*	2.48*	2.55*	2.73*	3.08*	3.18*	4.30*	3.65*
Isorhamnetin	181.13	206.52	241.66	313.75	500.84	529.20	607.14	719.71	727.77	2.72*
Okanin	211.80	343.41	427.35	429.31	443.64	1.45*	3.15*	8.36*	8.51*	4.86*
Pachypodol	979.64	988.78	1.75*	2.14*	3.01*	3.03*	1.55*	1.89*	5.03*	6.05*
Peonidin	463.43	467.71	732.53	1.55*	814.22	863.30	1.95*	2.01*	6.71*	12.05*
Robinetin	1.32*	1.38*	1.39*	1.42*	1.48*	1.49*	1.49*	14.35*	16.98*	27.45*
Tangeritin	933.05	1.84*	2.35*	2.39*	4.67*	2.18*	2.38*	2.57*	4.95*	6.57*
Epalrestat	397.18	457.96	469.23	1.12*	1.73*	1.76*	2.67*	4.98*	2.32*	6.57*

Table 3 Intermolecular energies of the compounds by the docking studies.

Compounds	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aromadedrin	-9.58	-9.45	-9.41	-9.37	-9.40	-9.26	-9.13	-8.97	-8.96	-7.96
Eriodictyol	-10.35	-10.34	-10.14	-9.25	-9.65	-9.12	-8.88	-8.85	-8.84	-8.66
Homoeriodictyol	-9.90	-9.79	-9.62	-9.14	-9.12	-9.08	-9.01	-8.99	-8.81	-8.91
Isorhamnetin	-10.99	-10.91	-10.82	-10.66	-10.38	-10.35	-10.27	-10.17	-10.16	-9.38
Okanin	-11.49	-11.21	-11.08	-11.07	-11.05	-10.35	-9.89	-9.31	-9.30	-9.64
Pachypodol	-9.99	-9.98	-9.64	-9.53	-9.32	-9.32	-9.71	-9.60	-9.02	-8.91
Peonidin	-10.43	-10.43	-10.16	-9.72	-10.10	-10.06	-9.58	-9.56	-8.85	-8.50
Robinetin	-9.81	-9.78	-9.78	-9.77	-9.74	-9.74	-9.74	-8.40	-8.30	-8.01
Tangeritin	-10.02	-9.61	-9.47	-9.46	-9.06	-9.51	-9.46	-9.42	-9.03	-8.86
Epalrestat	-10.52	-10.44	-10.42	-9.91	-9.65	-9.64	-9.39	-9.02	-9.48	-8.86

dedrin, robinetin. On the basis of the above study, all the selected flavonoids showed better ALR2 inhibitory activity than the standard. Among the selected flavonoids, isorhamnetin, okanin and eriodictyol showed excellent binding interactions with ALR2 enzyme than the standard. This may be attributed due to the differences in the position of the functional groups in the compounds.

CONCLUSION

These results clearly indicate that the flavonoids especially, isorhamnetin, okanin and eriodictyol showed excellent binding interactions with ALR2 enzyme than the standard. This *in silico* studies is actually an added advantage to screen the ALR2 enzyme inhibition. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes.

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