A study of anti-diabetic activity of gensinoside by assessing sodium-glucose symporter blocking effect in silico and in rat intestinal model

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Abstract

Diabetes mellitus has repeatedly attracted attention of researchers to design different therapeutic approaches to achieve an optimal treatment for this serious health challenge. The purpose of the study is to evaluate treatment option for controlling hyperglycemia at site of glucose absorption through designing computerized and in vivo models to test a sodium-glucose symporter based drug design. In silico protein data bank (pdb) model of SGLUT was processed and analyzed for docking with edited test glycoside. Another model included determination of the dose-response relationship in rat intestinal glucose/saline perfusion with test glycoside. Our result showed that Ginsenoside revealed a dose dependent SGLUT blocking activity in a saturation kinetics curve, which was agreed with in silico model results.

Keywords: SGLUT; Gensinoside; Perfusate glucose; ICM docking

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Introduction

Diabetes mellitus has become increasingly a global health challenge that has a progressive incidence and prevalence all over the world [1, 2]. It has multivariate pathological mechanisms and etiologies and hence has variable clinical pictures in regard severity and progressiveness [3]. However, concerning an arrangement for optimal treatment design, many target proteins may be rational candidate. There are

many mechanisms of treatment of diabetes mellitus like stimulation of pancreatic islets cells for secreting insulin, enhancing peripheral sensitizations of GLUT2 expressing cells for responding to insulin [4], in addition to diminishing the GIT absorption of glucose [5].

Several glycosides are good therapeutic options for modifying SGLUT function since they are carrying a structural analogy with beta-galactose and D-glucose [6, 7]. One of the common glycosides is phlorizin which is a glycoflavone derivative. Most of these glycosides are naturally occurring with a good spectrum of safety. The computerized model of docking test SGLUT blockers has many advantages in that it enables selecting the more efficacious analogue for further in vitro and in vivo assessment among many test agents. Bioassay model of assessing of rat intestinal absorption of glucose is a reliable tissue model for testing glucose absorption blocking effects of different natural products like glycoside analogue of galactose and glucose (7).

Method

The current research has been done in the Kufa College of Medicine/ Dep. Of Pharmacology and Therapeutics/ Therapeutics Researches Lab. 2014 and designed on a biotarget structure SGLUT (intestinal sodium glucose transporter) which was analyzed in two serial models:

1. In silico assessment
2. In vivo assessment

In the computerized model the SGLUT was processed and analyzed by protein internal coordinate mechanics ICM and drug docking parameters, figure 1. SGLUT of rat was introduced in pdb format for designing a docking project to calculate test glycoside energy index in blocking the target intestinal glucose symporter. Molsoft ICM pro software package was used.

Gensinoside was edited and converted into 3D format for interaction test. Molecular parameters important in molecular dynamics were calculated with the same editor program. Whereas SGLUT was processed for surface receptors and scaffold finding.

Gensinoside-SGLUT interaction trajectory was run to calculate blocking effect in form of hydrogen bond, Vander Waals bonds and ionic bond energy index. ICM embedded statistics were used automatically by the software.

Animal model involved designing a bioassay model

Westar rat was anesthetized with 0.1 ml diazepam i.m. and under wet and warm (25 C) conditions, the longitudinal abdominal wall was excised to expose and identify jejunum where maximal glucose absorption occurs. Care was taken to ensure intact mesenteric blood supply. Fifteen cm of rat jejunum which was canulated from both proximal and distal ends to collect and assess output perfused for the level of glucose. Repeated and sequential addition of the test glycoside was monitored for mathematically evaluating saturation kinetics which were taken as an indicator for SGLUT blocking activity.

Material

The test glycoside was the natural product; Ginsenoside (100 µg/L USA. Healthy Sense), Figure 2 and that has the characteristic properties obtained with ICM listed in the table 1.

Animal

A Wistar rat species of 200g body weight was anesthetized with an ip injection of 20 mg of Phenobarbital for induction of prolonged anesthesia [8]. A longitudinal abdominal incision was done through the midline to expose whole intestine. A piece of wet gauze is inserted to keep the intestine wet throughout the experiment. Rapid flow distilled water was done through the piece of jejunum to ensure a complete wash of intra-luminal contents to avoid artifacts. Then a glucose/saline solution is allowed to perfuse through the jejunum for one minute at a rate of 2drops. A serial addition of ginsenoside (50 µg) is assessed with wash cycle and perfused collection for each addition. Output perfuse is assessed for glucose.

As output glucose is an indirect indicator of GLUT1 blocking activity of ginsenoside, since the only route of D-glucose absorption in the intestine is GLUT1 which is a symporter with Na ions. The glucose absorption through SGLUT follows a saturated kinetics of the following formula:

J= Jmax.[Na]/Km+[Na]

Where J is glucose flux rate (in nMol/mg/min), Jmax is the maximum rate of glucose diffusion along the Na concentration through SGLUT it normally equals to 8 nM/mg/min, whereas Km is the concentration of Na that achieves half Jmax, it equals to 2 nMol. According to the formula above, a test SGLUT blocker will follows saturation kinetics.

Results

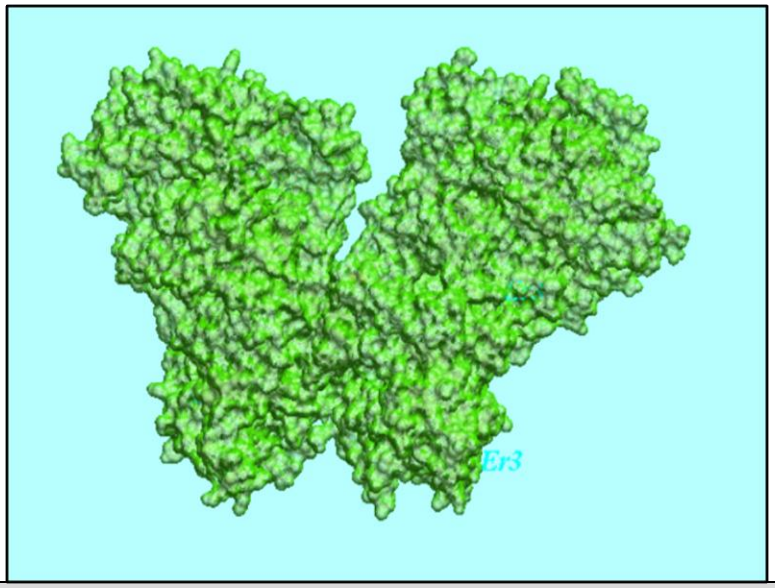

Figure 1.

The SGLUT receptor map setting and analysis with ICM.

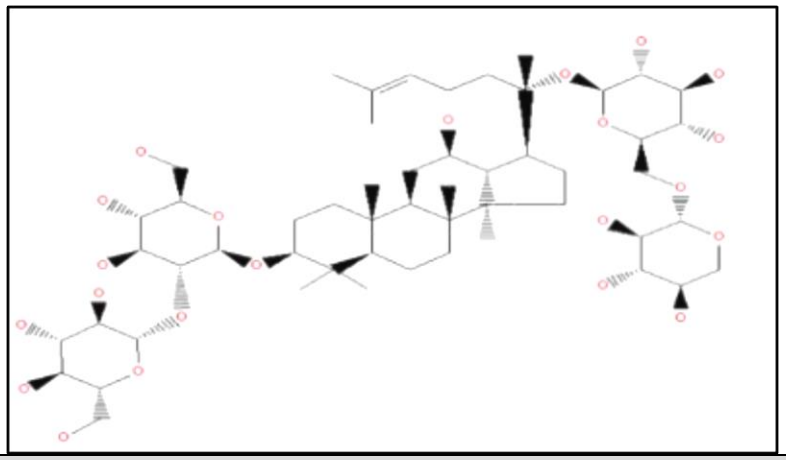

Figure 2.

Represented 2D structure of ginsenoside.

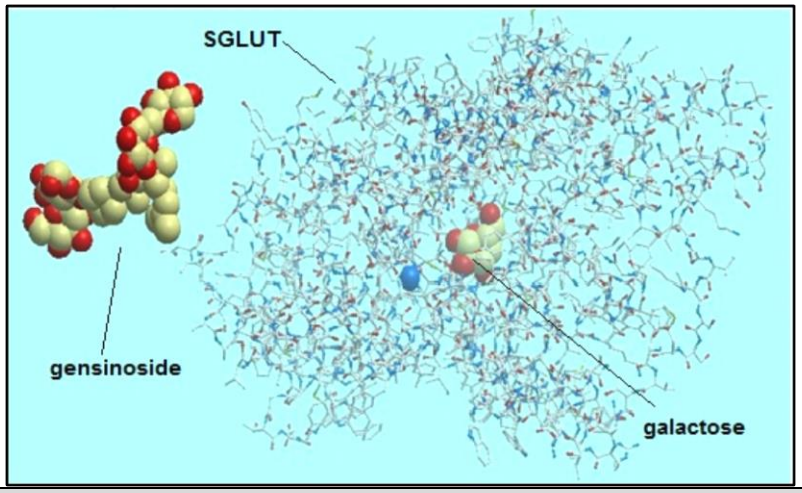

Figure 3.

The drug docking setting between a ginsenoside (left CPK presentation: space-filling) and sodium-glucose symporter (right with a central beta galactose and Na ion). There was an obvious structural analogue between ginsenoside sugar moiety and beta galactose.

	Name	Value	pVal
1	MolWeight	1078.59	0.01
2	HBA	22	0.01
3	HBD	14	0.01
4	RotB	15	0.06
5	DrugLikeness	0.24	0.40
6	MolHf	-979.39	0.00
7	MolLogP	-1.05	0.16
8	MolLogS	-6.98	0.03
9	MolPSA	284.92	0.02
10	Volume	1072.26	0.01
11	Formula	C53 H90 O22	
12	Smiles	CC(C)=CCC[C@@](	
13	Bad Groups		

Table 1.

The computerized estimation of gensinoside molecular parameters, where: Molecular Weight (MolWeight). Number of Hydrogen Bond Acceptors (HBA). Number of Hydrogen Bond Donators (HBD). Number of Rotatable Bonds (RotB). Drug Likeness value returns either 1 to -1. Prediction model build for 'delta Hf in gas' property. A low dHf value means that the compound is more 'stable.' Polar Surface Area (PSA). Bad ADME-Tox Groups

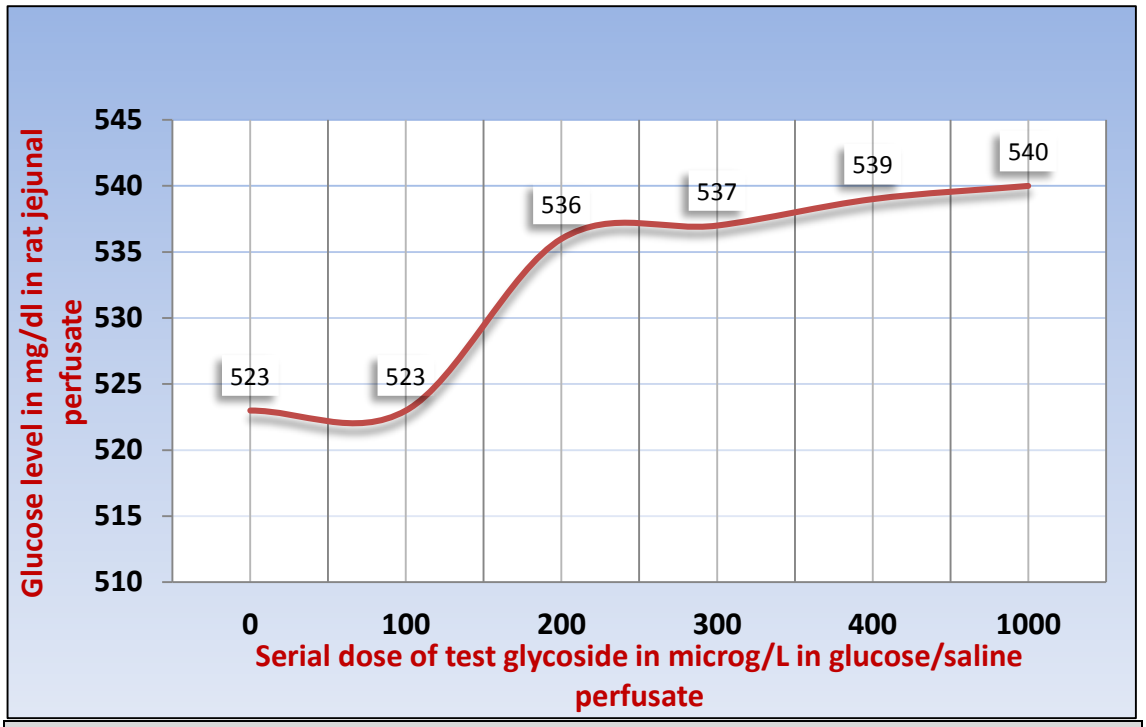

Figure 3.

The serial increase in the level of output perfuse glucose as a response to an increase in glycoside G concentration. This represents a dose dependent blocking effect on SGLUT since D-glucose unlike fructose is only absorbed through GLUT1 (SGLUT).

A. The SGLUT binding affinity was assessed with ginsenoside docking project settings, figure 3. Energy index can estimate a drug affinity to protein scaffold that transport beta galactose.

B. *In vivo results*: the blocking effect of the test glycoside was analyzed and revealed saturation kinetics in glycoside-SGLUT affinity. Curve fitting by MATLAB package statistics verifies saturation formula that correlates the dose-response relationship, figure 4.

Discussion

Intestinal sodium-glucose symporter is a very important target for modulation of glycemic control in patients with diabetes mellitus. Targeting GLUT1 blocking has several beneficial considerations, including suitability of use of such mechanism in both insulin dependent and non-insulin dependent DM. This mechanism can be effective in noncompliant patients like pediatric age groups. Moreover, blocking intestinal GLUT1

could be given as an adjunct with conventional and diabetic drugs. Targeting peripheral absorption of sugar is commonly used in Acarbose mechanism of action [5].

There was a considerable ginsenoside affinity for blocking the computerized model of SGLUT. This parameter is estimated by energy index of binding [9]. In silico results has agreed with in vivo model in that SGLUT blocking effect of ginsenoside was increasingly dependent on the concentration of the glycoside in a sigmoid shape indicating saturation kinetics similar studies using phlorizin glycoside showing a potent inhibition of SGLUT obtained with phlorizin [10, 11]. In addition that gensinoside prevents glucose absorption in dose dependent saturable response (figure 4), extensive studies showed antioxidant and anticoagulant action. These properties encourage use of this glycoside to prevent cardiovascular complications in patients with diabetes mellitus [12, 13].

In conclusion, there was a dose dependent blocking of glucose absorption through rat intestinal GLUT1 achieved with gensinoside that agreed with the ICM docking project. Further assessment of other glycosides analogues for higher energy indices to be assessed in animal models and clinical trials.

Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

All authors wrote, read and approved the final manuscript.

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