DOI 10.1007/s10517-017-3881-0
Bulletin of Experimental Biology and Medicine, Vol. 163, No. 5, September, 2017 METHODS

Effect of GRP119 Receptor Agonist, Compound MBX-2982, on Activity of Human Glucokinase

A. A. Spasov, V. A. Kosolapov, D. A. Babkov, and O. Yu. Maika

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 5, pp. 657-660, May, 2017
Original article submitted October 5, 2016

Validation of the method for studies of glucokinase activators by the spectrophotometric method in an *in vitro* test system is carried out. The advantage of NAD coenzyme *vs.* thio-NAD is proven. Manifest activation of glucokinase by MBX-2982 compound (GPR119 agonist) in a wide range of concentrations is demonstrated experimentally.

Key Words: *glucokinase; hexokinase; glucokinase activators; type 2 diabetes mellitus*

Despite a great choice of oral hypoglycemic drugs, creation of new effective and safe drugs for the treatment of patients with type 2 diabetes mellitus (DM2) remains a pressing problem. One of the promising targets for the creation of new antidiabetic drugs is glucokinase (GK), or type 4 hexokinase catalyzing stage 1 of glucose catabolism, formation of glucose-6-phosphate [4]. GK is extremely important for glucose homeostasis acting as a glucose "sensor" in pancreatic β cells and controlling the rate of glycogen synthesis in the liver [8]. Activators of GK are a new class of prospective antidiabetic drugs with a reliable hypoglycemic effect due to increase of GK activity in the liver and pancreas [7].

Compound MBX-2982 (Sanofi) is a potential antidiabetic drug that demonstrated good results in phase 2 clinical trials. It is a GPR119 receptor agonist (this receptor is located in the intestine and pancreas) with a double mechanism of action. By directly modulating activity of pancreatic β cells, it increases insulin production and stimulates the secretion of glucagon-like peptide 1 (GLP-1) in the intestine [5].

The MBX-2982 molecule contains aminopyrimidine and tetrazole fragments, this rendering it structural similarity to highly active GK activators of pyrazin derivative series, such as AZD1656 (Astra-Zeneca, EC_{50} 64 nm at 5 mM glucose), *etc.* (Fig. 1) [3,12]. On the other hand, the effects of MBX-2982 on GK activity have not been described.

We study the effects of MBX-2982 on human GK activity in an *in vitro* test system.

MATERIALS AND METHODS

Human GK 4IWW model [11] crystallized with low molecular activator from the RCSB PDB public repository was used in the study. Preparation of the protein structure for docking consisted in removal of ligands, inorganic ions, and solvent molecules. All hydrogen atoms were then added and Gasteiger partial atomic charges calculated. Preparation of the ligand models for docking was carried out automatically using AutoDockTools 1.5.6 software. Experimental docking of ligand models in GK allosteric center was carried out using AutoDock Vina 1.1.2 software [10]. Only the conformations with the maximum free binding energy gain were selected for subsequent analysis. At stage 1, docking of the original ligand was carried out for evaluation of the correctness of reproduction of crystallographic binding conformation. Docking of the studied ligand was then carried out with the use of the same parameters.

Analysis of GK activity was carried out at 37°C in a 96-well transparent flat bottom plate (Costar 9018). The incubation mixture contained 25 mM HEPES buffer (pH 7.2) (Sigma), 25 mM KCl (analytical grade), 5 mM D-glucose (Sigma), 1 mM ATP (Sigma), 1.8 mM NAD (Sigma), 2 mM $MgCl_2$ (chemi-

Volgograd State Medical University, Ministry of Health of the Russian Federation, Volgograd, Russia. ***Address for correspondence:*** aspasov@mail.ru. A. A. Spasov

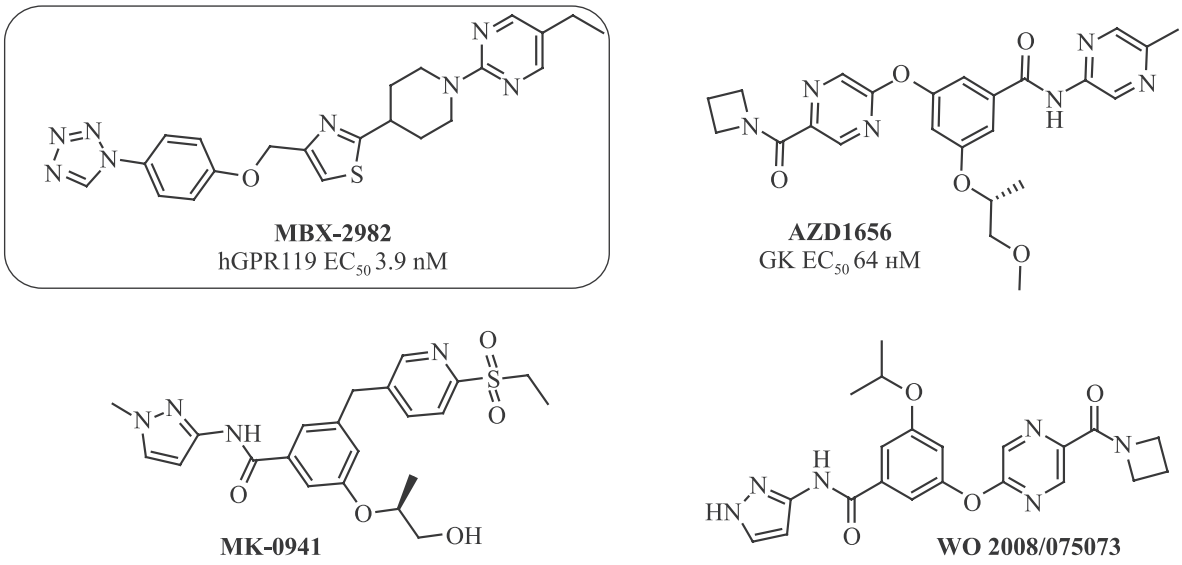

Fig. 1. Structure of GPR119 activator of compound MBX-2982 and of highly active activators of GK.

cally pure), 1 mM dithiotreitol (Sigma), 1.8 U/ml *L. mesenteroides* glucose-6-phosphate dehydrogenase (G6PDH; Sigma), and GK (recombinant human liver type 4 hexokinase expressed in *E. coli* in the form of protein fused with glutathione-S-transferase — GST-GK; Sigma). G6PDH and GK were added in amounts sufficient for attaining an optical density increase of 0.08-0.1 U within 30 min of incubation. The formation of NADH was evaluated by optical density increment at λ=340 nm using Infinite M2000 PRO microplate reader (Tecan) for 30 min of culturing.

Compound MBX-2982, 2-[1-(5-ethylpyrimidin-2-yl)piperidin-4-yl]-4-[[4-(tetrasol-1-yl)phenoxy] methyl]-1,3-thiasole (Sanofi), was chosen for the study [5]. The compound was added to experimental samples in 25 mM HEPES buffer (pH 7.2) containing 5% DMSO. To zero samples, the same volume of 5% DMSO solution in 25 mM HEPES buffer (pH 7.2) was added.

The results were processed using GraphPad Prism 6 software (GraphPad Inc.) with calculation of the basic statistical values: arithmetic mean±standard error. Statistical processing was carried out using Statistica 10.0 software with parametric Student's paired *t* test or nonparametric Mann—Whitney test. The effective concentration of the substance at which it activated the reaction by 50% (EC_{50}) was calculated using GraphPad Prism 6 software by the linear regression method.

RESULTS

Molecular docking experiments have been carried out for preliminary evaluation of the capacity of compound MBX-2982 to bind to GK allosteric center. The MBX-

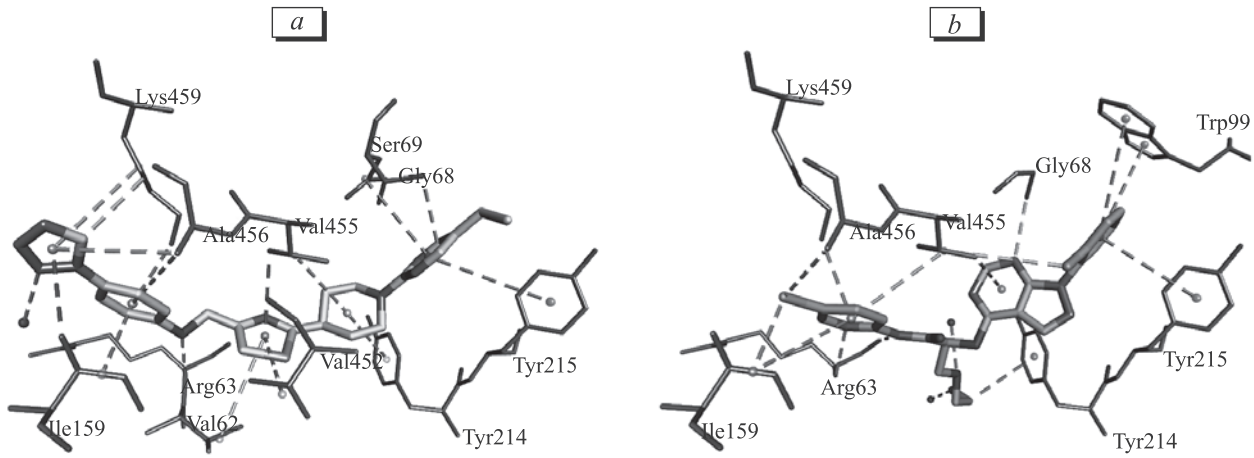

Fig. 2. Models of binding of MBX-2982 in GK allosteric center according to molecular docking results (*a*) and of original ligand according to roentgenostructural analysis (*b*).

TABLE 1. Convergence and Intermediate Convergence of Method for *In Vitro* Studies of GK Activity with the Use of MBX-2982 Compound in a Concentration of 0.1 mM (*M*±*m*)

Parameter	Optical density, arb. units	Variability coefficient of method, %
Convergence (*n*=9)	0.3370±0.0174*	5.154775
Intermediate convergence (*n*=24)	0.3360±0.0188*	5.600132

Note. *n*: number of repeats. *$p<0.05$ in comparison with zero samples.

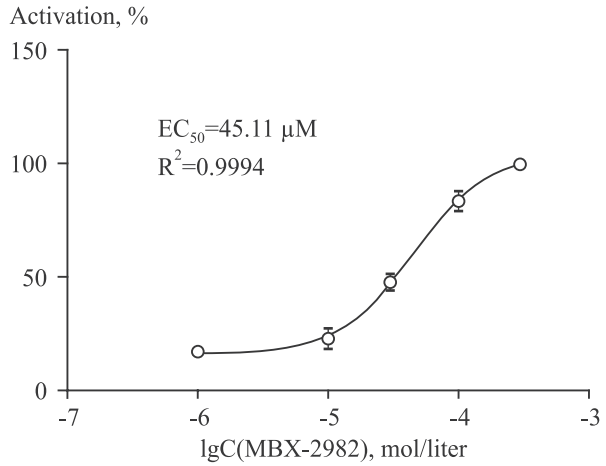

Fig. 3. Effects of MBX-2982 compound in various concentrations on GK activity.

2982 molecule forms a series of interactions characteristic of GK activators (Fig. 2). Hydrogen bond to the enzyme main chain in Arg63 and T-wise π—π stacking with Tyr215 aromatic radical are essential [6]. Numerous hydrophobic and π-alkyl interactions with the lateral chains of the binding pouch amino acids are found, which are realized at the expense of aromatic cycles in the MBX-2982 molecule. All these facts suggest specific binding of the compound to GK and hence, the probability of activation of the enzyme.

Today, activity of GK is studied *in vitro* by the G6PDH-conjugated method with substrates thio-NAD or NAD [2,9]. For this reason we studied in preliminary experiments the effect of the coenzyme on the course of analytical reaction. The use of NAD promoted a more rapid increment of optical density of the reaction mixture and a better signal/murmur proportion. In the presence of thio-NAD the optical density increased from 0.127±0.001 arb. units (min 0) to 0.173±0.001 arb. units (min 30), with NAD — from 0.052±0.001 arb. units (min 0) to 0.189±0.001 arb. units (min 30).

The next stage of the study was validation of the analytical method for GK activity evaluation. The convergence and intermediate (intralaboratory) convergence of the method with the use of MBX-2982 compound were evaluated. The convergence of the method was 5.15%, intermediate convergence 5.6% (Table 1), which indicated its high precision (reproducibility) [1].

Activity of GK in the presence of various concentrations of MBX-2982 was then evaluated. In concentrations of 1-300 μM the compound caused a significant increase of GK activity ($p<0.05$; Mann—Whitney test). The EC_{50} for MBX-2982 was calculated from the resultant data (Fig. 3).

Hence, validation of the method for GK activity evaluation with the use of NAD coenzyme showed 5.15% convergence and 5.6% intermediate convergence of the method, this recommending it for studies of GK activity of new compounds.

Compound MBX-2982, known as GPR119 agonist, has exhibited manifest activation of GK *in vitro*. Despite the fact that its activity is significantly inferior to the leader compounds on this class, the results extend our concepts on the mechanism of hypoglycemic effect and antidiabetic potential of MBX-2982.

The study was carried out within the framework of the State Task for Applied Research, Experimental Development, Organization, and Information Support of Public Health Activities "Search for New Glucokinase Activators and Study of Their Effects in Streptosotocin-Nicotinamide Diabetes Mellitus Model" (No. 115042410204).

REFERENCES

1. GOST R ISO 5725-1-2002. Accuracy (Correctness and Precision) of Methods and Results of Measurement. Moscow, 2009. Russian.
2. Desai UJ, Slosberg ED, Boettcher BR, Caplan SL, Fanelli B, Stephan Z, Gunther VJ, Kaleko M, Connelly S. Phenotypic correction of diabetic mice by adenovirus-mediated glucokinase expression. Diabetes. 2001;50(10):2287-2295.
3. Eiki J, Nagata Y, Futamura M, Sasaki-Yamamoto K, Iino T, Nishimura T, Chiba M, Ohyama S, Yoshida-Yoshimioto R, Fujii K, Hosaka H, Goto-Shimazaki H, Kadotani A, Ohe T, Lin S, Langdon RB, Berger JP. Pharmacokinetic and pharmacodynamic properties of the glucokinase activator MK-0941 in rodent models of type 2 diabetes and healthy dogs. Mol. Pharmacol. 2011;80(6):1156-1165.
4. Ishikawa M, Nonoshita K, Ogino Y, Nagae Y, Tsukahara D, Hosaka H, Maruki H, Ohyama S, Yoshimoto R, Sasaki K, Nagata Y, Eiki J, Nishimura T. Discovery of novel 2-(pyridine-

2-yl)-1H-benzimidazole derivatives as potent glucokinase activators. Bioorg. Med. Chem. Lett. 2009;19(15):4450-4454.
5. Jain S, Gupta N, Jindal R, Dubey T, Agarwal N, Siddiqui A, Wangnoo SK. Newer anti-hyperglycemic agents in type 2 diabetes mellitus — Expanding the horizon. Apollo Medicine. 2013;10(2):108-112. doi.org/10.1016/j.apme.2013.05.013.
6. Kamata K, Mitsuya M, Nishimura T, Eiki J, Nagata Y. Structural basis for allosteric regulation of the monomeric allosteric enzymehuman glucokinase. Structure. 2004;12(3):429-438.
7. Leighton B, Atkinson A, Coghlan MP. Small molecule glucokinase activators as novel anti-diabetic agents. Biochem. Soc. Trans. 2005;33(Pt. 2):371-374.
8. Matschinsky FM. Assessing the potential of glucokinase activators in diabetes therapy. Nat. Rev. Drug Discov. 2009;8(5):399-416.
9. Qian-Cutrone J, Ueki T, Huang S, Mookhtiar KA, Ezekiel R, Kalinowski SS, Brown KS, Golik J, Lowe S, Pirnik DM, Hugill R, Veitch JA, Klohr SE, Whitney JL, Manly SP. Glucolipsin A and B, two new glucokinase activators produced by Streptomyces purpurogeniscleroticus and Nocardia vaccinii. J. Antibiot. (Tokyo). 1999;52(3):245-255.
10. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010;31(2):455-461.
11. Waring MJ, Bennett S, Boyd S, Campbell L, Davies R, Hargreaves D, MacFaul P, Martin NG, Ogg DJ, Robb GR, Wilkinson G, Wood JM. Optimising pharmacokinetics of glucokinase activators with matched triplicate design sets — the discovery of AZD3651 and AZD9485. Med. Chem. Comm. 2013;4(4):663-668.
12. Waring MJ, Clarke DS, Fenwick MD, Godfrey L, Groombridge SD, Johnstone C, McKerrecher D, Pike KG, Rayner JW, Robb GR, Wilson I. Property based optimisation of glucokinase activators-discovery of the phase IIb clinical candidate AZD1656. Med.Chem. Comm. 2012;3(9):1077-1081