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Carbonaceous Fullerene Containing Nano Mineral Shungite. Properties for Purification of Water Detoxification of Human Body

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Abstract

Shungite is amorphous, uncrystallized, fullerene analogous carbon containing natural mineral. Shungite carbon is a fossilized organic material of sea bottom Precambrian sediments of high level of carbonization containing the fullerene-like regular structures. Shungite got its name after the village of Shunga in Karelia (Russian Federation), located on the shore of Onezhskoe Lake, where is located Zazhoginsky deposit. The total shungite reserves of Zazhoginsky deposit amount to approximately 35 million tons. The plant production capacity for the mining and processing of shungite makes up 200 thousand tons of shungite per year. We study the properties of shungite for purification of water and detoxification of human body. In the report the authors show the properties for purification of water. There are basic data for detoxification of human body with water solution of shungite.

Keywords: shungite, nanostructure, fullerenes, detoxification, water purification, NES, DNES.

1. Introduction

Shungite is mineral refers to new generation of natural mineral sorbents (NMS). Shungite is an intermediate form between the amorphous carbon and the graphite crystal containing carbon (30 %), silica (45 %), and silicate mica (about 20 %). As natural mineral shungite has unusually broad scope of application in industry. Shungite was used initially, mainly as a filler and substitute of the carbon coal coke (fuel) in blast furnace production of high-silicon cast iron, in ferroalloys melting, in the production of non-stick heat-resistant paints and coatings, and as filler in rubber production. Subsequently there were discovered other new valuable properties of shungite – adsorptional, bactericidal, catalytic, reduction-oxidation properties, as well as the ability of sungite minerals to screen off electromagnetic and radio radiations.

These properties have made the use of shungite in various branches of science, industry and technology, for creating on its basis a variety of new nanotechnological materials with nano-molecular structure. On the basis of shuntite have been created new conductive paints, fillers for plastic materials, rubber and carbon black substitutes, composite materials, concrete, bricks, stuccoing plasters, asphalts, as well as materials having bactericidal activity, and materials shilding off the radio and electromagnetic radiation. Adsorptional, catalytic, and reduction-oxydation

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properties of shungite favored its use in water treatment and water purification technologies, i.g. in treatment of sewage waters from many organic and inorganic substances (heavy metals, ammonia, organochlorine compounds, petroleum products, pesticides, phenols, surfactants, etc.). Moreover, shungite has a strongly marked biological activity and bactericidal properties.

Shungite is widely used in industry as a desiccant of gases and liquids, for treatment of drinking and sewage water from heavy metals, ammonia, phosphorus, as catalyst in petrochemical industry for benzene extraction, for production of detergents and for extracting of radionuclides in nuclear reprocessing. They are also used in medicine as nutritional supplements having antioxidant properties.

A wide range of properties of shungite and zeolite defines the search for new areas of industrial application of these minerals in science and technology that contributes to a deeper study the mechanism of interaction of these minerals with water. This paper deals with methods NES and DNES evaluating of mathematical model of interaction of shungite with water (Ignatov, Mosin, 2013).

2. Materials and Methods

2.1. Materials

The study was performed with samples of shungite obtained from Zazhoginsky deposit (Karelia, Russia). Samples were taken and analyzed in solid samples according to National standard of the Russian Federal Agency of Technical Regulation and Metrology. Samples were put into 100 cm3 hermetically sealed glass tubes after being washed in dist. H2O and dried in crucible furnace, and homogenized in homogenizer by mechanical grinding. For the decomposition of the shungate samples a system of microwave decomposition was used. Other methods of samples processing were waching with dist. H2O, drying, and homogenization on cross beater mill Retsch SK100 (“Retsch Co.”, Germany) and Pulverisette 16 (“Fritsch GMBH”, Germany).

2.2. Analytical Methods

The analytical methods were accredited by the Institute of Geology of Ore Deposits. Petrography, Mineralogy, and Geochemistry (Russian Academy of Sciences). Samples were treated by various methods as ICP-OES, GC, and SEM.

2.3. Gas-Chromatography

Gas-chromatography (GC) was performed at Main Testing Centre of Drinking Water (Moscow, the Russian Federation) on Kristall 4000 LUX M using Chromaton AW-DMCS and Inerton-DMCS columns (stationary phases 5 % SE-30 and 5 % OV-17), equipped with flame ionization detector (FID) and using helium (He) as a carrier gas.

2.4. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The mineral composition of shungite was studied by inductively coupled plasma optical emission spectrometry (ICP-OES) on Agilent ICP 710-OES (Agilent Technologies, USA) spectrometer, equiped witth plasma atomizer (under argon stream), MegaPixel CCD detector, and 40 MHz free-running, air-cooled RF generator, and Computer-optimized echelle system: the spectral range at 167–785 nm; plasma gas: 0–22.5 l/min in 1.5 l/min; power output: 700–1500 W in 50 W increments.

2.5. Elemental Analysis

The total amount of carbon (C_{total}) in sungtate was measured according to the ISO 29541 standard using elemental analyzer CHS-580 (“Eltra GmbH”, Germany), equipped with electric furnace and IR-detector by combustion of 200 mg of solid homogenized sample in a stream of oxygen at the temperature 1500 ^{o}C.

2.6. Transmission Electrom Microscopy (TEM)

The structural studies were carried out with using JSM 35 CF (JEOL Ltd., Korea) device, equiped with X-ray microanalyzer “Tracor Northern TN”, SE detector, thermomolecular pump, and tungsten electron gun (Harpin type W filament, DC heating); working pressure: 10^{-4} Pa (10^{-6} Torr); magnification: 300.000, resolution: 3.0 nm, accelerating voltage: 1–30 kV; sample size: 60–130 mm.

2.7. IR-Spectrospopy

IR-spectra of water samples, obtained after being contacted 3 days with shungite, were registered on Fourier-IR spectrometer Brucker Vertex ("Brucker", Germany) (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0.5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}); Thermo Nicolet Avatar 360 Fourier-transform IR (Chakarova); Non-equilibrium Spectrum (NES) and Differential Non-equilibrium Spectrum (DNES) (Antonov, 1995; Ignatov, 1998).

3. Results and Discussion

According to the last structural studies shungite is a metastable allotropic form of carbon with high level of carbonization (carbon metamorhism), being on prior to graphite stage of coalification.

Shungite differs in composition of mineral matrix (aluminosilicate, siliceous, carbonate), and the amount of carbon in shungite samples. Shungite minerals with silicate mineral basis are divided into low-carbon (5 % C), medium-carbon (5–25 % C), and high-carbon shungite (25–80 % C) (Kasatochkin et al., 1978). The sum (C + Si) in shungites of Zazhoginsky deposit (Karelia, Russian Federation) is varried within 83–88 % as shown in Figure 1.

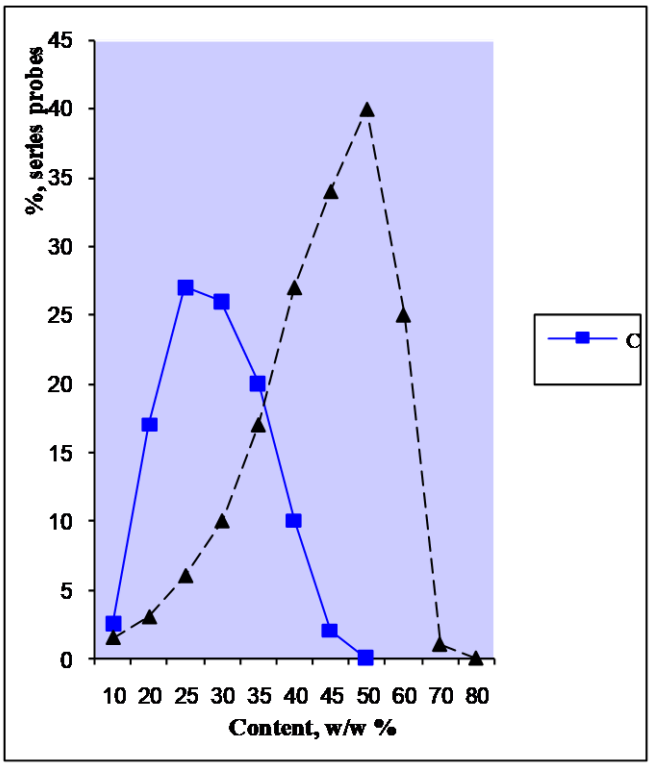

Fig. 1. The distribution (%) of carbon (C) (solid line) and silicon (Si) (dotted line) in shungite samples from Zazhoginsky deposit (Karelia, Russian Federation) according to atomic emission spectrometry (AES) Along with carbon the shungite, obtained from Zazhoginsky deposit in Karelia (Russian Federation) contains C (30.0 %), SiO_2 (57.0 %), TiO_2 (0.2 %), Al_2O_3 (4.0 %), FeO (0.6 %), Fe_2O_3 (1.49 %), MgO (1.2 %), MnO (0.15 %), K_2O (1.5 %), S (1.2 %) (Table 1).

Table 1. The chemical composition of shungite, Zazhoginsky deposit (Karelia, Russia), in % (w/w)

№	Chemical component	Content, % (w/w)
1	C	30.0
2	SiO_2	57.0
3	TiO_2	0.2
4	Al_2O_3	4,0
5	FeO	0.6
6	Fe_2O_3	1.49
7	MgO	1.2
8	MnO	0.15
9	K_2O	1.5
10	S	1.2

Physical and chemical properties of shungite have been sufficiently studied (Parfen'eva, 1994). Density of shungite 2.1–2.4 g/cm^3; porosity – up to 5%; the compressive strength – 1000–1200 kgf/cm^2; conductivity coefficient – 1500 SI/m; thermal conductivity coefficient – 3.8 W/m·K, the adsorption capacity up to 20 m^2/g.

The crystals of crushed, fine ground shungite possess strong bipolar properties. This results in a high adhesion, and the ability of shungite to mix with almost all organic and inorganic substances. Besides, shungite has a broad spectrum of bacterecidal properties; the mineral is adsorptive active against some bacterial cells, phages, and pathogenic saprophytes (Khadartsev, Tuktamyshev, 2002).

The unique properties of the mineral are defined by nanostructure and composition of its constituent elements. Schungite carbon is equally distributed in the silicate framework of fine dispersed quartz crystals having the size of 1–10 μm (Kovalevski, 1994; Mosin, Ignatov, 2013), asconfirmed by studying of ultra-thin sections of shungite by transmission electron microscopy (TEM) in absorbed and backscattered electrons.

The carbonaceous material of shungite is the product of a high degree of carbonization of hydrocarbons. Its elemental composition (%, w/w): C – 98.6–99.6; H – 0.15–0.5; (H + O) – 0.15–0.9 (Golubev, 2000). With virtually constant elemental composition of shungite carbonaceous matter is observed variability in its structure – both molecular and supramolecular, as well as surface, and porous structure. X-ray studies showed that the molecular structure of schungite carbon is represented by a solid uncristallized carbon, which components may be in a state close as to graphite and carbon black and glassy carbon as well, i.e. the maximally disordered (Kovalevski et al., 2001). Carbonaceous matter of shungite having a strongly marked structural anisotropy shows a significant increase in the diamagnetism at low temperatures that is characteristic for fullerites (Jushkin, 1994).

The basis of shungite carbon compose the hollow carbon fullerene-like multilayer spherical globules with a diameter of 10–30 nm, comprizing inclusive packages of smoothly curved carbon layers covering the nanopores. The globule structure is stable relative to shungite carbon phase transitions into other allotropic carbon forms. Fullerene-like globules (the content of fullerenes makes up 0.001%) may contain from a few dozen to a several hundred carbon atoms and may vary in shape and size (Reznikov, Polehovsky, 2000).

According to the data on adsorption capacity shungite loses effectiveness before the activated carbon filter in the first stage of filtration, during the first 24 h, further shungite began purifiy water with a high and constant speed. This is explaned by high catalytic properties of shungite and its ability to catalytically oxidize organic substances absorbed on the surface. The mechanism of interaction of shungite with water has not been completely understood. It is assumed that shungite can adsorb oxygen actively interacting with them as a strong reducing agent in water and in air. In this process is produced atomic oxygen, which is a strong oxidizing agent oxidizing adsorbed on shungit organic substanses to CO_2 and H_2O, thus, freeing the surface of shungite for new acts of adsorption. Atomic oxygen is produces in the process of electrolyses of water in anolyte with anti inflammatory and virucidal effects, (Ignatov et al., 2014). Overexposure of shungite in respect to dissolved metal cations in water as Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Fe^{3+} is explaned by the fact that the

metals are transferred by the catalytically active shungite into the form of insoluble carbonates due to the oxidation of organic matter to CO_2.

By the measurement of IR spectra in the range of vibrations in the crystal mineral framework one can obtain the information: a) on the structure of the framework, particularly type lattice ratio SiO_2/Al_2O_3, nature and location of cations and changes in the structure in the process of the thermal treatment; b) on the nature of the surface of the structural groups, which oftnen serve as adsorption and catalytically active sites.

The methods NES and DNES obtaining information about the average energy of hydrogen bonds in an aqueous sample is measuring of the spectrum of the water state (Antonov, 1995; Ignatov, Mosin, 2013).

The research of antioxidant properties of shungite in relation to organochlorine compounds, and free radicals have shown that shungite removes free radicals (Mosin, Ignatov, 2013) [28]. This is a very important factor, because the free radicals formed during water treatment with chlorine and its derivatives, have a negative impact on the human health that is the cause of many diseases. The research of with methods NES and DNES shows that water solution of shungite decreases the tumor cells as size and number (Ignatov, Mosin, 2013).

In 2017 from Koreahave performed interesting research that the redox profile of shungite-treated groups showed counterbalance of ROS/RNS and superoxide levels in serum and skin lysates. The team has confirmed the involvement of Nrf2- and MAPK-mediated oxidative stress pathways in the antioxidant mechanism of shungite. Collectively, the results clearly show that shungite has an antioxidant and anti-inflammatory action against UVB-induced skin damage in hairless mice (Ma. Easter Joy V. Sajo et al., 2017).

Our study shows anti inflammatory effect of shungite. For the value E=-0.1212 eV or λ=10.23 μm. there is local extremum corresponding to the re-structuring of hydrogen bonds among H_2O molecules for anti inflammatory effect of shungite. Anti inflammatory effect is part of process of detoxification of shungite with the following effects – absorption, catalytic, antioxidant, regenerative, antibacterial. Shungite$^-$ creates a negative charge by cations (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, etc.), in most cases, capable of cations exchange in solutions. There is permanent antioxidant activity of shungite on enzymes (Ignatov, Mosin, 2015). Our study shows connection between pH (7.17) and ORP (+175) and that water solution of shungite has positive role for microorganisms. Inhibition of development of tumor cells is influenced from anti inflammatory effects. Our proofs are for the value E = -0.1387 eV or λ = 8.95 μm there is local extremum, corresponding to the re-structuring of hydrogen bonds among H_2O molecules for inhibition of development of tumor cells of molecular level.

These positive qualities allow using shungite as an effective filter material for wastewater treatment and purification from organic and chlorinated organic substances (oil, pesticides, phenols, surfactants, dioxins, etc.). Thus shungite is able to purify wastewater from oil up to threshold limit value (TLV) of water discharge into the water reservoir. Shungit adsorbs on its surface up to 95 % of contaminants, including organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, etc., removes turbidity and color, and gives the water good organoleptic qualities, additionally saturating it with micro-and macro-elements (Table 2). Thus, adsorption activity of shungite relative to phenol makes up 14 mg/g , while for thermolysis resins – 20 mg/g, for oil products – more then 40 mg/g. Model experiments showed that heavy metals (copper, cadmium, mercury, lead), boron, phenol and benzenecontained in water in concentrations being in 10–50 times higher than the TLVs, after the treatment by shungite in stationary or dynamic conditions on the shungite filter units, the content of these pollutants in water is reduced below the established levels of regulatory documents. In this case into the water does not enter any toxic elements from shungite adsorbents.

Table 2. Indicators of performance of filters based of mineral shungite

№	Common water pollutants	The removal degree, %
1	Fe^{2+}/Fe^{3+}	95
2	Zn^{2+}	80
3	Pb^{2+}	85
4	Cu^{2+}	85

5	Cs^{2+}	90
6	St^{2+}	97
7	Radionuclides	90
8	Fluorine	80
9	Ammonia	90
10	Chlorine and organochlorine compounds	85
11	Phenols	90
12	Dioxins	97
13	Helminth's eggs	90
14	Smell	85
15	Turbidity	95

From a practical point of view, carbonate-shale shungite is of interest because it provided the largest decline in chlorides (1.7 %) and the smallest increase in sulphates (13.5 %). Use of all shungite has a beneficial health effect on the process of water purification, as coliform bacteria were not found in experimental samples (Turkaeva et al., 2017).

Owing to the unique porous structure the natural mineral shungite is ideal absorbent and filler (Gorshteyn et al., 1979), and as sorbents have a number of positive characteristics:

- High adsorption capacity, characterized by low resistance to water preasure;
- Mechanical strength and low abrasion resistance;
- Corrosion-resistance;
- Absorption capacity felative to many substances, both organic (oil, benzene, phenol, pesticides, etc.) and inorganic (chlorine, ammonia, heavy metals);
- Catalytic activity;
- Relatively low cost;
- Environmental friendliness and ecological safety.

In addition, owing to adsorption activity of shungite against pathogenic microflora shungite has strong bactericidal properties that allow carrying out the efficient disinfection of drinking water by this mineral in water treatment and water purification technologies. It is observed the bactericidal activity of shungite against pathogenic saprophytes and Protozoa. There is evidence that after the passage of water containing bacterium *E. coli*, through shungite filter there is an almost complete removal of this bacterium (the viral titer varries from 2300 cells /l in initial water up to 3 cells/l in treated water) (Mosin, Ignatov, 2013). Of 1785 cells/l of protozoa (ciliates, rotifers and crustaceans) contained in the initial water after the treatment by shungite were observed only a few exemplars (5 cells/l). In addition to these qualities, shungite has biological activity.

Owing to all these positive properties shungite may find its application for the preparation of drinking water in flow-through systems of any capacity for industrial and domestic purposes, as well as in the wells in order to improve the quality characteristics of water to return water its beneficial properties.

Especially effective and technologically justified is the use of complex filter systems based of the mixtures of shungite with activated carbon or zeolite, with subsequent regeneration of the absorbents (Podchaynov, 2007). When adding to the treatment scheme to shungite other natural absorbents (zeolite, dolomite, glauconite) purified water is enriched to a physiologically optimal levels by calcium, magnesium, silicon and sodium ions.

4. Conclusion

Shungite can find wide practical applications in many branches of science and industry, and can be used as an alternative to activated carbon the natural mineral absorbent in water treatment. Efficiency of using of shungite is stipulated by the high range of valuable properties (absorption, catalytic, antioxidant, regenerative, antibacterial), high environmental safety and relatively low cost of filters based on shungite.

Our study shows anti inflammatory effect of shungite. For the value E=-0.1212 eV or λ=10.23 μm. there is local extremum corresponding to the re-structuring of hydrogen bonds among H_2O molecules for anti inflammatory effect of shungite. Anti inflammatory effect is part of process of detoxification of shungite with the following effects – absorption, catalytic, antioxidant,

regenerative, antibacterial. Shungite- creates a negative charge by cations (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, etc.),, in most cases, capable of cations exchange in solutions. There is permanent antioxidant activity of shungite on enzymes (Ignatov, Mosin, 2015).

Our study shows connection between pH (7.17) and ORP (+175) and that water solution of shungite has positive role for microorganisms. Inhibition of development of tumor cells is influenced from anti inflammatory effects. Our proofs are for the value E = -0.1387 eV or λ = 8.95 μm there is local extremum corresponding to the re-structuring of hydrogen bonds among H_2O molecules for inhibition of development of tumor cells of molecular level.

The research of Mosin and Ignatov show different applications of shungite.

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Investigation of the Effect of Polyphenol Euphorbin on the Transport of L Glutamate and Calcium Channels to Synaptosomes of Rat Brain

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Abstract

Background: The purpose of this study was to determine the effect of L glutamate and polyphenol euphorbin on the transport of NMDA-receptor mediators in rat's brain synaptosomes. This makes it possible to adjust the transport of antagonists and agonists NMDA-receptors brain synaptosomes in rats.

Methods: The study was carried out using the Weilers method. Synaptosomes were isolated from the brain of rats by a two-step centrifugation method. The entire isolation procedure was carried out at 4°C. To measure the amount of cytosolic Ca^{2+} synaptosomes were calculated by the Grinkevich equation.

Results: Increase in the concentration of $[Ca^{2+}]_{in}$ caused by L glutamate, primarily due to activation of membrane permeability, movement of Ca^{2+} into the cell and release of Ca^{2+} from intracellular stores. The two-phase L glutamate process of induced release of protons from synaptic vesicles of rat brain nerve terminals is correlated with a two-step increase in the concentration of calcium under the influence of L glutamate. Euphorbin competes with L glutamate for glutamate binding site of NMDA-receptors. L glutamate partially reduces the action of euphorbin, which may indicate that part of the external calcium comes under the influence of euphorbin also through the open L glutamate binding site and in place of calcium channels NMDA-receptors.

Conclusion: In these studies, it was found that euphorbin slightly increases the fluorescence and the level of $[Ca^{2+}]_{in}$, respectively, in the synaptic membranes compared with the control. The obtained results indicate a possible competition between euphorbin and L glutamate for the site of regulation of the opening of ion channels of NMDA-receptors. It was found that the effect of euphorbin responsible for the opening of calcium channels with other sites of NMDA-receptors against the background of magnesium ions, argiolobatin and nifedipine, a change in the level of $[Ca^{2+}]_{in}$ synaptosomes was not observed.

Keywords: NMDA-receptors, synaptosomes, L glutamate, Euphorbin.

1. Introduction

Calcium plays an important role in the process of releasing the neurotransmitter and performing the function of transferring excitation and inhibition of the brain nerves. All this is closely related to the movement of calcium ions in nerve cells (Hardingham et al., 2010).

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Calcium is a key signaling ion involved in many different intracellular and extracellular processes ranging from synaptic activity to cell-cell communication and adhesion. The exact definition at the molecular level of the versatility of this ion has made overwhelming progress in the past several years and has been extensively reviewed.

Calcium ions act as a universal intracellular messenger in the cells of all living organisms. In addition, calcium ions play a key role in the work of excitable cells. For example, in nerve cells, calcium ions play an important role in the secretion and transduction of the signal of neurotransmitters.

The concentration of intracellular Ca^{2+} in neurons is a homeostatic parameter and under physiological conditions the transmembrane calcium exchange is regulated by several mechanisms. On the one hand, Ca^{2+} concentration increases as a result of the discovery of ligand-controlled and potential-controlled calcium channels, and the release of Ca^{2+} bound by intracellular depots upon activation of IP3 or ryanodine receptors of the endoplasmic reticulum. On the other hand, the excess concentration of intracellular Ca^{2+} is counteracted by ATP-dependent mechanisms of Ca^{2+} "pumping" through the plasmolemma and sequestration in the endoplasmic reticulum, Ca^{2+}/Na^{+} transmembrane exchange and other buffer and/or Ca^{2+}-binding processes. Coordinated management of these mechanisms controls the level of $[Ca^{2+}]_{in}$, allowing it to fluctuate within certain limits and with a certain spatio-temporal pattern to provide a variety of Ca^{2+}-dependent processes of intracellular signal transduction.

The rise in intracellular calcium levels upon synaptic activity triggers the activation of several kinases critical for the induction and expression of LTP. These include the calcium/calmodulin-regulated protein kinases CaMKII and CaMKIV (Wayman et al., 2008), the cAMP-dependent protein kinase A (PKA) (Abel et al., 2008), PKC (Malinow et al., 1989; Saito et al., 2002) and MAPK/ERKs. A broad range of evidence from molecular, cellular, and transgenic animal studies established CaMKII as a key factor in LTP. Postsynaptic injection of CaMKII inhibitors or genetic deletion of a critical CaMKII subunit blocked the ability to generate LTP and impaired learning in mice (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992).

Brain functions are manifested at specific synapses through release of neurotransmitters inducing a number of biochemical signaling events in postsynaptic neurons. One of the most prominent of these events is a rapid and transient rise in calcium levels. This local increase in calcium concentrations results in a number of short-term and long-term synapse-specific alterations. These include the insertion or removal of specific calcium channel subunits at or from the membrane and the post-translational modification or degradation of synaptic proteins (Catterall et al., 2008; Greer et al., 2008; Higley et al., 2008).

The violation of calcium homeostasis in nerve cells is accompanied by many brain diseases. For example, in cerebral ischemic strokes, an avalanche-like increase in the concentration of calcium in the cytoplasm of neurons plays a major role in the chain of pathological disorders that lead to cell death by apoptosis, which causes all processes occurring in ischemic brain tissue to be termed the “calcium hypothesis of ischemic cell death”.

Neurotransmitters are types of hormones in the brain that transmit information from one neuron to another. They are synthesized by amino acids. Neurotransmitters control the body's main functions: movement, emotional reactions, physical ability to feel pleasure and pain. The most famous neurotransmitters affecting the regulation of nerve receptors are L glutamate, serotonin, noradrenaline, dopamine, acetylcholine and GABA.

With calcium deficiency, the release of the neurotransmitter is blocked, the excitation and inhibition mechanisms are violated.

L glutamate in neurons can develop neurodegenerative processes associated with violation of Ca^{2+} regulation, which trigger intracellular signaling cascades leading to the death of neurons (Khodorov, 2004). It is known that the neurotoxicity of L glutamate is involved in the pathogenesis of such socially important neurological diseases as epilepsy, ischemic stroke, migraine, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease. In this regard, the study of the mechanisms of neurotoxic action of L glutamate and agonists of its receptors is one of the most topical directions in modern neuroscience.

Calcium homeostasis perturbations in neurodegenerative diseases. Perturbations in calcium homeostasis were observed in several neurodegenerative disorders including Alzheimer's disease (AD) (Mattson, 2004; Selkoe, 2001; Bezprozvanny et al., 2008; Green et al., 2008; Mattson, 2007),

Parkinson's disease (PD) (Thomas et al., 2007; Hallett et al., 2004; Surmeier,2007), Huntington's disease (HD) (Ramaswamy et al., 2007; Nakamura et al., 2007; Fan et all 2007; Bezprozvanny, 2007), and amyotrophic lateral sclerosis (ALS) (Rowland et al., 2001; Strong et al., 2005; Alexianu et al., 1994; von Lewinski et al., 2005). Calcium homeostasis disruption implicates several mechanisms, such as alterations of calcium buffering capacities, deregulation of calcium channel activities, or excitotoxicity. Rare examples support a direct causative role of calcium homeostasis deregulation in neurodegeneration. However, compelling evidence supported by an increasing number of publications on this topic, highlights the importance of calcium deregulation in the neurodegenerative process (Bezprozvanny, 2008; Wojda et al., 2008). We will focus in this section on how calcium homeostasis is affected in neurodegenerative disorders by taking non exhaustive examples in AD, PD, HD, and ALS.

In the brain, calcium is fundamental in the control of synaptic activity and memory formation, a process that leads to the activation of specific calcium-dependent signal transduction pathways and implicates key protein effectors, such as CaMKs, MAPK/ERKs, and CREB. Properly controlled homeostasis of calcium signaling not only supports normal brain physiology but also maintains neuronal integrity and long-term cell survival. Emerging knowledge indicates that calcium homeostasis is not only critical for cell physiology and health, but also, when deregulated, can lead to neurodegeneration via complex and diverse mechanisms involved in selective neuronal impairments and death. The identification of several modulators of calcium homeostasis, such as presenilins and CALHM1, as potential factors involved in the pathogenesis of Alzheimer's disease, provides strong support for a role of calcium in neurodegeneration. These observations represent an important step towards understanding the molecular mechanisms of calcium signaling disturbances observed in different brain diseases such as Alzheimer's, Parkinson's, and Huntington's diseases.

It should be noted that the vast majority of data on the effect of L glutamate on neurotransmission processes were obtained in electrophysiological experiments in which the main criterion for evaluating the effect of activation of presynaptic L glutamate receptors was the change in the frequency and amplitude of the registered synaptic currents in postsynaptic structures. The extremely small geometric dimensions of most nerve terminals are a serious obstacle to the successful conduct of direct measurements of the corresponding phenomena in presynaptic formations. In this regard, information on those intracellular processes developing in the presynaptic nerve structures was carried out using fluorescent probes.

The study of the mechanisms of calcium homeostasis regulation in excitable cells, the search for biologically active substances and physical factors that affect this homeostasis is one of the most urgent tasks of modern science.

Purpose: The effects of L glutamate and euphorbin polyphenol on the transport of NMDA-receptor mediators in synaptosomes rats brain.

2. Material and methods

Experiments were conducted on 20 outbred male albino rats weighing (200-250 g) contained in a standard vivarium ration. All experiments were performed in accordance with the requirements of "the World Society for the Protection of Animals" and "European Convention for the protection of experimental animals" (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. 1986). Synaptosomes isolated from rat brain by a two-step centrifugation (Weiler et al., 1981). The whole procedure of selection was carried out at 4°C. To measure the amount of cytosolic Ca^{2+} was calculated from the equation of Grinkevich (Grynkiewicz et al., 1985) in synaptosomes isolated from brain of rats placed in an environment similar to, the one that was used to isolate cells were added 20 μM of chlortetracycline (CTC). Incubated for 60 min to achieve maximal interaction with the membrane - CTC Ca^{2+} as in plasma, and intracellular membranes. CTC excitation wavelength – 405 nm, recording – 530 nm. Results are expressed as a percentage, taking 100 % of the difference between the maximum value of fluorescence intensity (fluorescence dye, a saturated Ca^{2+}) and its minimum value (in the absence of fluorescence of the indicator of Ca^{2+}) obtained after adding ethylene-glycol-bis-amino-ethyl-tetra-acetate EGTA.

Statistical analysis

The measurements were made using a universal spectrometer (USB-2000). Statistical significance of differences between control and experimental values determined for a number of data using a paired t-test, where the control and the experimental values are taken together, and unpaired t-test, if they are taken separately. The value of P <0.05 indicated a statistically significant differences.

The results obtained are statistically processed to Origin 7,5 (Origin Lab Corporation, USA).

3. Results and discussion

Investigation of the effect of L glutamate on the level of cytoplasmic calcium in brain synaptosomes of rats.

Synaptosomes obtained from rat brain were used in the work, which is an adequate and convenient model for studying presynaptic processes. The activity of L glutamate was judged by the change in the intensity of the fluorescent signal, by the change in the cytoplasmic levels of free calcium $[Ca^{2+}]_{in}$.

A fluorescence ratio excited by light at 340 and 380 nm (F_{340}/F_{380}) in synaptosomes was established with the help of the Ca^{2+} -sensory chlortetracycline probe (CTC). When Ca^{2+} was removed from the extracellular medium, preincubation of EGTA resulted in a 10% decrease in fluorescence. In the presence of EGTA in the incubation medium (Figure 1), L glutamate in concentrations of (10-100 μM) dose-dependently increases the level of fluorescence by 15-25 %, which indicates an increase in $[Ca^{2+}]_{in}$ concentration caused by L glutamate, primarily due to activation of membrane permeability, displacement of Ca^{2+} into the cell and release of Ca^{2+} from intracellular depots (Figure 2).

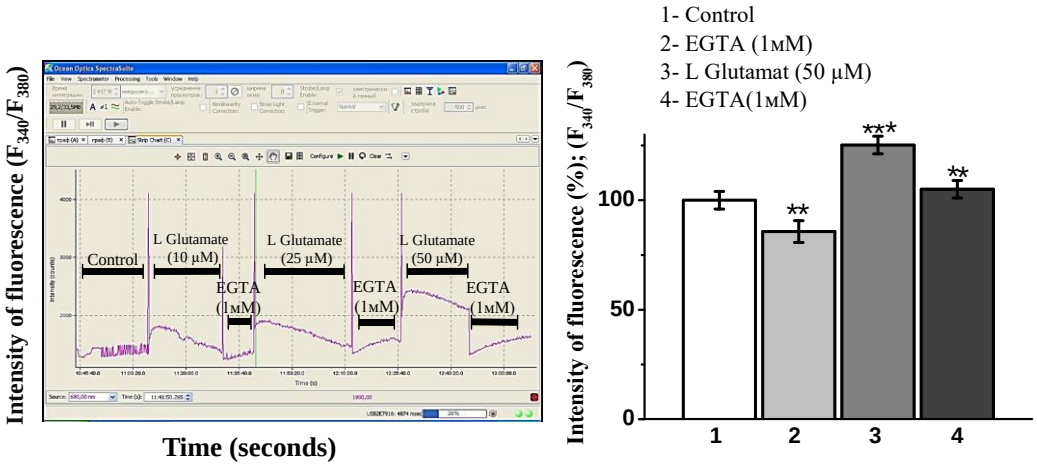

Fig. 1. Fluorescence intensity change with L glutamat (10-50 μM) when incubated with rats of brain tumor synaptosomes EGTA (1 mM). Increased fluorescence intensity induced by L glutamat (50 μM). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

In the following experiments, it was shown that when 100 μM L glutamate was added to the synaptic suspension, a change in the intensity of the fluorescent signal was clearly indicated, which clearly indicated the two-phase nature of the process. With the addition of 100 mM L glutamate, the first ("fast") phase was a sharp increase in fluorescence intensity of the CTC (within 5-10 s), followed by its attenuation down to the initial level. The first phase of the response to the action of L glutamate was similar to that observed when 30 mM KCl was added. This effect, which leads to the depolarization of the plasma membrane, stimulates the process of exocytosis in the calcium containing medium. Depolarization of nerve endings in the presence of Ca^{2+} caused a rapid increase in $[Ca^{2+}]_{in}$, which occurred in two stages. The nature of the initial phase of the response to the action of L glutamate suggests that the primary response to the activation of L glutamate presynaptic receptors is the stimulation of the process of exocytosis.

After the completion of the first, a second, more "slow" phase began to develop, which was characterized by a gradual increase in the intensity of the fluorescent signal (Figure 3).

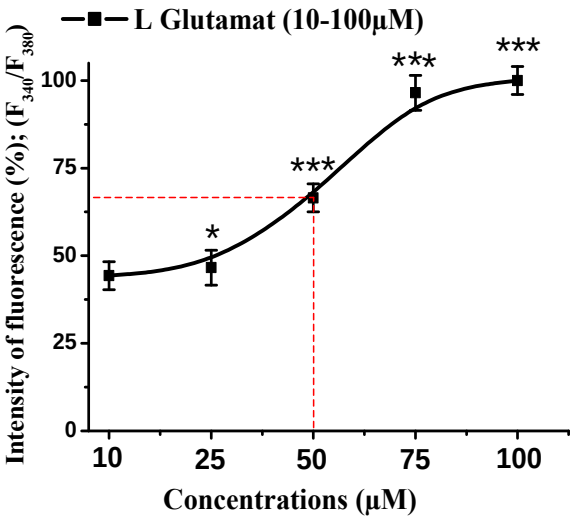

Fig. 2. The dose-dependent effect of L glutamate on the level of intracellular calcium in the brain synaptosomes of rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

Analysis of the dose response of the effect of L glutamate revealed that the magnitude of only the first ("fast") phase of the response is directly proportional to the concentration of the agonist (Figure 3). With an increase in the L glutamate concentration to 100 μM, the amplitude of the "burst" of the fluorescent signal increased, which may be due to the involvement of more synaptic vesicles in the exocytosis process. An increase in the concentration of L glutamate in this case led to a decrease in the period lag between the two phases.

As a result, at an agonist concentration of 100 μM after a primary increase in fluorescence intensity, an inverse change in the fluorescent signal was not observed at all.

Since the nature of the development of the first phase of the response to the action of L glutamate suggested that this phase reflects the process of exocytosis, it was logically justified to try to clarify the role of calcium in the development of this process.

Thus, the two-phase process of L glutamate observed by us, induced release of protons from synaptic vesicles of rat brain nerve terminals, correlates with a two-step increase in the concentration of calcium under the influence of L glutamate.

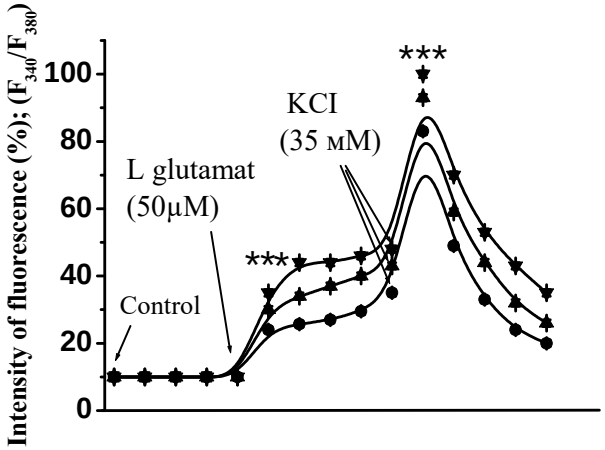

Fig. 3. Comparative effect of rats of the L-glutamate acid (50 μM) and KCl (35mM) on the fluorescence intensity of the rats synaptosomes suspension. Effect of L Glutamate (50 μM) and KCl (35 μM) solution on time-dependent fluorescence intensity. Ordinate axis - the intensity of fluorescence expressed in percent (%), the abstractions on the axis - time (min). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 4).

A significant contribution to the maintenance of an elevated Ca^{2+} level in the cytosol can be caused by the activation of potential-dependent Ca^{2+} channels and the inversion of Na^{+}/Ca^{2+}-transmembrane exchange (Siesjo et al., 1989).

In addition to increasing the level of intracellular free Ca^{2+} due to entry from outside the cell, the processes of maintaining its high concentration in the cytosol due to the release of calcium from the membranes of the endoplasmic reticulum and mitochondria, as well as the disturbance of the processes of its sequestration, are of great importance.

It is known that the change in calcium transport by presynaptic membranes is accompanied by an increase in glutamatergic transmission, which is due to an increase in the release of L glutamate. Excitatory neurotransmitter L glutamate can cause damage and death of DA neurons, and therefore the damaging effect of glutamate on neurons is indicated by the term "toxicity of excitatory amino acids", or “excitotoxicity”.

The L glutamate excitotoxicity is mediated by NMDA-receptors, named for a specific N-methyl-D-aspartate antagonist. When the L glutamate interacts with these receptors, the ion channels of the neuronal membrane open and the L glutamate enters the neuron. The extensive binding of L glutamate with NMDA-receptors leads to an increase in the current of Ca^{2+} to the neuron through NMDA-receptor channels. Due to the fact that Ca^{2+} current amplification is one of the leading mechanisms of neuron death, it can be assumed that the mechanism of excitotoxicity of L glutamate in Parkinson's disease (BP) is associated with a massive entrance of Ca^{2+} into DA-neurons of a black substance. The violation of glutamatergic transmission is now also considered as a leading factor in the pathogenesis of diseases such as epilepsy, Alzheimer's disease, etc. (Choi, 1995; Stout et al., 1998; Nicholls et al., 2000; Vergun et al., 2001).

The effect of polyphenol euphorbin (*1-O-galloyl-6-bisgalloyl-2,4-valoneoyl-β-D-glucose*) isolated from the plant (*EUPHORBIA HIMUFUSA*) on the glutamatergic neurotransmitter system in rat brain synaptosomes was studied.

Preincubation of Euphorbin (10-100 μM) with the complex of the CTC-synaptosomes increases the fluorescence and accordingly, the level of $[Ca^{2+}]_{in}$ difference from L glutamate (Figure 4).

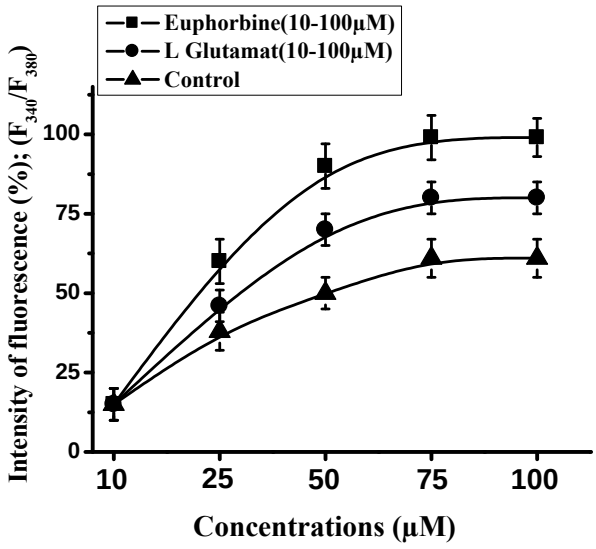

Fig. 4. Effects of Euphorbine and L glutamate in concentrations (10-100 μM) on the intensity of CTC fluorescence of brain synaptosomes suspension in rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

Euphorbin (50 μM) reduced the fluorescence and accordingly the level of $[Ca^{2+}]_{in}$ against the background of L glutamate (50 μM) on the complex of CTC-synaptosomes (Figure 5).

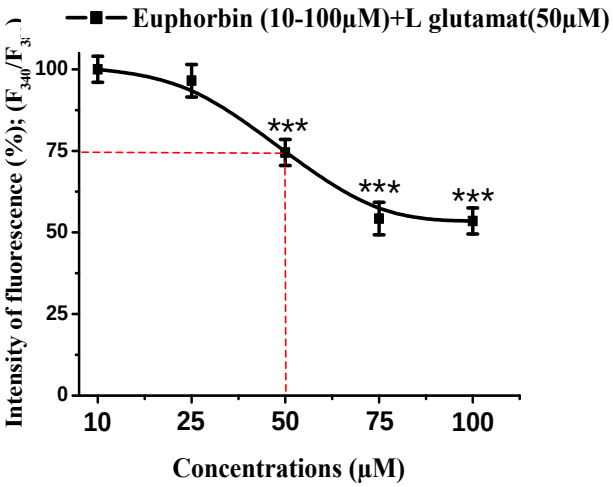

Fig. 5. Effect on fluorescence intensity in synaptosomes suspension in conditions of incubation with euphorbin (10-100 μM) L glutamate (50 μM). Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

The preliminary preincubation of euphorbin (10 μM) with synaptic membranes, then the addition of CTC- L glutamate resulted in a decrease in fluorescence and a level of $[Ca^{2+}]_{in}$, respectively. A dose-dependent increase in euphorbin concentration to (10-100 μM), respectively, resulted in a dose-dependent decrease in the effect of L glutamate (Figure 5).

The effect of L glutamate was observed depolarization of the synaptic membrane and an increase in intracellular calcium without an appreciable change in the concentration of internal sodium ions. Increase in synaptosomal calcium was inhibited by the addition of L glutamate. Activation of L glutamate receptors causes the opening of calcium channels ionotropic receptors, calcium influx into synaptosomes and depolarization of the synaptosomal plasma membrane, followed by the release of amino acid neurotransmitters.

L Glutamate partially reduces the action of euphorbin, which may indicate that part of the external calcium comes under the influence of euphorbine also through the open glutamine site and in place of calcium channels NMDA-receptors.

Even the preliminary addition of L glutamate does not completely abolish the action of euphorbin, which may indicate that euphorbin has several mechanisms of action on rat brain neurons, the result of which is an increase in $[Ca^{2+}]_{in}$.

From the literature data it is known that, Mg^{2+} ions selectively block the activity of NMDA-receptors. Glycine enhances NMDA-receptor responses by increasing the frequency of channel opening. In the complete absence of glycine, the receptor is not activated by L glutamate.

Indeed, the addition of glycine to the incubation medium (5 μM) enhanced the L glutamate-dependent increase in fluorescence by 15-22 %. At the same time, Mg^{2+} ions (50 μM) inhibited L glutamate-induced Ca^{2+} release from intracellular depots (Figure 6).

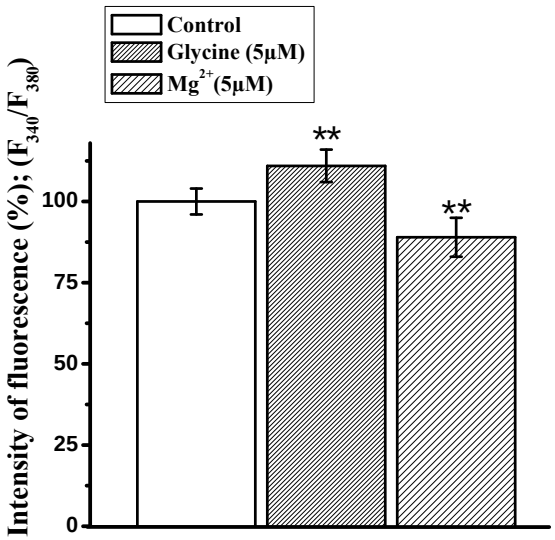

Fig. 6. Effect of glycine and Mg^{2+} ions on L glutamate-inducible Ca^{2+} intracellular depot. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 5)

It is known that glycine stimulating effects of L glutamate and competitive receptor antagonists such as AP_5, AV-2-1 toxin can prevent activation of L glutamate. Other drugs and Mg^{2+} ions may block the open channel through the non-competitive antagonism. These medications include experimental neuroprotective drug MK-801 and argiolobatin (Martin et al., 1977).

In order to identify, possible interaction with polyphenol euphorbin areas over stimulation NMDA-receptor responsible for the opening of calcium channels, investigated its effect on the background of the non-competitive antagonists such as magnesium ions, argiolobatin and calcium channel blockers – nifedipine

It is shown that magnesium ions in millimolar concentrations significantly inhibit the fluorescence of the L glutamate-CTC-synaptosomes complex. The inhibitory effect of magnesium ions against the background of euphorbin (50 μM) of the fluorescence of the CTC-synaptosomes complex did not change.

In these studies, it was shown that in the presence of euphorbin, the inhibitory effect of magnesium ions (50 μM) was not observed. This is probably due to the fact that there is no competition between Mg^{2+} and euphorbin over sites that stimulate the opening of ion channels. It has also been shown that the action of argiolobatin (10 μM) on the calcium channels of the NMDA-receptor in the presence of euphorbin (50 μM) does not change (Figure 7).

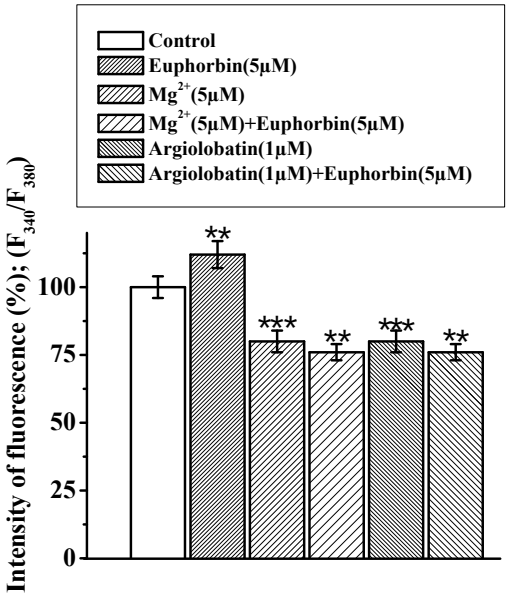

Fig. 7. Effect of non-competitive NMDA-receptor antagonists Mg^{2+} and argiolobatin on the background of euphorbin on fluorescence intensity and the level of $[Ca^{2+}]_{in}$ in the brain synaptosomes of rats. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

When investigating the effect of euphorbin on calcium-dependent NMDA-receptor processes were studied against the background of the blocker of the L-type Ca^{2+} channels of nifedipine in the brain synaptosomes of rats.

Preincubation of nifedipine (0.01 μM) with the suspension complex of the CTC-synaptosomes resulted in a decrease in fluorescence. Preincubation of euphorbin (50 μM) with the suspension complex of the CTC-synaptosomes, no decrease in fluorescence. Preincubation of euphorbin (50 μM) against a background of nifedipine (0.01 μM) with a complex of CTC-synaptosomes did not result in a change in fluorescence (Figure 8), indicating that there is no competition between euphorbin and nifedipine for the site of regulation of dihydropyridine-sensitive calcium channels.

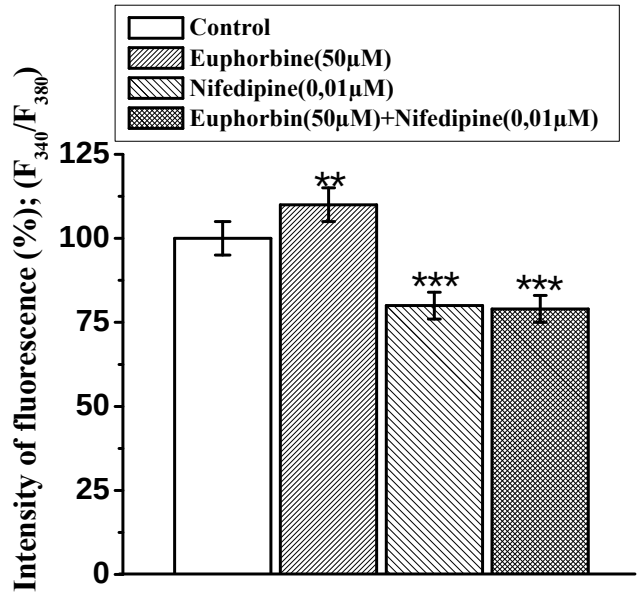

Fig. 8. Effect of euphorbin on calcium-dependent NMDA-receptor processes on the background of nifedipine. Reliability level * - P <0,05; ** - P <0.01; *** - P <0.001. (n = 6)

This is explained by the fact that, euphorbin does not work for the site of regulation of the dihydropyridine-sensitive calcium channels of the rat brain synaptosomes membrane.

4. Conclusion

In these studies, it was found that euphorbin slightly increases the fluorescence and the level of $[Ca^{2+}]_{in}$, respectively, in the synaptic membranes compared with the control. The results obtained indicate a possible competition between euphorbin and L glutamate for the site of regulation of the opening of ion channels of NMDA-receptors.

It was found that the effect of euphorbin responsible for the opening of calcium channels with other sites of NMDA-receptors against the background of magnesium ions, argiolobatin and nifedipine, a change in the level of $[Ca^{2+}]_{in}$ synaptosomes was not observed.

The results indicate the possibility of using euphorbin, as an exciting neurotransmitter in neurodegenerative diseases.

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Pharmacokinetic and Pharmacodynamic Interactions of Sulfonylurea Antidiabetics

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Abstract

Sulfonylureas are useful to treat type 2 diabetes patients. Apart from sulfonylureas, the patients with diabetes may use many other medications to treat various concomitant illnesses such as high blood pressure, higher lipids, infections, pain, etc. The probability of interactions increases with the number of drugs used concomitantly. Most of the adverse drug interactions of sulfonylureas result in hypoglycemia, which can be life threatening. Sulfonylureas are primarily metabolized by CYP2C9 enzyme, which paves the way for most of their pharmacokinetic drug interactions. Drugs inhibiting CYP2C9 enzyme are expected to elevate the plasma concentrations of sulfonylureas and subsequent hypoglycemic complications. Some drugs potentiate the hypoglycemic activity of sulfonylureas pharmacodynamically too. The prescribers and pharmacists must be aware of the adverse drug interactions of sulfonylureas to prevent hypoglycemic episodes.

Keywords: Sulfonylurea, Pharmacokinetic interactions, Pharmacodynamic interactions, CYP2C9.

1. Introduction

Diabetes is a group of metabolic disorders occurring due to decreased insulin secretion/activity or both (ADA, 2014). According to first WHO Global report on diabetes, 422 million adults are living with diabetes worldwide and the number is increasing (WHO, 2016). The prevalence of diabetes is increasing and it is estimated that 552 million adults would be affected by diabetes by the year 2030 (Whiting et al., 2011), 592 million by the year 2035 (Guariguata et al., 2014) and 642 million by the year 2040 (Ogurtsova et al., 2017), globally.

Sulfonylureas are insulin secretagogues and may be used as second-line drugs to treat type 2 diabetes patients, in certain patients (Zhang et al., 2014). Sulfonylureas include first-generation drugs (Tolbutamide, Chlorpropamide, etc.), second-generation drugs (Gliclazide, Glipizide, Glibenclamide) (Sola et al., 2015) and third-generation drug (Glimepiride) (Ma et al., 2010; Inukai et al., 2005; Ueba et al., 2005). The second and third generation sulfonylureas are more potent than first-generation drugs (Melander, Wåhlin-Boll, 1982). Sulfonylureas bind to Sulfonylurea receptors leading to the closure of ATP-sensitive K^+-channel, inhibition of K^+ efflux, depolarization of cell membrane and opening of voltage-gated calcium channels. Increased intracellular calcium concentrations leads to the release of insulin (Henquin, 2017; Panten et al., 1996; Ashcroft, 1996).

Interference of effects of one drug by the coadministered drug(s), herb(s) or food is referred as Drug interaction (Baxter, 2010). Cigarette smoking and alcohol consumption too affect the fate of drugs. Drugs' effects are also affected by chronic disorders including liver and kidney diseases. Concurrent administration of two or more drugs results either in elevated risk of adverse effects or

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decreased therapeutic efficacy (Rowland, Matin, 1973). The drug interaction results in undesirable effects is termed “Adverse Drug Interaction”.

2. Methods

The databases such as Medline/PMC/PubMed, Google Scholar, Science Direct, Directory of open access journals (DOAJ) and reference lists were searched to identify related articles using the keywords Drug Interactions, Sulfonylureas, Pharmacodynamic Interactions, Pharmacokinetic Interactions and CYP2C9 enzyme.

3. Results and Discussion

Sulfonylurea antidiabetics have been identified to interact with various drugs pharmacokinetically or pharmacodynamically.

Pharmacokinetic drug interactions:

Increasing or decreasing the concentration of one drug in the system by another coadministered drug through the changes in absorption, distribution, metabolism, or excretion, is known as Pharmacokinetic interaction (Cascorbi, 2012). The bioavailability, volume of distribution, peak concentration, metabolism, clearance and half life, etc. of drugs are affected by pharmacokinetic drug interactions leading to changes in plasma concentrations. Various drugs have been identified to interact with sulfonylureas pharmacokinetically (Table 1).

Absorption Interactions:

The absorption of sulfonylureas altered by the concomitant use of drugs such as antacids containing magnesium salts and bile acid sequestrants.

Magnesium salts containing antacids:

Sulfonylureas are weakly acidic drugs and they are not ionized at gastric pH. However, administration of magnesium containing antacids elevates the gastric pH and increases solubility and absorption of sulfonylureas, which may result in hypoglycemia. To avoid this interaction, it is advised to administer sulfonylureas at least 1 hour before taking antacids (Neuvonen, Kivistö, 1994).

Bile acid sequestrants:

Sulfonylureas undergo enterohepatic circulation and the presence of cholestyramine in the gastrointestinal tract interrupts the enterohepatic circulation and enhances the elimination of sulfonylureas resulting in decreased intestinal absorption of sulfonylureas (May, Schindler, 2016; Kivisto, Neuvonen, 1990) and it is recommended to take sulfonylureas before 1-2 hours of administration of cholestyramine. In addition, the patients are advised to take glyburide 4 hours prior to colesevelam (Brown et al., 2010; Takebayashi et al., 2010).

Metabolism interactions:

Cytochrome P450 2C9 (CYP2C9) enzyme is primarily involved in the metabolism of sulfonylureas (Holstein et al., 2012). To a lesser extent, CYP3A4 enzyme is also involved in the sulfonylurea metabolism (Holstein, Beil, 2009). The drugs inducing or inhibiting CYP2C9 or CYP3A4 enzymes expected to result in decreased therapeutic efficacy or increased incidence of sulfonylurea-associated hypoglycemia.

CYP enzyme Inducers:

CYP enzyme inducers such as rifampicin and St John's Wort may decrease the plasma concentrations of sulfonylureas and hence their therapeutic efficacy. The blood glucose levels required to be monitored and the dosage adjustments may be necessary during concomitant use of sulfonylureas and CYP enzyme inducers.

Rifampicin:

Rifampicin is an inducer of CYP enzymes including CYP2C9 and CYP3A4, which usually metabolise sulfonylureas (Glaeser et al., 2005; Kanebratt et al., 2008). Administration of rifampicin in patients taking sulfonylureas may result in decreased exposure and reduced therapeutic efficacy of sulfonylureas, moderately (Niemi et al., 2001; Park et al., 2003). Monitoring

of blood glucose and dosage adjustments of sulfonylureas may be required if these drugs used concurrently (Surekha et al., 1997).

St John's Wort (*Hypericum perforatum)*:

St John's Wort is an antidepressant herb and it has the ability of inducing CYP enzymes (Wang et al., 2001). St John's Wort may accelerate the metabolism of sulfonylureas and reduce their plasma concentrations through the induction of CYP enzymes (Xu et al., 2008). Caution is advised in patients taking sulfonylureas and St John's Wort together.

CYP enzyme Inhibitors:

CYP2C9 enzyme inhibitors such as fibrates, azole antifungals, sulfonamides, isoniazid, metronidazole, cimetidine, fluvoxamine, and warfarin may elevate the plasma concentrations of sulfonylureas and subsequent hypoglycemic risk (Figure 1). It is recommended to advise the patients to monitor the signs and symptoms of hypoglycemia, while using sulfonylureas and CYP inhibitors concurrently.

Fibrates:

Fibrates such as gemfibrozil and fenofibrate can inhibit CYP2C9 enzyme and increase the plasma concentrations of sulfonylureas (Niemi et al., 2001). The plasma concentrations of sulfonylureas may also be elevated by reduced hepatic clearance of sulfonylureas resulting from fibrates induced inhibition of organic anion transporter polypeptides (OATPs) mediated hepatic uptake (Schelleman et al., 2014). Fibrates also found to be weak agonists of Peroxisome Proliferator-Activated Receptor (PPARα) and they can improve insulin resistance by affecting lipid and lipoprotein metabolism (Gross, Staels, 2007). The hypoglycemic risk is enhanced in patients taking sulfonylureas and fibrates concomitantly (Leonard et al., 2016).

Azole antifungals:

Azole antifungals such as voriconazole, miconazole, ketoconazole, fluconazole, etc. can inhibit CYP enzymes like CYP2B6, CYP2C9, CYP2C19 and CYP3A4 (Jeong et al., 2009; Hyland et al., 2003). Azole antifungals can interfere with the metabolism of sulfonylureas by inhibiting CYP enzymes and the risk of hypoglycemia is enhanced in diabetics taking sulfonylureas and azole antifungals concurrently (Shobha, Muppidi, 2010; Schelleman et al., 2010; Lomaestro, Piatek, 1998). Exercise caution in patients taking sulfonylureas and azole antifungals concomitantly (Kumar et al., 2013).

Sulfonamides:

Sulfonamides like sulfaphenazole, sulfadiazine, sulfamethizole, sulfisoxazole, sulfaphenazole, and sulfamethoxazole are potent inhibitors of CYP2C9 (Komatsu et al., 2000). Hypoglycemia risk is enhanced by the coadministration of sulfonamides with sulfonylureas (Tan et al., 2014). Patients taking sulfonylureas and sulfonamides together should be monitored for the signs and symptoms of hypoglycemia (Ho, Juurlink, 2011).

Isoniazid:

Isoniazid is a potent inhibitor of cytochrome P450 isozymes such as CYP2C9, CYP2C19 and CYP2E1 (Self et al., 1999). The risk of hypoglycemia may be enhanced in patients taking sulfonylureas and isoniazid concurrently (Boglou et al., 2013). The patients using sulfonylureas and isoniazid should be monitored for signs and symptoms of hypoglycemia.

Metronidazole:

Metronidazole is a CYP2C9 inhibitor (Covvey, Lewis, 2010). Administration of metronidazole in patients taking sulfonylureas may result in increased plasma levels of sulfonylureas and subsequent hypoglycemia (Parekh et al., 2014).

Cimetidine:

Cimetidine is an inhibitor of hepatic cytochrome P450 (CYP) enzymes (Levine, Bellward, 1995) and it's concomitant use with sulfonylureas may result in decreased metabolism of sulfonylureas and subsequent rise of plasma concentrations and hypoglycemia (Kubacka et al.,

1987). Monitoring of blood glucose and dosage adjustments are recommended (Archambeaud-Mouveroux et al., 1987).

Fluvoxamine:

Fluvoxamine is a Selective Serotonin Reuptake Inhibitor (SSRI) and it can inhibit the CYP2C9-mediated drug metabolism (Hemeryck et al., 1999). Concomitant use of fluvoxamine and sulfonylureas may result in hypoglycemia due to the inhibition of CYP2C9-mediated metabolism of sulfonylureas by fluvoxamine (Madsen et al., 2001). Monitor signs and symptoms of hypoglycemia if fluvoxamine and sulfonylureas used concurrently (Schmider et al., 1997).

Warfarin:

S-Warfarin is a substrate of CYP2C9 enzyme (Shaik et al., 2016). The risk of hypoglycemia is elevated in patients taking sulfonylureas when warfarin is added. The plasma drug concentrations of sulfonylureas elevated by warfarin, which displaces the sulfonylureas from protein binding and larger doses of warfarin, inhibits CYP2C9 mediated metabolism of sulfonylureas resulting in hypoglycemia (Romley et al., 2015).

Phenytoin:

Phenytoin is metabolized by CYP2C9 enzyme (Bajpai et al., 1996). Sulfonylureas can inhibit the metabolism of CYP2C9 substrate phenytoin (Kim, Park, 2003). Concomitant use of sulfonylureas and phenytoin may result in phenytoin toxicity including severe bradycardia and hypotension (Srinivasan et al., 2015; Beech et al., 1988).

Clopidogrel:

Clopidogrel is a prodrug and it's bioactivation depends on CYP enzymes including CYP2C9 (Brandt et al., 2007). Coadministration of sulfonylureas with clopidogrel may result in decreased clopidogrel bioactivation and reduced platelet inhibition (Harmsze et al., 2011). Ticagrelor can be substituted with clopidogrel if the patients need sulfonylureas and antiplatelet therapy together (Wang et al., 2015).

Clarithromycin:

Sulfonylureas including glibenclamide are the substrates of P-glycoprotein transporters (Golstein et al., 1999) and Clarithromycin is found to be the potent inhibitor of P-glycoprotein transporters (Eberl et al., 2007). Concurrent use of clarithromycin and sulfonylureas resulted in hypoglycemia (Bussing, Gende, 2002; Jayasagar et al., 2000).

Pharmacodynamic drug interactions:

The change of effect of one drug in presence of other drug(s) acting at the same site, same organ or different organ is called pharmacodynamic interaction. It can be additive, synergistic, potentiation or antagonistic interactions (Hinder, 2011). Sulfonylureas may interact pharmacodynamically with the drugs having some hypoglycemic potential (Figure 2 and Table 2).

ACE Inhibitors:

ACE inhibitors like captopril, enalapril, etc. increase the insulin sensitivity in presence of sulfonylureas (Rave et al., 2005). The incidence of hypoglycemic episodes reported to be higher in patients with type 2 diabetes taking ACE inhibitors and sulfonylureas concurrently (Herings et al., 1995; Shorr et al., 1997; Girardin, Raccah, 1998). Monitoring of blood glucose is recommended (Thamer et al., 1999).

Beta-adrenergic blockers:

Non-selective beta-adrenergic blockers such as propronolol, nadolol, etc. potentiate the hypoglycemic effects of sulfonylureas through the inhibition of glycogenolysis, gluconeogenesis and lipolysis and the stimulation of glucose uptake (Aziz et al., 1996; Groop, Neugebauer, 1996; Gaafar et al., 1994). Cardioselective beta blockers such as atenolol, metoprolol, etc. are preferred in patients with diabetes (Sinclair et al., 1990). Beta-blockers can mask the important signs and symptoms of hypoglycemia such as tachycardia, tremors and shakes.

Disopyramide:

Disopyramide is a class Ia antiarrhythmic drug and it is indicated for the treatment of ventricular and supraventricular arrhythmias. The blood glucose may be reduced by disopyramide through the inhibition of ATP-sensitive K^+-channel of β-cells and stimulation of insulin release. More potent and almost complete inhibition of K^+-channels occurs when disopyramide and sulfonylureas are administered together which may result in elevated risk of hypoglycemia. Caution is advised and monitoring of blood glucose is warranted when disopyramide and sulfonylureas are used concomitantly (Negishi et al., 2009).

Aspirin:

Aspirin is an antiplatelet drug and daily use of aspirin is recommended in high-risk patients to prevent heart attacks, strokes and blood clots. Aspirin may increase the effectiveness of sulfonylureas and elevate risk of hypoglycemia. Concomitant use of aspirin and sulfonylureas warrants monitoring of blood glucose (Patel et al., 2014; Fendrick et al., 2008; Cattaneo et al., 1990; Arena et al., 1978).

Phenylbutazone:

Phenylbutazone is a NSAID (Non-steroidal anti-inflammatory drug) and is not widely used due to its dangerous adverse effects such as agranulocytosis (Etess, Jacobson, 1953), hepatic lesions (Benjamin et al., 1981), and renal complications (Weisman, Bloom, 1955; Lipsett, Goldman, 1954), etc. However, some dietary supplements promoted for the treatment of arthritis and back pain may contain phenylbutazone as undeclared ingredient (Ries, Sahud, 1975). The elimination of sulfonylureas such as Acetohexamide, Chlorpropamide, Tobutamide, etc. can be decreased and their hypoglycemic activity potentiated by the administration of Phenylbutazone (Nomura et al., 1990; Shah et al., 1984; Szita et al., 1980; Ober, 1974).

Fluoroquinolones:

Fluoroquinolone antibacterials such as gatifloxacin, levofloxacin, etc. are able to enhance the insulin secretion (Bansal et al., 2015; Ghaly et al., 2009). The hypoglycemic risk is higher in patients taking fluoroquinolones and sulfonylureas together. The blood glucose level should be monitored closely and the dose of sulfonylureas needed to be adjusted during initiation and discontinuation of a fluoroquinolone (Garber et al., 2009; Lin et al., 2004; LeBlanc et al., 2004; Roberge et al., 2000).

4. Conclusion

The number of patients affected by diabetes is increasing yearly and the diabetics are prescribed with many medications to treat comorbidities such as hypertension, hyperlipidemia, etc., along with antidiabetic drugs. The patients may also take medications to treat infections, pain, etc. and some herbal supplements to help reducing blood sugar. The probability of drug interactions is higher in patients taking many medications. Most of the adverse drug interactions of sulfonylureas result in hypoglycemia, which can be life threatening. Pharmacokinetic drug interactions of sulfonylureas may occur mainly due to the inhibition of CYP2C9 mediated metabolism of sulfonylureas. Drugs such as Fibrates, Azole antifungals, Sulfonamides, Isoniazid, Metronidazole, Cimetidine, Fluvoxamine, etc. inhibit CYP2C9 enzyme and increase the plasma concentrations of sulfonylureas and the risk of subsequent hypoglycemic complications. Some drugs like Pioglitazone, Dulaglutide, ACE inhibitors, Beta blockers, Aspirin, Disopyramide, Fluoroquinolones, etc. potentiate the hypoglycemic activity of sulfonylureas pharmacodynamically. The prescribers and pharmacists must be aware of the adverse drug interactions of sulfonylureas to prevent hypoglycemic episodes. They may consider using alternative drugs and if concomitant use is necessary, the patients should be monitored for signs and symptoms of hypoglycemia including sweating, restlessness, confusion, irritability, palpitations, dizziness, blurred vision, seizures, unconsciousness, etc.

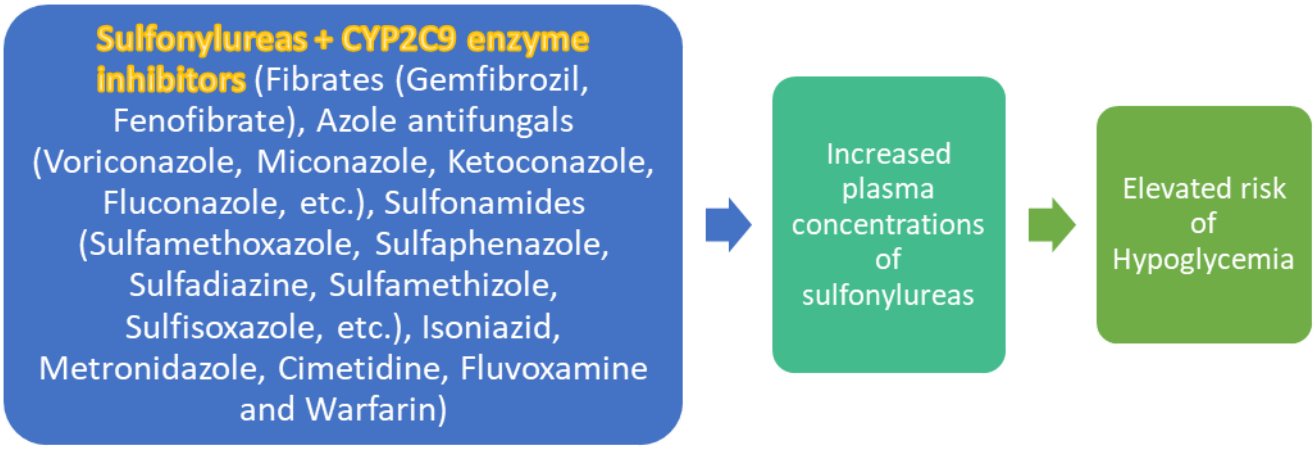

Fig. 1. Pharmacokinetic Drug Interactions of Sulfonylureas

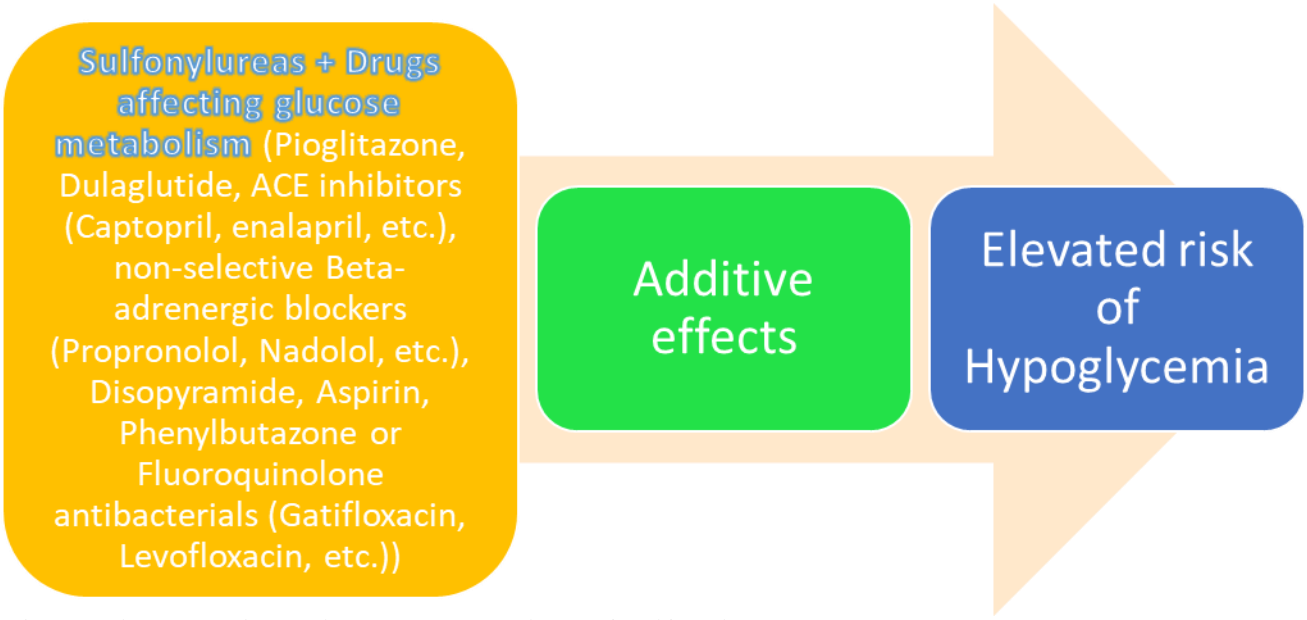

Fig. 2. Pharmacodynamic Drug Interactions of Sulfonylureas

Table 1. Pharmacokinetic interactions of Sulfonylureas

Interacting Drugs	**Mechanism of Interaction**	**Comments**
Magnesium containing **antacids**	Magnesium containing antacids elevate the gastric pH and increase solubility and absorption of sulfonylureas (Neuvonen et al., 1994).	Administer sulfonylureas at least 1 hour before taking antacids to avoid hypoglycemia.
Bile acid sequestrants (Cholestyramine)	Cholestyramine interrupts the enterohepatic circulation and decreases the intestinal absorption of sulfonylureas (Kivisto, Neuvonen, 1990).	Take sulfonylureas before 1-2 hours of administration of cholestyramine.
Rifampin	The therapeutic efficacy of sulfonylureas may be decreased by rifampin,	Monitoring of blood glucose and dosage adjustments of sulfonylureas may be required (Surekha et al., 1997).

	which induces CYP2C9, CYP3A4 and P-glycoprotein (Niemi et al., 2001; Park et al., 2003).	
St John's Wort	St John's Wort may reduce the plasma concentrations of sulfonylureas through the induction of CYP enzymes (Xu et al., 2008).	Monitor the patients closely for the possible signs of reduced sulfonylureas efficacy.
Fibrates (Gemfibrozil, Fenofibrate)	Fibrates such as can inhibit CYP2C9 enzyme and increase the plasma concentrations of sulfonylureas (Niemi et al., 2001a).	The risk of hypoglycemic is enhanced in patients taking sulfonylureas and fibrates concomitantly (Leonard et al., 2016).
Azole antifungals (Voriconazole, Miconazole, Ketoconazole, Fluconazole, etc.)	Azole antifungals can interfere with the metabolism of sulfonylureas by inhibiting CYP enzymes (CYP2C9 and CYP3A4) (Shobha JC, Muppidi MR, 2010 ; Schelleman H et al., 2010 ; Lomaestro BM, Piatek MA, 1998).	Exercise caution in patients taking sulfonylureas and azole antifungals concomitantly (Kumar et al., 2013).
Sulfonamides (Sulfamethoxazole, Sulfaphenazole, Sulfadiazine, Sulfamethizole, Sulfisoxazole, etc.)	Sulfonamides enhance the plasma concentrations of sulfonylureas by inhibiting their CYP2C9 mediated metabolism (Tan et al., 2014).	Patients taking sulfonylureas and sulfonamides together should be monitored for the signs and symptoms of hypoglycemia (Ho, Juurlink, 2011).
Isoniazid	Isoniazid can inhibit CYP2C9 mediated metabolism of sulfonylureas and elevate their plasma concentrations (Boglou et al., 2013).	The patients using sulfonylureas and isoniazid should be monitored for signs and symptoms of hypoglycemia.
Metronidazole	Metronidazole is a CYP2C9 inhibitor and administration of metronidazole in patients taking sulfonylureas may result in increased plasma levels of sulfonylureas (Covvey, Lewis, 2010).	Monitor the patients for signs and symptoms of hypoglycemia.
Cimetidine	Cimetidine is an inhibitor of hepatic cytochrome P450 (CYP) enzymes and its concomitant use with sulfonylureas may result in decreased metabolism of sulfonylureas and subsequent rise of plasma	Monitoring of blood glucose and dosage adjustments are recommended (Archambeaud-Mouveroux et al., 1987).

	concentrations and hypoglycemia (Kubacka RT et al., 1987).	
Fluvoxamine	Fluvoxamine can inhibit the CYP2C9-mediated metabolism resulting in hypoglycemia (Madsen et al., 2001).	Monitor signs and symptoms of hypoglycemia if fluvoxamine and sulfonylureas used concurrently (Schmider et al., 1997).
Warfarin	S-Warfarin is a substrate of CYP2C9 enzyme and the risk of hypoglycemia is elevated in patients taking sulfonylureas when warfarin is added (Shaik AN et al., 2016).	It is recommended to monitor the signs and symptoms of hypoglycemia.
Phenytoin	Sulfonylureas can inhibit CYP2C9 mediated metabolism of phenytoin (Kim, Park, 2003).	Concomitant use of sulfonylureas and phenytoin may result in phenytoin toxicity including severe bradycardia and hypotension (Srinivasan et al., 2015; Beech E et al., 1988).
Clopidogrel	Sulfonylureas inhibit CYP2C9-mediated bioactivation of clopidogrel resulting in reduced platelet inhibition (Harmsze et al., 2011).	Ticagrelor can be substituted with clopidogrel if the patients need sulfonylureas and antiplatelet therapy together (Wang et al., 2015).
Clarithromycin	Clarithromycin can elevate the plasma levels of sulfonylureas by inhibiting P-glycoprotein transporters (Bussing, Gende, 2002; Jayasagar et al., 2000).	Concurrent use of clarithromycin and sulfonylureas resulted in hypoglycemia.

Table 2. Pharmacodynamic interactions of Sulfonylureas

Interacting Drugs	**Mechanism of Interaction**	**Comments**
ACE inhibitors (Captopril, Enalapril, etc)	ACE inhibitors can increase the insulin sensitivity in presence of sulfonylureas (Herings et al., 1995 ; Shorr et al., 1997 ; Girardin, Raccah, 1998).	The incidence of hypoglycemic episodes reported to be higher in patients with type 2 diabetes taking ACE inhibitors and sulfonylureas concurrently. Monitor the blood glucose (Thamer et al., 1999).
Non-selective **beta-adrenergic blockers** (Propronolol, Nadolol, etc.)	Non-selective beta-adrenergic blockers potentiate the hypoglycemic effects of sulfonylureas through the inhibition of glycogenolysis, gluconeogenesis and lipolysis and the stimulation of glucose uptake (Aziz et al., 1996; Groop, Neugebauer, 1996; Gaafar et al., 1994).	Cardioselective beta blockers such as atenolol, metoprolol, etc. are preferred in patients with diabetes (Sinclair et al., 1990).

Disopyramide	The blood glucose may be reduced by disopyramide through the inhibition of ATP-sensitive K^+-channel of β-cells and stimulation of insulin release. More potent and almost complete inhibition of K^+-channels occurs when disopyramide and sulfonylureas are administered together which may result in elevated risk of hypoglycemia (Negishi et al., 2009).	Caution is advised and monitoring of blood glucose is warranted when disopyramide and sulfonylureas are used concomitantly (Negishi et al., 2009).
Aspirin	Aspirin may increase the effectiveness of sulfonylureas and elevate risk of hypoglycemia (Patel et al., 2014; Fendrick et al., 2008; Cattaneo et al., 1990; Arena et al., 1978).	Concomitant use of aspirin and sulfonylureas warrants monitoring of blood glucose.
Phenylbutazone	The elimination of sulfonylureas such as Acetohexamide, Chlorpropamide, Tobutamide, etc. can be decreased and their hypoglycemic activity potentiated by the administration of Phenylbutazone (Nomura et al., 1990 ; Shah et al., 1984; Szita et al., 1980; Ober, 1974).	Monitor blood glucose if concomitant use is necessary.
Fluoroquinolone antibacterials (Gatifloxacin, Levofloxacin, etc.)	Fluoroquinolone antibacterials are able to enhance the insulin secretion. The hypoglycemic risk is higher in patients taking fluoroquinolones and sulfonylureas together (Bansal et al., 2015; Ghaly et al., 2009).	The blood glucose level should be monitored closely and the dose of sulfonylureas needed to be adjusted during initiation and discontinuation of a fluoroquinolone (Garber et al., 2009; Lin et al., 2004; LeBlanc et al., 2004; Roberge et al., 2000).

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Biomarkers of Diabetic Nephropathy Progression

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Abstract

Diabetic nephropathy is a leading cause of morbidity and mortality and leads to an end stage renal disease (ESRD). Standard biomarkers including serum creatinine, estimated glomerular filtration rate, and albuminuria do not directly measure renal tissue injury and they are relatively insensitive to small changes in renal function. Therefore, research focuses on discovering and validating additional biomarkers that improve risk stratification for future renal function decline and end-stage renal disease in patients with diabetes, along with already established biomarkers. In view of this, the utility of urinary biomarkers reported in the literature is discussed in this brief review.

Keywords: diabetic nephropathy, end stage renal disease, biomarkers.

1. Introduction

In 2010, worldwide adult population with diabetes mellitus was estimated to be about 285 millions, and by 2030 it is predicted to have an increase by 54 % totaling to about 439 millions (Tramonti, 2013). Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease (ESRD) in developed countries and is becoming more prevalent globally due to the rise in the incidence of obesity and type 2 diabetes (International Diabetes Federation, 2017). Diabetic nephropathy develops along with generalized microvascular disease, most often concomitant with macrovascular disease including cardiovascular, cerebrovascular, and peripheral arterial disease (Satirapoj, 2014; Satirapoj, 2015). Patients with DN have a higher risk of mortality, (mostly cardiovascular complications) than diabetic patients without nephropathy (Afkarian, 2013). DN is a progressive kidney disease caused by alterations in the glomerular capillary and tubular structure and function induced by the disturbed glucose homeostasis (Berkman, 1973). It affects all renal cellular elements: glomerular endothelia, mesangial cells, podocytes and tubular epithelia. Glomerular damage results in proteinuria, due to both increased permeability of plasma proteins, such as albumin and transferrin, that are normally not freely filtered through the glomerulus and increased synthesis of extracellular matrix (ECM) proteins (Tisher, 1976). DN severity is accessed by measuring urine albumin levels (albumin-to –creatinine ratio). Persistent microalbuminuria

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(between 30-300 mg/24hr) or macroalbuminuria (levels >300mg /24hr) is considered a marker and predictor of DN and its progression to ESRD (Zdler, 2003; Palmer, 2007). However, due to the inability of microalbuminuria to adequately predict diabetic kidney disease, especially in young patients or in non-albuminuric diabetic nephropathy, additional biomarkers of glomerular and/or tubular injury have been proposed to identify early renal dysfunction and structural lesions, even before microalbuminuria occurs.

The aim of this article is to update, through review of the relevant medical literature, the most promising biomarkers for early DKD detection. In recent years there has been an active growing interest in alternative biomarkers that might provide a more sensitive and rapid mean of detecting progression of diabetic nephropathy.

2. Results

Current biomarkers

In clinical practice, most commonly used markers of renal disease and progression of DN include serum creatinine, estimated glomerural filtration rate (eGFR), blood urea and proteinuria, or albuminuria. GFR measures the rate at which the glomeruli filter the plasma and remove waste products from it. If the kidney is injured the GFR gradually declines and the glomerular function can be estimated by measuring the GFR. GFR estimation is still largely creatinine based. It is the best index available to assess kidney function; yet, GFR reflects late functional changes in the kidney (Currie, 2014).

Clinically, microalbuminuria is considered the earliest manifestation for the onset of diabetic nephropathy (Mogensen, 1985). However, a large proportion of renal impairment occurs in a nonalbuminuric state or before the onset of microalbuminuria. Several studies have shown that diabetic patients can still develop DN without any change in their urinary albumin levels. Recent studies have raised growing concerns about the value of microalbuminuria as a very predictable marker of progression to ESRD. These data suggest that microalbuminuria may represent an initial reversible phase of kidney damage rather than the inevitability of progression to ESRD (Perkins, 2010). In patients with type 1 diabetes and new onset microalbuminuria the development of advanced chronic kidney disease may not display progressive proteinuria (Perkins, 2007).

Creatinine has been found to be a fairly reliable indicator of kidney function because a high creatinine level in the blood is associated with poor clearance of creatinine by the kidneys. The use of serum creatinine as an indirect filtration marker is limited by its biological variability because several factors influence serum creatinine level other than renal factors, including age, race, gender, pregnancy, muscle mass, drug metabolism, protein intake, hydration medications(corticosteroids),drugs. These intraindividual variabilities compromise the generalizability of the eGFR equations.

The next section links important aspects of DN pathogenesis including the processes of oxidative stress, tubular damage, and renal inflammation with some of the promising new biomarkers in serum and urine.

Oxidative stress markers

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. A significant correlation between the content of 8-oxo-7, 8-dihydro-2 ' -deoxyguanosine (8-OHdG), a product of oxidative DNA damage, in urine or leucocytes and the severity of diabetic nephropathy and retinopathy has been reported (Hinokio, 2002). Several studies showed that urinary 8-OHdG is increased in the urine of diabetic patients with nephropathy and tends to increase with the severity of the glomerular lesions. Naito et al demonstrated that the urinary albumin levels increased in diabetic mice in parallel with the increase in urinary 8-OHdG levels (Naito, 2004). Because the increased oxidative stress has a primary role in the pathogenesis of DN the 8-OHdG in urine may be a useful clinical biomarker to predict the development and progression of DN in diabetic patients.

Pentosidine is one of the best chemically characterized AGE compounds. The intracellular formation of advanced glycation end products (AGEs) which accumulate in the kidney and are excreted in the urine, is another pathogenetic aspect of diabetes. In patients with both type 1 and 2 diabetes, a significant association between the degree of albuminuria and urinary AGE-modified

proteins was found (Coughlan, 2011). Plasma pentosidine level was significantly influenced by the quality of glycemic control and renal function (Sugiyama, 1988).

There is evidence that uric acid (UA) is involved in varius stages of DN onset and progression. There is a paradox concerning UA function. Studies have shown that UA is one of the major antioxidants of the plasma. On the other hand, once UA enters the cell, it can induce oxidative stress, endothelial dysfunction and cytokine activation. An elevated serum UA level predicts the development of DN. Randomized controlled trials have demonstrated that chronic kidney disease progression can be decreased by lowering serum UA levels in diabetic patients (Jalal, 2013). Uric acid is a potential target for therapeutic intervention in diabetes.

Biomarkers of tubular/glomerular damage

Recently, certain biomarkers which were initially identified in acute kidney injury (AKI) also have been reported to confer value in evaluating patients with CKD. Biomarkers such as cystatin C, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), angiotensinogen, periostin and monocyte chemoattractant protein-1 (MCP-1) reflect tubular injury.

Cystatin C

Cystatin C is a low-molecular weight protease inhibitor, produced by all nucleated cells in the body and is reabsorbed and catabolized by the proximal tubule. A prospective observational study showed that urine cystatin is an independent predictor of CKD progression in type 2 diabetes (Kim, 2013). Elevated cystatin C is a better early predictor compared with serum creatinine-based formulae. Numerous studies have validated cystatin C as a marker of renal function. In addition, cystatin C levels not only correlate with progression of nephropathy but also show a more sensitive marker of early DN when eGFR remains>60mL/min.

Neutrophil – gelatinase associated lipocalin (NGAL)

Neutrophil –gelatinase associated lipocalin (NGAL) also known as oncogene 24p3 is a secreted glycoprotein. It is a member of lipocalin family of proteins that transport small hydrophobic ligands. NGAL is expressed in renal tubular epithelium and a rise in urinary concentrations may provide an indication of acute renal injury. It is a prognostic biomarker in numerous diseases such as malignant neoplasms and provides protection against bacterial infection (Bolignano, 2008). NGAL is also considered to be a sensitive and more accurate early predictor of acute renal damage before the rise in serum creatinine concentration. Higher urinary NGAL has been associated with the decline in GFR in type 2 diabetic patients with micro- or macroalbuminuria (Bolignano, 2008; Nauta, 2011). Models of acute kidney injury (AKI) to chronic kidney disease (CKD) transition have implicated its role as a potential biomarker of chronically injured kidney. In a related study carried out among patients with diabetic nephropathy, elevated urine NGAL level was reported to be associated with the progressive course of the disease leading to ESRD (Yang, 2009).

Kidney Injury Molecule-1 (KIM-1)

KIM-1 is a membrane protein expressed on the apical membrane of renal proximal tubule cells and reflects tubular damage in the most advanced stages of the renal disease in diabetic patients (Han, 2002).This biomarker is undetected when the kidneys are normal. Therefore, KIM-1 is considered a potential novel urinary biomarker in the early detection of AKI. From a cross-sectional descriptive study, urine KIM-1 increased in type 2 diabetes mellitus with normoalbuminuria and midly increased albuminuria. Serum and urine KIM-1 predicted the rapid decline of GFR (de Carvahlo, 2016; Nielsen, 2012). In a kidney biopsy study of 74 patients with CKD having various etiologies, KIM-1 was primarly expressed at the luminal side of dedifferentiated proximal tubules in areas with fibrosis and iflamation (van TImmeren, 2007). This ectodomain protein segment has been suggested to be a quantitative marker of AKI.

Liver-fatty acid-binding protein (L-FABP)

Liver-fatty acid-binding protein (L-FABP) is expressed in the cytoplasm of human renal proximal tubules. Renal L-FABP expression is up-regulated and urinary excretion of renal L-FABP is increased by various stressors, such as urinary protein, hyperglycemia, tubular ischemia, toxins and salt-sensitive hypertension, which lead to the progression of kidney disease. Urinary L-FABP

levels reflect the degree of tubulointerstitial damage and are strongly correlated with the prognosis of CKD patients in clinical studies. In patients with type 1 or type 2 diabetes, urinary L-FABP levels seem to be higher in patients with normal levels of urinary albumin than in those with microalbuminuria. Urinary L-FABP may be useful for the early detection of diabetic nephropathy (Kamijo,2004; Kamijo, 2011).

Angiotensinogen

Renal angiotensinogen is formed primarly in proximal tubular cells and is secreted in tubular fluid. The intrarenal angiotensin aldosterone system (RAS) was recently proposed to be involved in the progression of renal injury in models of hypertension and in kidney diseases (Suzaki, 2007). Recent studies revealed that high urinary level of angiotensinogen/creatinine was associated with lower eGFR and hypertension (Hayne, 2015). Angiotensinogen might be useful as an early biomarker of the activation of the RAAS in diabetic nephropathy.

Pigment Epithelium-Derived Factor (PEDF), Fibroblast Growth Factor 21 (FGF-21)

FGFs are multifunctional proteins with a wide variety of effects. Today FGFs are classified as intracrine, paracrine and endocrine FGFs by their action mechanisms (Itoh, 2015). Endocrine FGFs comprise FGF-19, FGF-21 and FGF-23. FGF-21 is a hepatoadipokine with pleiotropic metabolic regulatory actions.

Pigment epithelium-derived factor (PEDF) and fibroblast growth factor 21 (FGF-21) are two potential biomarkers of progression in diabetic nephropathy (Hui, 2014). PEDF is a secreted circulating glycoprotein with anti-oxidative, anti-inflamatory and anti-angiogenic properties, whereas FGF-21 is a hormone predominantly secreted from the liver and possess multiple metabolic regulatory properties. In subgroups of diabetic patients with relatively well-preserved kidney function, with an eGFR>60 mL/min/1,73m2 and normoalbuminuria, serum PEDF and FGF-21 levels were idependently associated with the progression to micro- or macroalbuminuria and eGFR decline respectively, even after adjusted for baseline eGFR levels. The elevation in both serum PEDF and FGF-21 levels reflect on the severity of the underlying renal inflammation and injury in type 2 diabetes which would contribute to the development and progression of diabetic nephropathy (Lee, 2015).

Fibroblast Growth Factor 23 (FGF-23)

Several tissues express FGF-23, such as bone tissue, bone marrow vessels ventrolateral thalamic nucleus, thymus and lymph nodes[35]. Its principal target is kidney, where it regulates phosphate reabsorption and production of 1,25(OH)2D3 (Feldman, 2003). Data from Chronic Renal Insufficiency Cohort (CRIC) study suggested that FGF-23 is superior to existing markers as a sensitive screening test to identify which patients are developing disordered mineral metabolism in early CKD (Zhang, 2011). Several other studies identified FGF-23 as a risk factor for CKD progression. Even though there was however no association between FGF-23 levels and the severity of AKI, in some AKI models FGF-23 rised more quickly than phosphate levels or NGAL (Zhang, 2011; Christov, 2013).

Biomarkers of renal inflammation

TNF-a is an important cytokine produced under high glucose conditions by macrophages, renal tubular cells and glomerular mesangial cells. Apart from being a major participant in promoting inflammation, TNF-a is known to induce apoptosis and accumulation of ECM in glomerular and tubular regions leading to alteration of glomerular filtration, tubular permeability and reabsorption (Donate-Correa,2015; Navarro, 2006). Clinical studies have reported higher serum and urinary levels of TNF-a in diabetic patients with renal dysfunction, which further increase with progression of the disease (Navarro, 2006). In terms of excretion of inflammatory cytokines, urinary concentrations of 27 cytokines in type 2 diabetic patients with normo- and micro-albuminuria have been evaluated. The inflammatory cytokines act as pleiotropic polypeptides that regulate inflammatory and immune responses, thus providing important signals in varius pathologic and physiologic processes, including diabetic nephropathy (Chen, 2008). Urinary levels of IL-6, IL-8, IP-10, MCP-1, G-CSF, EOTAXIN, RANTES in microalbuminuric patients were significantly increased compared to those in normo-albuminuric patients or controls.

Monocyte Chemoattractant Protein-1 (MCP-1)

MCP-1 is a cytokine and as a member of the CC chemokine family is a major factor influencing macrophage accumulation in animal and human models with renal impairment (Segere, 2000). MCP-1 is upregulated and expressed in the diabetic glomerular and renal tubular epithelium and a rise in urinary MCP-1 levels correlates with the extent of interstitial inflammatory infiltrate. A number of studies indicated that urinary detection of MCP-1 is a reliable early marker of DN over other conventional markers.

3. Conclusion

Diabetic nephropathy is the leading cause of CKD. Thus, estimation of renal functions is a crucial task in the management of patients with diabetes.

Over the past few years, a better understanding of DN pathogenesis has revolutionized and improved the approaches used for treating the patients with diabetes and its associated renal complications. The identification of biomarkers of early stages of DN, and progression toward ESRD, is of critical importance. In this review, we have summarized the novel biomarkers, based on the pathogenesis of the DN and presented a list of several putative prognostic biomarkers.

4. Conflict of interest

None.

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