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Saxagliptin: A novel antiparkinsonian approach

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ABSTRACT

The emergence of glucagon-like peptide-1 as a crucial contender in modifying neurodegenerative diseases in the preclinical studies has instigated interest in investigating the antiparkinsonian effect of dipeptidyl peptidase (DPP)-4 inhibition. Notably, saxagliptin (SAX), the DPP-4 inhibitor, recently showed efficacy in ameliorating streptozotocin-induced Alzheimer's disease; however, its effect on Parkinson's disease (PD) has not yet been elucidated. In a rat rotenone (ROT) model, SAX prominently improved motor performance as well as muscle coordination and corrected akinesia. Moreover, SAX preserved substantia nigra pars compacta tyrosine hydroxylase (TH) immunoreactivity while halting the reduction in the striatal TH, dopamine (DA) and complex I. Meanwhile, SAX prevented the ROT-induced increment of striatal DPP-4 and the decline in cAMP, ATP/ADP and brain-derived neurotropic factor levels. Improvement in striatal energy level was associated with partial hindrance of ROT-induced body weight reduction. In addition, through its anti-inflammatory potential, SAX decreased the ROT-induced nuclear factor-κB, inducible nitric oxide synthase, tumor necrosis factor-α, intracellular adhesion molecule-1 and myeloperoxidase. The antiapoptotic marker B-cell lymphoma-2 was enhanced by SAX, versus reduction in caspase-3 and its intrinsic apoptotic activator cytochrome C. Furthermore, SAX amended alterations induced by ROT in the thiobarbituric acid reactive substances and the transcriptional factor Nrf-2 level. In conclusion, SAX can be introduced as a novel approach for the management of PD based on the remarkable improvement in motor functions denoting antiparkinsonian efficacy via antioxidant, anti-inflammatory, antiapoptotic, neuroprotective and neurorestorative mechanisms. These effects were linked to DPP-4 inhibition, reduced neurodegeneration and enhanced DA synthesis.

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder that affects around 2% of adults over 60 years (Schapira and Olanow, 2004). The hallmark of this morbid disease is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and striatum that results in motor disorders (Dauer and Przedborski, 2003). A causal relation between dopamine (DA) and energy deficits stems from the mitochondrial dysfunction and consequently the decline in adenine nucleotides in PD (Reale et al., 2012). Furthermore, other factors appear to act in synchrony leading to neurodegeneration including apoptosis, free radicals and inflammation (Schapira and Olanow, 2004; Mounsey and Teismann, 2010). However, the PD traditional DA replacement therapy, L-DOPA, eventually loses efficacy associated with marked motor complications (Carlsson et al., 2006; Gardian and Vecsei, 2010).

Central glucagon-like peptide (GLP)-1 receptor stimulation has been proven to play an important role in regulating neuronal plasticity as well as survival (Kim et al., 2009). Furthermore, both

Abbreviations: 6-OHD, 6-hydroxydopamine; AD, Alzheimer's disease; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma-2; BDNF, brain-derived neurotopic factor; cAMP, cyclic adenosine monophosphate; Casp-3, caspase-3; Cyto C, cytochrome C; DA, dopamine; DDP-4, dipeptidyl peptidase-4; EX-4, exenatide; GLP-1, glucagon-like peptide -1; ICAM-1, intracellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NADH, reduced nicotinamide adenine dinucleotide; NAD^+, oxidized nicotinamide adenine dinucleotide; NF-κB, nuclear factor-kappa B; Nrf-2, nuclear factor (erythroid-derived 2)-like; PASS, Prediction of Activity Spectra for Substances; PD, Parkinson's disease; ROS, reactive oxygen species; ROT, rotenone; SAX, saxagliptin; SNpc, substantia nigra pars compacta; TBARS, thiobarbituric acid reactive substances; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor-alpha.

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anti-inflammatory (Pugazhenthi et al., 2010) and neurogenic potentials (Hunter and Holscher, 2012) have been ascribed to GLP-1 receptor activation. Noteworthy, these receptors are widely expressed in various brain areas of humans and rodents (Alvarez et al., 2005; Merchenthaler et al., 1999) including the SNpc (Perry and Greig, 2005). Indeed, innovative experimental approaches utilized exenatide (EX-4), the GLP-1 receptor agonist, in ameliorating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine induced PD (Bertilsson et al., 2008; Harkavyi et al., 2008; Li et al., 2009). In another experimental model, Li et al. (2009) reported that the striatum of ischemic reperfused rats was less vulnerable to damage following EX-4 treatment. From a different prospective, GLP-1 activity may be extended via utilization of dipeptidyl peptidase (DPP)-4 inhibitors (Shannon, 2013), leading to a two-to three-fold increase in basal levels of this incretin which is a blood brain barrier (BBB) permeable ligand (Kastin et al., 2002). Peptidases like DPP-4 regulate a variety of biological processes related to inflammation such as T cell activation, immune responses and inflammation-related disorders (Aroor et al., 2013; Sedo and Malik, 2001; Varga et al., 2011). Moreover, the DPP-4 enzyme plays a pivotal role with respect to growth regulation and cytokine production (Ansorge et al., 2011).

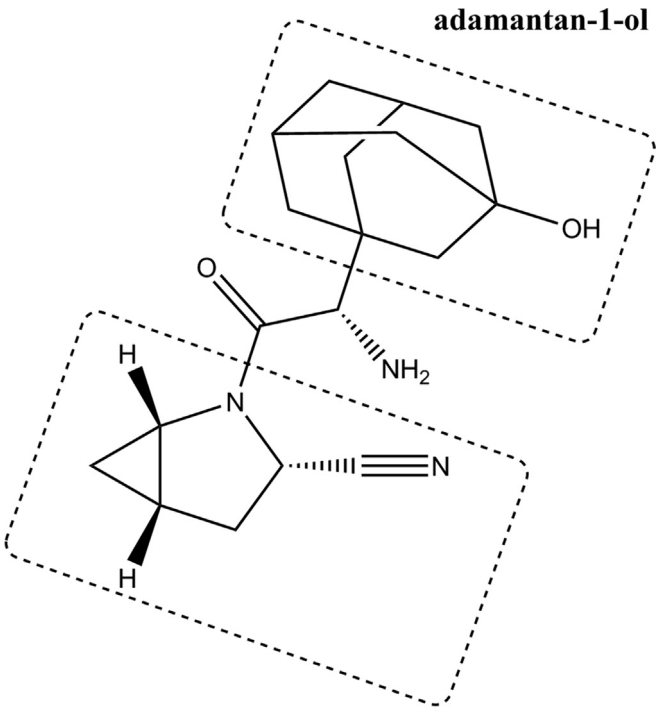
Saxagliptin (SAX), [(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile], a drug approved for the treatment of type-2 diabetes slows the rapid inactivation of GLP-1 through DPP-4 inhibition, extending the short half-life of the endogenous peptide due to the active metabolites of SAX (Kania et al., 2011). The neuroprotective effect afforded by SAX in Alzheimer's disease (AD) (Kosaraju et al., 2013) indicates that DPP-4 inhibition promises to be a novel approach for management of PD.

The utilization of the software Prediction of Activity Spectra for Substances (PASS) which provides the estimation of biological activities based on the organic compound structure, was exploited in an unprecedented attempt to test a probable antiparkinsonian activity for SAX. Therefore, the antiparkinsonian and free radical scavenging abilities of SAX and its structural moieties, namely adamantan-1-ol and (1S,3R,5S)-2-azabicyclo[3.1.0]hexane-3-carbonitrile (Scheme 1) were compared to the existing data base of the PASS program. Since this program verified antiparkinsonian efficacy and free radical scavenging ability to both entities of SAX, it was suggested that this drug might possess a positive role against PD. To this end, the effect of SAX was evaluated on PD motor deficits in a rotenone (ROT) rat model. Since its antiparkinsonian efficacy was confirmed in the current work, the potential molecular mechanisms involved in mediating such an effect were targeted. Before attempting to pursue SAX mechanistic action, it was imperative to first verify the possible alteration in DDP-4 by ROT in the striatum. The study was further extended to evaluate the potential role of SAX in intercepting the deleterious effects of ROT on complex I activity, SNpc tyrosine hydroxylase (TH) immunoreactivity, DA, cellular energy, apoptosis, neurogenesis as well as oxidative stress and inflammation pathways involved in PD pathology.

2. Material and methods

2.1. PASS program

Since no previous data reported an antiparkinsonian effect of SAX, the current study utilized the PASS software to predict the pharmacological activities of SAX as well as its structural moieties. This program is used for the prediction of different types of pharmacological activities for organic substances based on previous published data to provide insight into the possible mechanistic actions of drugs (Ekins et al., 2007; Khurana et al., 2011; Lagunin et al., 2000). The PASS docking program gives both Pa (probability to be *pharmacologically* active) and *Pi (probability to be pharmacologically inactive)* values to predict a molecule's pharmacological activity.



(1*S*,3*S*,5*S*)-2-azabicyclo[3.1.0]hexane-3-carbonitrile

Scheme 1. Saxagliptin [(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile] and its structural moieties adamantan-1-ol and (1S,3R,5S)-2-azabicyclo[3.1.0]hexane-3-carbonitrile.

2.2. Ethics statement

The study was performed in accordance with the ethical procedures and policies approved by Research Ethical Committee of Faculty of Pharmacy, Cairo University (Cairo, Egypt) and complies with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996).

2.3. Animals

Adult male Wistar rats (280 ± 20 g) obtained from El Nile Pharmaceutical Company (Cairo, Egypt) were used. Rats were allowed one week acclimatization period at the animal facility of the Faculty of Pharmacy, Cairo University. Animals were housed in groups at constant temperature (23 ± 2 °C), humidity (60 ± 10%) and a light/dark (12/12 h) cycle with lights on at 5:00 am. They were allowed free access to food and water throughout the experimental period. All behavioral experiments were carried out in separate and isolated laboratories. The experiments were performed at the same time-period of the day (09:00 am–12:00 pm).

2.4. Experimental design

At the end of the experimental period, a total of 112 rats were allocated into 4 groups (28 rats each) and divided in 3 subsets that were utilized for further assays. The vehicle-treated animals received 11 subcutaneous injections of dimethysulfoxide (DMSO; Sigma–Aldrich, St. Louis, MO, USA) in saline [1:1 (v)] on ROT corresponding days. Rats were daily given SAX (1 mg/kg, p.o.; Bristol-Myers Squibb and AstraZeneca, Indiana, USA) (Kosaraju et al., 2013) for 21 days to serve as the drug control group. In the ROT group, animals received ROT in DMSO/saline (1.5 mg/kg/48 h/11 doses, s.c., Sigma–Aldrich) based on a pilot study and as modified from Abd El-Gawad et al. (2004). The pilot study was intended to verify the behavioral effects induced by 6 injections of ROT over 11 days since the previous report from our lab had not employed behavior examinations to the animals. Based on the preliminary findings, the 6 injections did not induce proper behavioral alterations; accordingly the number of injections was extended to 11 to verify obvious parkinsonian-like behavioral changes. The last group of rats received ROT as described for the ROT-treated animals, in addition to daily protective doses of SAX. The regimen was administered 1 h before ROT on the days of the insecticide injection.

2.5. Body weight change and behavioral assessments

Each rat was weighed at the beginning of the experimental protocol and 24 h after the last ROT injection and weight changes were calculated. Rats were