Research Article

Salts of 5-hydroxynicotinic acid as potential cardioprotectors: Study of the structure and pharmacological evaluation

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ABSTRACT

Introduction: Impact on oxidative stress is one of the promising approaches in cardioprotection. In this regard, experimental verification requires the study of the cardioprotective properties of the salt of 5-hydroxynicotinic acid compounds under laboratory code CCK-497. **Objective:** Study of the chemical structure and cardioprotective effects of magnesium 5-hydroxynicotinate. **Materials and Methods:** Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) spectra of compound CCK-497 were measured on an Agilent MR400 broadband pulse spectrometer in DMSO-D6 solutions using the σ-scale of chemical shifts (ppm). The study of the cardioprotective properties of CCK-497, as well as the comparison drug Mexidol, was carried out on 30 rabbits using the model of coronary occlusion myocardial infarction. The cardioprotective effect of CCR-497 was assessed by determining the necrosis zone and biochemical marker Tn I, the plasma concentration of which was determined after 2 h on a Triage MeterPro immunofluorescence device (Biosite, USA); in addition, the products of malondialdehyde (MDA) and diene conjugate (DC) lipid peroxidation in the homogenizer were evaluated hearts. **Results:** When confirming the structure of CCK-497, a high sensitivity of ^{1}H NMR spectroscopy was revealed, confirming the chemical structure of the compound. Compound CCK-497 at a dose of 37.2 mg/kg showed high cardioprotective activity in a model of coronary occlusion infarction in rabbits by limiting the necrosis zone, preventing ST-segment elevation and reducing the increase in biochemical markers of Tn I, MDA, and DC myocardial damage. **Conclusion:** Magnesium 5-hydroxynicotinate (CCK-497) potential cardioprotector for the correction of ischemic/reperfusion myocardial injuries was found.

KEY WORDS: Cardioprotection, Ischemia/reperfusion, Magnesium 5-hydroxynicotinate, Myocardium, Nuclear magnetic resonance spectroscopy

INTRODUCTION

In recent years, increased attention has been paid to clarifying the role of free radical oxidation in normal and pathological conditions.[1] Free radicals are formed in the body as a result of the metabolism of oxygen dissolved in tissues, and the resulting active forms of oxygen reactive oxygen species (ROS) cause oxidation of membrane lipids, proteins, polysaccharides, and nucleic acids.[2,3] The damaging effect of free radicals is opposed by a multilevel endogenous antioxidant system,[4] which balances free radical oxidation and antioxidant systems. With the intensive formation of ROS and with insufficient activity of the antioxidant system, a condition occurs, accompanied by an increase in destructive processes in tissues accompanied by oxidative stress.[5] This process is involved in the pathogenesis of a significant number of diseases.[6] In case of oxidative stress, the use of pharmacological correction of free radical oxidation-exogenous antioxidants is pathogenetically substantiated.[7-10]

For pharmacological correction of oxidative stress is widely used drug for various chemical.[11,12]

The chemical structure of the substance determines its target action in the process of correction of oxidative stress.[13,14]

Accordingly, taking this relationship into account can be useful in the search for new groups of drugs under oxidative stress. From a chemical point of view, the prerequisites for the high efficiency of the compound as an antioxidant are: The presence of an aromatic or

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heteroaromatic cycle of low molecular weight; the presence of one hydroxyl group in the aromatic cycle or side chain to ensure lipophilicity and antiradical activity; good solubility in an environment where free radicals are generated (hydrophilicity); and the presence in the aromatic cycle of a saturated or unsaturated alkyl chain as a prerequisite for the integration of the compound into the cell membrane.[15]

Salts of 5-hydroxynicotinic acid possess the above-listed properties, which have shown high antioxidant activity and effectiveness in the treatment of a number of diseases in domestic experimental and clinical studies. In addition, a number of drugs have been synthesized based on 5-hydroxynicotinic acid for the treatment of cardiovascular, oncological diseases, and AIDS.[16] A wide range of pharmacological activity and low toxicity determine the relevance of the search and development of new original drugs in the series of 5-hydroxynicotinic acid.[17]

Preclinical studies[18] at the cellular, molecular,[19] organ,[20,21] systemic and organismic levels,[22-24] including specific activity[25,26] and toxic studies in combination with bioequivalence studies,[27] and therapeutic equivalence and effectiveness[28] are an integral part of the study of innovative drugs.

Objectives of the Study

Increasing the effectiveness of pharmacological cardioprotection of ischemic/reperfusion injuries of the myocardium using salts of 5-hydroxynicotinic acid.

MATERIALS AND METHODS

The study was carried out in accordance with the requirements of the Law of the Russian Federation "On the Protection of Animals from Cruel Treatment" of June 24, 1998, the rules of laboratory practice in conducting preclinical studies in the Russian Federation (GOST 3 51000.3-j96 and GOST R 53434-2009), directives of the European Community (86/609 EU), the rules of the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental research (1997), and the rules of Laboratory Practice adopted in the Russian Federation (order of the Ministry of Health of the Russian Federation No. 708 dated 08.29.2010).

At the first stage, studies were carried out confirming the structure of SSK-497 by the hydrogen-1 nuclear magnetic resonance (^{1}H NMR) physicochemical method to prove the presence and place of preferred chemical groups, which is especially important for compounds selected for an in-depth study of pharmacological properties. ^{1}H NMR spectra were measured on an Agilent MR400 broadband pulsed spectrometer, in DMSO-D6 solutions; the σ-scale of chemical shifts (ppm) was used. The Agilent 400 operating radio frequency range varies from 20 to 410 MHz, which makes it possible to convert NMR signals from 1H, 2H, 13C, 31P, 19F, and 15N nuclei, as well as nuclei of compounds of inorganic origin.

The study of the survival of ischemic myocardium was carried out on laboratory rabbits weighing 2–2.5 kg. The method is ligation of the anterior descending branch of the left coronary artery in anesthetized rabbits located on controlled breathing. Myocardial infarction (MI) was modeled under the control of electrocardiogram recording in standard II lead.

Twenty minutes before the coronary artery ligation, test compounds were introduced into the rabbit ear vein. After 30 min after coronary occlusion, the ligature was removed, and myocardial reperfusion was performed for 90 min. The size of the necrosis zone was determined practically; previously, heart sections were placed in 1% TTC (2,3,5-Triphenyltetrazolium chloride, Sigma-Aldrich, USA), kept for 15 min at a temperature of 37.0°. Stained sections were scanned, images were transferred to a computer, the results were processed using Photoshop CS2, and the areas (in pixels) were calculated. Given the mass of each section, the mass of the necrosis zone was calculated for each section as a whole.

To evaluate the biochemical markers of MI, Tn I was chosen. Blood was taken to determine it from the right ventricle into a disposable vacuum tube with an anticoagulant. Its plasma concentration was determined after 2 h on a Triage MeterPro immunofluorescence device (Biosite, USA). The level of primary products of peroxidation malondialdehyde and diene conjugate (MDA and DC) was evaluated by generally accepted methods.[29]

RESULTS AND DISCUSSION

The new compound CCK-497 was obtained by the interaction of 5-hydroxynicotinic acid with magnesium oxide in an aqueous medium when heated, the general formula $C_{12}H_{16}N_2O_{10}Mg$ [Figure 1].

To obtain information on the structure of compounds, nuclear magnetic resonance spectroscopy is most often used at present, which was carried out in our study. Figure 2 shows the ^{1}H NMR spectrum of compound CCK-497:

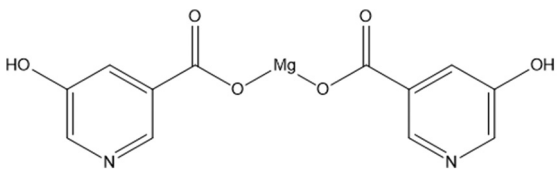

Figure 1: Chemical formula CCK-497

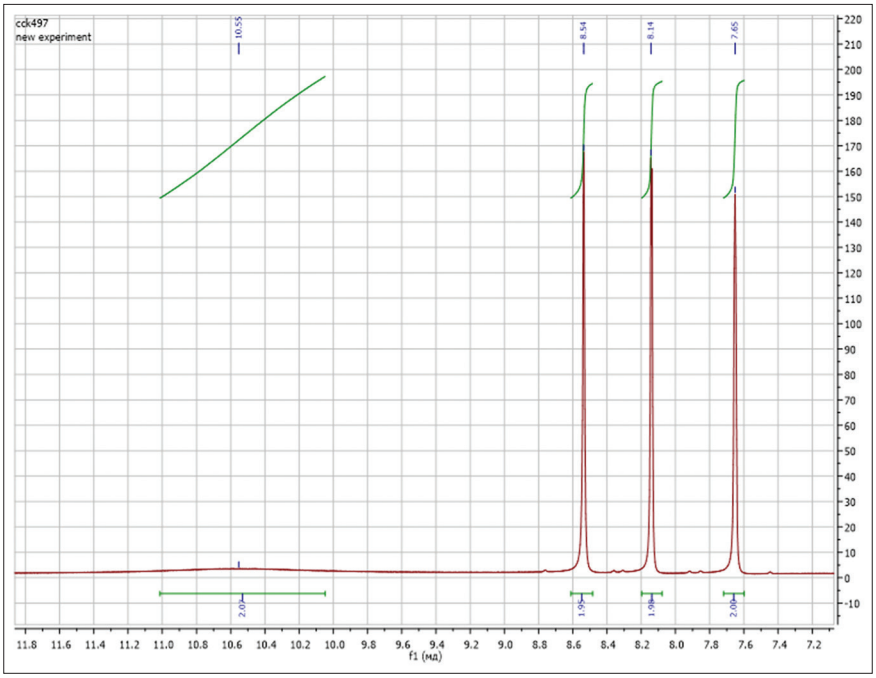

Figure 2: Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) spectrum of the compound CCK-497, ^{1}H NMR (400 MHz, DMSO) δ 10.55, 8.54, 8.14, and 7.65

The ^{1}H NMR spectrum of compound CCK-497 shows the signals of protons of pyridine nuclei in the form of three singlets with an integrated intensity of two protons each; with chemical shifts of 8.54, 8.14, and 7.65 ppm corresponding to protons at positions 2, 6, and 4 of the pyridine nucleus. The singlet of protons of hydroxyl groups at position five of the pyridine nuclei is strongly broadened and weakly visible in the survey spectrum, but it is clearly manifested in the analysis of integral intensities in the region of 10 ppm.

Conducted studies ^{1}H NMR spectroscopy confirmed the chemical composition of the substance CCK-497.

To confirm anti-ischemic activity, the effect of CCK-497 on the myocardial necrosis zone was studied when modeling coronary occlusion MI.

In the group of animals to which the derivatives of CCK-497 were injected 30 min before ischemia, a decrease in ST-segment elevation was detected in post-reperfusion, and by the 90th min it significantly decreased by 25%, relative to the control. However, complete normalization of the ST-segment level did not occur [Table 1].

In the study of antifibrillator activity under the conditions of modeling coronary occlusion infarction, it was found that the compound CCK-497 (37.2 mg/kg) prevented the appearance of reperfusion fibrillations. The reference drug Mexidol limited the incidence of fibrillation to 42.1% of cases (66.7% in the control).

In addition, magnesium 5-hydroxynicotinate CCK-497 at a dose of 37.2 mg/kg showed a statistically significant decrease in the necrosis zone, which was 19.2 ± 3.5%, which is 2.4 times less than in the control group (46.3 ± 8.1%). The cardioprotective effect of CCK-497 was confirmed by a 2.7-fold restriction in the growth of the marker of myocardial damage Tn I and a decrease in the products of lipid peroxidation (LPO) – MDA and DC [Table 2].

Thus, magnesium 5-hydroxynicotinate under laboratory code CCK-497 at a dose of 37.2 mg/kg showed high cardioprotective activity in a model of coronary occlusion infarction in rabbits by limiting the necrosis zone, preventing ST-segment elevation and reducing the increase in biochemical markers of Tn I myocardial damage, MDA, and DC. The reference drug Mexidol at a dose of 85.7 mg/kg was inferior in cardioprotective activity.

At the beginning of prolonged ischemia, two separate pathological processes occur. The first process is tissue damage caused by ischemia *per se.* The second is the biochemical changes that occur during ischemia and contribute to a surge in ROS generation and the infiltration of pro-inflammatory neutrophils and other immunocytes when molecular oxygen is re-introduced into tissues during reperfusion.[30]

Despite the fact that reperfusion restores the delivery of oxygen and substrates necessary for the generation of aerobic ATP and normalizes extracellular pH, the reoxygenation effect disrupts the oxygen recovery process in the ischemic myocardium, which leads to the accumulation of significant amounts of ROS.[30] Moreover, this inevitably leads to the activation of

Table 1: Effect of CCK-497 on the dynamics of ST-segment elevation at the 30-min ischemia stages followed by 90-min myocardial reperfusion in rats with a single intravenous administration 30 min before modeling myocardial infarction (M±m)

No.	Experimental group	Exodus	Ischemia, 30 min	Reperfusion	
				5 min	90 min
1.	Л/о, *n*=6	0.11±0.03	0.12±0.03	0.12±0.03	0.12±0.02*
2.	I/Rcontrol, *n*=8	0.09±0.02	0.69±0.08*	0.52±0.05*	0.50±0.03*ý
3.	I/R+CCK-497 (37.2 mg/kg), *n*=8	0.12±0.04	0.63±0.06*	0.43±0.06*	0.34±0.04*#ý
4.	I/R+Mexidol (87.5 mg/kg), *n*=8	0.10±0.03	0.64±0.06*	0.46±0.05*ý	0.40±0.04*#ý

L/o–falsely operated; the differences are statistically significant compared to: *–outcome, ý–with ischemia of 30 min in their group, #–to the "I/R" group at $P<0.05$

Table 2: The effect of CCK-497 on the size of the zones of myocardial necrosis, the content of Tn I in blood plasma and MDA and DC in the heart homogenate (M±m)

Experimental group	Sizes of a zone of a necrosis (% in relation to heart)	MDA (nMol/g of myocardial tissue)	DC (ΔE_{232}/gtissue	Tn I (ng/ml)
Л/о, *n*=6	0.00±0.00	97.07±5.3*	0.87±0.05*	3.4±0.9*
I/R, control, *n*=8	46.3±8.1#	169.41±11.7#	1.89±0.05#	16.6±3.2#
I/R+CCK-497 (37.2 mg/kg), *n*=8	19.2±3.5#*	103.4±6.4*	1.06±0.03*	6.0±1.2#*
I/R + Mexidol (85.72 mg/kg), *n*=8	31.2±4.1#*	121.4±7.1*	1.62±0.03*	12.2±2.4#*

L/o–falsely operated; the differences are statistically significant compared to: *–outcome, ý–with ischemia of 30 min in their group, #–to the "I/R" group at $P<0.05$. MDA: Malondialdehyde, DC: Diene conjugate

LPO.[31] LPO activation has a number of adverse effects due to postischemic myocardial hyperoxia and the influx of pro-oxidants, increased activity of membrane-bound and solubilized phospholipases, as well as the destructive effect of the amphiphilic compounds formed (free fatty acids, lysophospholipids, and LPO products), which penetrates into the lipid bilayer membrane organelles due to the accumulation of cations in them Na^+ and Ca^{2+}.[32] Since it is ROS that is considered one of the main factors that trigger the death mechanism during ischemic/reperfusion injury of the myocardium, it is obvious that the prevention of ROS synthesis or their neutralization will be crucial for the survival or restoration of cardiomyocyte function.[3] The pharmacological group used for this purpose may be drugs that exhibit high antioxidant activity. This group combines drugs of various nature and mechanisms of action, which ultimately affect the processes of free radical oxidation of cell structures and biomolecules, primarily peroxidation of membrane phospholipids.

However, the search continues for new compounds with pronounced cardioprotective activity targets in various groups of derivatives.

The prerequisites for the high efficiency of the compound may be the presence of privileged chemical groups, in particular, hydroxyl, which can increase the lipophilicity and antiradical activity of the compound. We found the presence of such a group by ^{1}H NMR in magnesium hydroxynicotinate CCK-497.

Further, to check the cardioprotective activity of the compound CCK-497, its cardioprotective effectiveness was evaluated on the model of coronary occlusion MI. It was shown that CCK-497 has the ability to significantly reduce the size of the necrosis zone, biochemical markers of Tn I and LPO products of MDA and DC. The reference drug was inferior to compound CCK-497.

CONCLUSION

The data obtained allow us to recommend the connection under the laboratory code CCK-497 for further preclinical studies as a means of cardioprotective activity. In particular, experimental verification requires studying the mechanism of action of the compound CCC-497, identifying the relationship between cardioprotective activity and the dose of the compound.

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