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Synthesis and biological evaluation of novel hydrogen sulfide releasing nicotinic acid derivatives

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ABSTRACT

Twelve novel hybrids of slowly releasing hydrogen sulfide donor ADT-OH combined with nicotinic acid were synthesized. All of their structures had been confirmed by ^{1}H NMR, ^{13}C NMR and MS spectra. The target compounds were evaluated for their neuroprotective effects on hippocampal neuron HT22 cells against glutamate-induced injury at the concentrations of 1–100 μM with MTT assay, and their toxicity on HT22 cells untreated by glutamine at the concentration of 100 μM. The active compound was further investigated for its effect on ischemic infarct volume by intraperitoneal injection at 3 h after ischemia in mice models of permanent middle cerebral artery occlusion (pMCAO). The results showed that all the compounds significantly protected HT22 cells from glutamate-induced damage at most of the experimental concentrations, and had no or little neurotoxicity on normal HT22 cells at the high concentration. More importantly, compound **A6** significantly reduced infarct volume in the pMCAO model. These results suggested that compound **A6** may be promising for further evaluation for the intervention of cerebral ischemic injury.

1. Introduction

Stroke is the third largest killer after heart disease and malignant tumor and the leading cause of disability in adults[1], which brings a major socioeconomic burden. Cerebral ischemic stroke accounts for about 70% of stroke. Ischemic stroke is a central nervous system disease triggered by thrombotic occlusion of cerebral arteries blood, involved in many complex pathological mechanisms, including excitatory toxicity of glutamate, release of a large number of free radicals, inflammatory response, neuronal necrosis and apoptosis.[2] The result that they reinforce and promote each other leads to the severe damage of central nervous system, and even to death. Selective thrombolysis via artery is common clinical treatment for ischemic stroke, such as tissue-type plasminogen activator tPA. But its use remains limited by a narrow therapeutic window only within 4.5 h after ischemic stroke, and the cerebral ischemic injury will further aggravate by the release of large free radicals and glutamic acid produced during the blood reperfusion process after thrombolysis.[3] Many neuroprotective agents reduced ischemic damage in animal models of stroke. However, most of them ended in failure in clinical trials to date. One of the reasons is that the ischemic cascade is so complex in the human that targeting a single pathway may be ineffective. Therefore, it is very important to search for new drugs with multiple pharmacologic actions for the treatment of cerebral ischemic stroke.

Hydrogen sulfide (H_2S) is the third gaseous signal molecule in vivo. Many studies have proved the cytoprotective effects of H_2S in vitro and/or in vivo ischemic injury. Administration of NaHS, an exogenous donor for H_2S, at the dose of 0.2 or 0.4 μmol/kg after a 24 h global cerebral ischemia–reperfusion (I/R) significantly decreased the apoplexy index, neurological symptom scoring, and brain infarcted area as compared to the I/R group in a dose dependent manner.[4] However, Kurokawa reported that NaHS at 0.1–1 mM induced neuronal death in mouse fetal cortical neurons, and that the neurotoxicity of NaHS is independent of NMDA receptors, NOS and T-type calcium channels, known as targets for H_2S.[5] Eizo Marutani reported H_2S releasing NMDAR antagonist S-memantine exerted lower neurotoxicity and prevented ischemic neuronal death more markedly than conventional H_2S releasing compound (ACS48) or memantine alone.[6] ADT-OH [5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione] is a slowly releasing hydrogen sulfide donor.[7] Our previous studies found that ADT-OH suppressed neuroinflammation by AMPK activation in BV2 microglial cells,[8] protected hippocampal neuronal HT-22 cells from damage

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induced by glutamate.[9] Moreover, its derivative (ADT-OCH_3) treated at 3 h after MCAO (middle cerebral artery occlusion) significantly decreased cortical and hemispheric infarction and protected the blood brain barrier integrity at 48 h following MCAO.[10]

Vitamin B3 has two forms: nicotinic acid (niacin, pyridine-3-formic acid) or nicotinamide (pyridine-3-formamide) reciprocally converting in the human body. Niacin takes part in the process of lipid metabolism, reduces the concentration of plasma triglyceride and very low density lipoprotein in plasma and increases the level of HDL. Nicotinic acid also can dilate blood vessels, therefore it is used in clinic for the treatment of hyperlipidemia, headache, vascular migraine and cerebral arterial thrombosis forming, etc. However, nicotinic acid has some adverse reactions such as skin flush, itching and gastrointestinal discomfort. Recent studies suggested niacin played a role as a neuroprotective agent in stroke. Nicotinamide (500 mg/kg) given intravenously or intraperitoneal, 2 h after in the model of permanent MCAO, significantly improved neurological behavioral scores and reduced the focal infarct volume of rats.[11,12] Moreover, treatment with nicotinamide given at three doses (500 mg/kg, intravenously) further enhanced the extent and duration of neuroprotection and conferred a complete motor recovery up to two weeks from the onset of focal cerebral ischemia in rats.[13] However, nicotinamide showed neuroprotection against focal cerebral ischemia at dose of 500 mg/kg/day. Large dose of medication could cause human gastrointestinal upset, skin flush and pruritus. Therefore, niaspan, an extended-release formulation of niacin, has been widely used to increase HDL cholesterol and to prevent cardiovascular diseases and stroke. Niaspan also induced neuroprotection after stroke. Combination treatment with low-dose niaspan and tPA administered 4 h after embolic stroke in a rat model reduced infarct volume and provided neuroprotection.[14,15] Mechanisms of niacin in protection of cerebral ischemia included inhibiting excessive activation of poly ADP ribose polymerase[16] and increasing synaptic plasticity and axon growth.[17] Niaspan increased TNF-α converting enzyme and promoted angiogenesis after stroke.[18,19]

Thus, based on the idea of 'a drug for multi-mechanism', we are now seeking novel drugs that can control many factors in the pathological mechanisms of ischemic stroke by multi-pathway simultaneously. In this study, we hypothesized that H_2S-releasing derivatives of niacin could display synergistic effects on neuroprotection exerted by both nicotinic acid and ADT-OH or ADT-OH ether derivatives. Thereby, a series of hybrids from nicotinic acid and ADT-OH were designed, synthesized and evaluated as potential neuroprotection agents in vitro and in vivo.

2. Results and discussion

2.1. Chemistry

In this study, twelve nicotinic acid derivatives were synthesized. The synthetic routes of compounds **A1**–**8** were shown in Scheme 1. Compounds **A1**–**8** were synthesized from compound 1 and nicotinic acid (**3**). Nicotinic acid was esterified with ADT-OH catalyzed by DCC to obtain **A1**. Esterification of nicotinic acid with dibromoalkane in the presence of anhydrous K_2CO_3 afforded the corresponding esters **4a**–**g**. Compounds **A2**–**8** were obtained by reacting **4a**–**g** with ADT-OH respectively in acetone.

Compounds **B1**–**4** were synthesized followed the route illustrated in Scheme 2. Nicotinoyl azide (**7**) was synthesized according to the method proposed by Qin et al.[20] Nicotinamide reacted with 50% hydrazine monohydrate in water, and then reacted with $NaNO_2$ and concentrated hydrochloride, and finally reacted with amino acid. Nicotinoyl azide was reacted with four amino acids respectively to obtain corresponding compounds **8a**–**d** (yield 56–71%).

The esterification of ADT-OH with Br $(CH_2)_4$Br in refluxing acetone in the presence of anhydrous K_2CO_3 afforded compound **9** (yield 76%). Compound **9** was esterified with **8a**–**d** respectively in the presence of anhydrous K_2CO_3 in refluxing acetone to give the corresponding compounds **B1**–**4**.

All the nicotinic acid hybrids were reported for the first time. Their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR and HR MS.

2.2. The effects of target compounds on glutamate-damaged HT22 cells

The synthesized nicotinic acid derivatives were evaluated for their neuroprotective effects on mouse hippocampal HT22 neuronal cells damaged by glutamate via MTT assay. Because the reported cell viability stimulated with glutamate at 5 mM ranged 15–60%.[21–23] The results are influenced by many factors, specially serum.[24] Therefore, in order to ensure accuracy of the results, we set glutamate alone group as control in the experiment when we evaluated every target compound. The results were summarized in Table 1. When HT22 cells were exposed to 5 mM glutamate in the absence of tested compounds, the survival rates of HT22 cells in every group were less than 43%, which indicated that glutamate-induced injury model was established. When HT22 cells were exposed to 5 mM glutamate in the presence of tested compounds, most of the target compounds significantly increase their survival rates at the experiment concentration 1–100 μM (p <0.01, p <0.05), compared to glutamate group. When glutamate-damaged HT22 cells were treated with compounds **A6**–**8** or **B1**–**4** at 1–100 μM, the survival rates of HT22 cells increased and ranged from 64.73% to 108.49% (p <0.01, p <0.05), which showed their significant neuroprotective effects on the glutamate-damaged HT22 cells. Specially, compounds **A6**–**8** or **B1**–**4** had more potent neuroprotective effects on glutamate-damaged HT22 cells than their parent compound niacin at 1–50 μM and ADT-OH at 1–10 μM. Especially, the survival rate of the HT22 cells, treated with compound **A8** at 1 μM or 10 μM, was increased to 100%. Moreover, compound **A6** increased about six-fold cell viability rates at experimental concentration, compared to glutamate group. The structure–activity relationship suggested that the target compounds with longer linker (**A6**–**8**, **B1**–**4**) had preferably neuroprotection on glutamate-damaged neurons at lower concentrations.

2.3. The influences of target compounds on the HT22 cell untreated with glutamate

The influences of target compounds **A1**, **2**, **3**, **7**, **8** and **B1**–**4** on the survival rates of HT22 cells untreated with glutamate (control group) were shown in Figure 1. The cell viabilities of HT22 cells treated with compounds **A1**–**3**, **5**–**7** and **B2**–**4** at the concentration of 100 μM were about 100%, which indicated that these compounds had no toxicity to HT22 cells. However, compounds **A4**, **A8** and **B1** decreased the survival rates of HT22 cells to 52.9%, 70.4% and 68.0% respectively at high concentration, which indicated that these compounds had some toxicity to neuron cells.

2.4. The protective effects of compound A6 on permanent MCAO mice

We examined whether these nicotinic acid derivatives could protect mice against focal cerebral ischemia in mice models of pMCAO. Compound **A6** (100 mg/kg) given intraperitoneally, 3 h after MCAO, significantly reduced the infarct volume of cortex