Gastrodia-Uncaria water extract and tissue plasminogen activator for treating embolus-induced cerebral ischaemia: abridged secondary publication

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KEY MESSAGES

1. The treatment potential of Gastrodia-Uncaria water extract on cerebral ischaemia was demonstrated in terms of reduction of brain infarct volume of the brain, improvement of the motor behaviour recovery, stimulation of anti-oxidative enzyme, inhibition of matrix metalloproteinase, induction of neurotrophins, and maintenance of brain tissue integrity.
2. Intravascular administration of tissue plasminogen activator is well tolerated with oral administration of Gastrodia-Uncaria water extract, which may reduce the risk of tissue plasminogen activator–induced intracranial haemorrhage.

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Introduction

Stroke is the third cause of death worldwide and leads to disability. The recombinant tissue plasminogen activator (tPA) is the only approved drug to treat acute ischaemic stroke. However, it has a narrow treatment window and increases the risk of haemorrhagic transformation. Neuroprotection is thus explored to reduce brain injury. The 11-herb Gastrodia-Uncaria decoction is a commonly prescribed Chinese herbal medicine for stroke. Gastrodia Rhizome and Uncaria Ramulus are the two main components in the decoction. They may act synergistically in protecting neurons from oxygen glucose deprivation/reperfusion through inhibiting oxidative stress and apoptosis.[1] We aimed to study the neuroprotective effect of Gastrodia-Uncaria water extract (GUW) with or without tPA post-onset of ischaemia. The efficacy of GUW plus tPA versus tPA alone and GUW alone on cerebral ischaemia and their drug interaction were also investigated.

Methods

A rat model of embolic-induced middle cerebral artery occlusion was used. Neurological deficits, behavioural rearing, and brain infarct volume were tested for the effect of GUW. The efficacy of GUW and tPA and their drug interaction were evaluated using in vivo molecular imaging, histology, immunohistochemistry, and gene expression.

Results

The use of GUW post-stroke significantly reduced brain infarct volume and improved motor behaviour recovery. It enhanced efficacy of tPA against embolic-induced cerebral ischaemia and suppressed tPA-induced matrix metalloproteinases (MMP) activity. After middle cerebral artery occlusion, GUW increased superoxide dismutase activity but tPA did not. GUW upregulated gene expression of neutrophins and overcame downregulation induced by tPA.

When GUW was applied after stroke, similar results were demonstrated. GUW showed neuroprotective potential against cerebral ischaemia in terms of brain tissue integrity, reduction of infarct volume, suppression of MMP activity, and enhancement of the motor behaviour recovery. GUW stimulated both catalase and superoxide dismutase activities. The use of both GUW and tPA increased the activity of catalase, superoxide dismutase, and GPx. GUW upregulated gene expression of some neutrophins (BDNF, GDNF, and NGF). It also increased BDNF and GDNF expression when tPA was applied.

Discussion

Stroke is a leading cause of death and disability worldwide. Its treatment and research are challenging because of its clinical variability in terms of duration, localisation, and severity of

ischaemia, as well as patient age and comorbid systemic diseases.[2] Oxidative stress is a major cause leading to brain damage and a potential contributor to the pathophysiological consequences following cerebral ischaemic stroke. Oxidative stress leads to oedema and destructs the blood-brain barrier (BBB). Reactive oxygen species triggers oxidative damage of lipids and proteins, leading to excitotoxic stimulation during reperfusion of ischaemic tissue. Furthermore, excessive free-radical production increases the risk of BBB disruption, which may contribute to more serious consequences such as brain oedema. Under normal conditions, the anti-oxidative enzymes including superoxide dismutase, catalase, glutathione, glutathione reductase/glutathione peroxidases (GR/GPX) are up-regulated to reduce harmful effects of oxidative stress parenchyma. However, tPA may suppress antioxidant (eg, vitamins) or free-radical scavengers (eg, edaravone) and result in more damage in brain parenchyma.

Gastrodia elata and its active ingredients exhibit anti-oxidative effect against focal cerebral ischaemia. Gastrodin is a major compound to protect against cerebral ischaemia by improving anti-oxidant and anti-inflammation activities. In our previous study, GUW treatment was found to upregulate the anti-oxidative pathway against oxygen-glucose deprivation-induced injury on PC12 cells in a rat model of middle cerebral artery occlusion.[1] Nonetheless, the changes in metabolite profiles of GUW and even *Gastrodia elata* during the neuroprotective events are not known. The use of GUW post-stroke increased the activity of anti-oxidative enzyme significantly. Furthermore, malondialdehyde concentration was reduced in GUW groups. This implies that GUW enhances the antioxidant mechanism to protect the cerebral ischaemia rats from reactive oxygen species–induced oxidative damage.

During cerebral ischaemia, MMP activities were increased. MMPs disrupt BBB integrity via digestion of endothelial basal lamina. Both MMP-2 and MMP-9 mainly participate in ischaemic damage processes. They are further activated by the reperfusion phase following cerebral ischaemia when blood clots are digested by endogenous or exogenous tPA. The BBB disruption may contribute to serious consequences such as brain oedema and intracerebral haemorrhage.

In our study, GUW inhibited the activity of MMPs and reduced BBB impairment. It rescued the neuronal cells from cell death and hence protected animal against ischaemic injury. The inhibitory effect of GUW on MMPs may repair the ischaemic brain, particularly during angiogenesis and reestablishment of cerebral blood flow. During cerebral ischaemia, reactive oxygen species and reactive nitrogen species are responsible for the activation of MMP-9. The formation of nitrous oxide during ischaemia facilitates the activation of MMP-9 by S-nitrosylation. Based on the anti-oxidative, anti-nitrosative, and anti-inflammatory effect of *Gastrodia elata* and *Uncaria rhynchophylla*, GUW attenuated cerebral ischaemia/reperfusion injury might be partly via inhibition of MMP activities.

Intravascular administration of tPA is the only Food and Drug Administration approved medical therapy for cerebral ischaemia. However, tPA may penetrate through the permeabilised BBB and lyse the blood vessels in brain. Excess tPA activity also accumulates free radicals and increases MMP activities, leading to neuronal damage, mainly involving inflammation and oxidative stress pathway. Nonetheless, inadequate application of tPA leads to life-threatening brain oedema and haemorrhage in patients during the reperfusion phase.[3] The haemorrhage may associate with thrombin activation of MMP-9 in astrocytes through protease-activated receptor 1. There are no available medications providing neuroprotective effect to neurons in the reperfusion phase to treat such cerebral oedema and haemorrhage.

In our study, the MMPs expression was significantly increased in the reperfusion phase after tPA administration. However, GUW suppressed tPA-induced MMP activities in this reperfusion phase. This suggests that the treatment of GUW might maintain BBB integrity through down-regulation of MMP activities that involve inflammation after cerebral ischaemia onset. Collectively, GUW significantly reduced the MMP activities in the brains of rats. The integrated therapy of tPA with GUW can provide protection in both ischaemic and reperfusion phase and reduce the risk of haemorrhage.

Neurotrophins are a family of growth factor proteins including BDNF, NGF, and GDNF. They are important in neuronal development and function and are therapeutic options for brain injury. NGF has demonstrated neuroprotection following neonatal rat hypoxia-ischaemia. GDNF has shown to have neuroprotective effects following ischaemic brain injury when introduced to the brain by viral vectors or GDNF-expressing cells. BDNF is a promising therapeutic candidate.[4] These growth factors potently prevent cell death and stimulate neuronal function.[5] Our results showed that GUW increased the neurotrophins' gene expression that provided neuroprotection effect against ischaemic damage.

Together with the anti-oxidant, anti-MMP, and enhancing neurotrophin effect, GUW treatment reduced brain infarct volume. Histological results showed an increase in tissue integrity in the ischaemic region. Thus, rats subjected to GUW treatment showed improvements in neurological deficit score.

Conclusion

GUW has treatment potential on cerebral ischaemia. Intravascular administration of tPA is well tolerated with oral administration of Gastrodia-Uncaria water extract, which reduces the risk of tPA-induced intracranial haemorrhage.

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Disclosure

The results of this research have been previously published in:

1. Xian JW, Choi AY, Lau CB, Leung WN, Ng CF, Chan CW. Gastrodia and Uncaria (tianma gouteng) water extract exerts antioxidative and antiapoptotic effects against cerebral ischemia in vitro and in vivo. Chin Med 2016;11:27.
2. Huan T, Xian JW, Leung WN, Li L, Chan CW. Cerebrospinal fluid metabolomics after natural product treatment in an experimental model of cerebral ischemia. OMICS 2016;20:670-80.

References

1. Xian JW, Choi AY, Lau CB, Leung WN, Ng CF, Chan CW. Gastrodia and Uncaria (tianma gouteng) water extract exerts antioxidative and antiapoptotic effects against cerebral ischemia in vitro and in vivo. Chin Med 2016;11:27.
2. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. Neuron 2010;67:181-98.
3. Zhang Y, Wang Y, Zuo Z, et al. Effects of tissue plasminogen activator timing on blood-brain barrier permeability and hemorrhagic transformation in rats with transient ischemic stroke. J Neurol Sci 2014;347:148-54.
4. Larpthaveesarp A, Ferriero DM, Gonzalez FF. Growth factors for the treatment of ischemic brain injury (growth factor treatment). Brain Sci 2015;5:165-77.
5. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nat Rev Drug Discov 2011;10:209-19.