The possible role of advanced glycation endproducts (AGEs) in experimentally-induced ischemic brain damage.

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Abstract

Stroke is the second leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide. Advanced glycation endproducts (AGEs) are known to be increased in several chronic diseases and induce inflammation and protein intercalation. The only drug approved for the treatment ischemic stroke is recombinant tissue plasminogen activator (r-TPA). The current study was designed to investigate the protective effect of benfotamine (70 mg/kg/day), perindopril (2 mg/kg/day) and alagebrium (2 mg/kg/day) on experimentally-induced ischemic brain damage. All drugs were administered daily for one week before and 2 days after middle cerebral artery occlusion (MCAO). Benfotamine, perindopril and alagebrium ameliorated the deleterious effects of MCAO as indicated by the improvement in the performance of the animals in initiation of walking test and improvement of the deteriorated brain histology. This was associated with normalization of the level of AGEs that was increased following MCAO. The result of the current study represent a new indication for benfotamine, perindopril, alagebrium in the management of ischemic stroke.

1. Introduction

Stroke is the second leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide (Barakat et al., 2013). Despite advances in understanding the pathophysiology of cerebral ischemia, therapeutic options for acute ischemic stroke remain very limited (Woodruff et al., 2011). Theoretically, acute occlusion of the cerebral arteries results in immediate loss of oxygen and glucose to the core region of the affected brain tissue (Eltzschig et al., 2011), hence, a complex cascade involving a series of biochemical reactions is rapidly activated that includes inflammatory mechanisms causing further cell death and functional deficits (Lakhan et al., 2009).

Advanced glycation endproducts (AGEs) accumulate as permanent adducts and cross-linking structures on long-lived body proteins as a function of age and glucose concentration (Makita et al., 1994). AGEs have been implicated in the development of diabetic complications such as accelerated atherosclerosis, renal dysfunction, and neuropathy (Vlassara et al., 1994).

Vascular wall stiffness and endothelial dysfunction may be linked to the formation of advanced glycation endproducts (AGE) that alter vascular structure and function (Basta et al., 2004). AGEs receptor-mediated responses mediate capillary leakage, cytokine production, enhanced procoagulant activity on the endothelial surface, and increased generation of reactive oxygen intermediates (Vlassara et al., 1994)(Yan et al., 1994)(Vlassara et al., 1992).

A variety of ligands, including, high mobility group box 1 (HMGB1), and S100 protein, bind to RAGE (Kim et al., 2005), leading to the activation of several
intracellular inflammatory pathways as nuclear factor-κB (Lotze et al., 2005). RAGE-ligand interactions are implicated in the development and progression of various disorders including vascular diseases (Kalea et al., 2009).

Therefore, targeting the AGEs-RAGE axis is promising to treat several diseases. Benfotiamine is a synthetic derivative of vitamin B1 which was recently shown to block AGEs formation (Balakumar et al., 2010) that lead to several diabetic complications (Hammes et al., 2003). Perindopril is a long-acting, ACE inhibitor used as anti-hypertensive agent (Yamada et al., 2011). Perindopril was shown to increase the plasma level of the soluble decoy receptor sRAGE (Forbes et al., 2002 and 2005) which acts as a decoy receptor to compete with RAGE for ligand binding and consequently blocks RAGE-associated intracellular signaling (Kalea et al., 2009).

Advanced glycation crosslink breakers, such as alagebrium chloride (ALT-711), have been previously shown to reduce vascular stiffening in aged and hypertensive experimental models (Vaitkevicius et al., 2001).

The current study was designed to investigate the possible effect of agents that decrease AGEs signaling (benfotiamine that reduces AGEs formation, perindopril which increases sRAGE level or alagebrium which cleaves AGEs-induced protein crosslinks) on brain damage induced in mice by MCAO.

2. Materials and methods
2.1. Animals
Pulb C mice (18–24 g) purchased from Theodor Bilharzs Research Center, Cairo, Egypt were used in all experiments. The mice were kept under standard environmental and nutritional conditions throughout the investigation. All experimental procedures were performed in accordance with the guidelines of the Ethical Committee for Animal Handling at Zagazig University (ECAHZU).

Mice were randomly distributed into 5 major groups (n=10) as follows: Sham operated mice, Ischemic mice, Ischemic mice treated with benfotiamine (100 mg/kg/day), perindopril at a sub-hypotensive dose (1 mg/day), or alagebrium (20 mg/kg/day). All treatments were started 7 days before MCAO and continued for 2 days later then the animals were sacrificed.

2.2. Induction of ischemia
Middle cerebral artery occlusion, (MCAO) was performed according to the method described previously (Barakat et al., 2009)( Neubert et al., 2011). After 48 h, the animals were anesthetized, and perfused with 50 ml Ringer's solution through the heart.

2.3. Behavioral test
Mice were assessed for the latency time to move before and 48 hours after MCAO. The experiment was performed 3 times and the mean-time was calculated (Hattori et al., 2000).

2.4. Determination of histological changes following MCAO and treatment using Hematoxylin and Eosin (H & E) staining
The brain was carefully isolated then stored at –80 °C. 20–30 µm sections were prepared using cryostat (SLEE, Mainz, Germany). The whole brain was cut from olfactory bulb to the cerebellum. The distance between sections was 400 µm. Brain sections were used for the determination of infarct volume using H & E staining as described previously (Lillie et al., 1965)(Avwioro et al., 2011).

2.5 Determination of AGEs level
AGEs level was quantified using ELISA kits supplied by Cell Biolabs, Inc. OxiSelect™.

2.6 Statistical analysis
Data are expressed as mean ± standard error of the mean. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by
Tukey's post Hoc test using Graph pad Prism software version 5. For all analysis, the level of statistical significance was set at p<0.05.

3. Results

3.1 Effect of MCAO and treatment with benfotiamine (70mg/kg/day), perindopril (2mg/kg/day) and alagebrium (2mg/kg/day) on behavior:

Fig. 1 shows the initiation of walking test results, sensorimotor deficits were induced by MCAO. Ischemic mice were significantly slower than pre-ischemic to start walking (3.37 vs zero seconds). Treatment with benfotiamine, perindopril and alagebrium, significantly improved the time needed for the mice to initiate walking compared with ischemic reaching, 0.96, 0.81 and 0.88 vs 3.37s, respectively.

![Graph](image_url)

**Fig.1.:** Effect of MCAO and treatment with benfotiamine, perindopril and alagebrium on Performance in initiation of walking test.
Data are expressed as mean ± S.E.M., n=10.
* Significantly different from the sham group,
# Significantly different from the ischemic group at P<0.05 using ANOVA followed by Tukey's post Hoc test.

3.2 Effect of MCAO and treatment with benfotiamine (70mg/kg/day), perindopril (2mg/kg/day) and alagebrium (2mg/kg/day) on brain histology:

Mice subjected to MCAO showed histological changes in the brain that was evidenced by multiple foci of leukocyte infiltration and large areas of edema. Treatment with benfotiamine, perindopril and alagebrium improved the histology of the brain compared to the ischemic group, where benfotiamine showed few aggregates of inflammatory cells mainly lymphocytes between the ganglion cells and neurofibrillary material, while, perindopril showed mild areas of edema around the ganglion cells and few pyknotic nuclei of ganglion cells tissue and alagebrium showed foci of infiltration within the brain tissue as shown in fig 2.
3.3 Effect of MCAO and treatment with benfotiamine (70mg/kg/day), perindopril (2mg/kg/day) and alagebrium (2mg/kg/day) on AGEs level:

After MCAO, the expression of AGEs was significantly increased reaching 6.13 vs 0.84 compared to sham group, while treatment with benfotiamine, perindopril and alagebrium caused a significant decrease in AGEs expression compared to the ischemic group reaching 1.82, 1.37 and 1.68 vs 6.13 respectively as shown in Fig. 3.

<table>
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<tr>
<th>Treatment</th>
<th>Fig 2: Effect of MCAO and treatment with benfotiamine, perindopril and alagebrium on brain histology using H &amp; E staining (400X).</th>
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<td>Sham</td>
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**Fig 3: Effect of MCAO and treatment with benfotiamine, alagebrium, perindopril on AGEs level.**

Data are expressed as mean ±SE, n=10.

* Significantly different from the sham group,

# Significantly different from the ischemic group at P<0.05 using ANOVA followed by Tukey's post Hoc test.

### 4. Discussion

Few neurological conditions are as complex and devastating as stroke (Moskowitz et al., 2010). Currently, thrombolysis with tissue plasminogen activator (t-PA) is the only effective therapy, but due to its narrow therapeutic window and safety concern; fewer than 5% of stroke patients receive this treatment (Woodruff et al., 2011). It is crucial to expand the narrow therapeutic opportunities for this devastating condition.

Previous studies have shown sensorimotor dysfunction following MCAO (Tu et al., 2010). In this study, ischemia was associated with delayed performance in the initiation of walking test which indicates a deficit in neurological function of the ischemic animals.

In this study, treatment of mice with benfotiamine, perindopril, and alagebrium showed a significant decrease the time of initiation of walking.

Accumulation of advanced glycation endproducts (AGEs) have been implicated in the development of diabetic complications such as accelerated atherosclerosis, renal dysfunction, and neuropathy (Brownlee et al., 1988; Vlassara et al., 1994).

High levels of AGEs may enhance tissue damage by binding to AGE-specific receptors present on macrophages, endothelial cells, and other cell types (Kirstein et al., 1992; Schmidt et al., 1994).

AGE-mediated tissue damage may also occur when circulating AGE-proteins or AGE-peptides react directly to covalently cross-link with basement membrane proteins in the subendothelial space (Vlassara et al., 1994). AGE-modified proteins or peptides are neurotoxic factors that amplify the volume of permanent damage and necrosis following focal cerebral ischemia. It was previously shown that administration of AGEs converted a typically small cerebral infarction following MCAO into a significantly larger stroke (Zimmerman et al., 1995).

In this study, ischemic group showed significant increase in AGEs level compared to sham group. However, treatment of ischemic mice with benfotiamine, perindopril and alagebrium caused
significant decrease in AGEs level compared to ischemic group.

Angiotensin-converting enzyme (ACE) inhibitors as perindopril, have been found to lower BP without decreasing cerebral blood flow in patients with hypertension (Minematsu et al., 1987), ischemic stroke (Dyker et al., 1997), and (Hatazawa et al., 2004), and carotid stenosis or occlusion (Walters et al., 2001).

Alagebrium can break carbon–carbon bonds and impair AGE-formation and has important implications for treating age related cardiovascular dysfunction (Kass et al., 2003). Studies have demonstrated enhanced cardiac compliance and cardiac output (Asif et al., 2000) and improved arterial compliance (Kass et al., 2001) following treatment with Alagebrium.

After stroke there is a breakdown of BBB with an associated increase in vascular permeability which often results in hemorrhage and edema, resulting in neuronal cell death (Rosenberg, 1995).

In the present study, mice subjected to MCAO showed significant brain edema associated with deterioration in brain histology. These results confirm the results obtained by (Kahle et al., 2009).

Leukocytes are responsible for the progression from tissue ischemia to cerebral infarction (Lipton et al., 1999; Frijins and Kappelle et al., 2002; Huang et al., 2006). Huang et al., (2006) found that arterial occlusion upregulates the expression of cytokines as IL-1, and IL-6 that act on the vascular endothelium to increase the expression of adhesion molecules which promote leukocyte adherence and accumulation. These inflammatory signals then promote leukocyte transmigration across the endothelium and mediate inflammatory cascades leading to further cerebral infarction.

In the current study, mice subjected to MCAO showed significant leukocyte infiltration in the ipsilateral hemisphere while sham operation did not show leukocyte infiltration. These results are in harmony with those obtained by (Tu et al., 2010) who found that MCAO caused a significant increase of neutrophil infiltration in infarcted brain tissue.

On the other hand, treatment with benfotiamine, perindopril and alagebrium reduced brain edema and leukocyte infiltration and improved brain tissue histology compared to the ischemic group.

Conclusion

In the light of the present study, it could be concluded that AGEs play an important role in mediating ischemic brain injury and agents that hinder AGEs pathway as benfotiamine, perindopril and alagebrium suppressed AGES expression and improved the outcome of ischemic brain damage as indicated by the improved performance of animals in the latency to walk test and brain histology. Therefore, such agents might be useful in the treatment of stroke following suitable clinical trials.

References


The potential role of advanced glycation end products (AGEs) in the pathophysiology of human ischemic stroke.