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"Combination of LT-12 and TM-03 drugs is a Highly Effective Treatment of Stroke and Cerebral Palsy: From Experimental to Clinical Studies"

By

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Abstract:

Stroke and cerebral palsy are common ischaemic brain problems in adult and neonate over the world. Both constitute one of the most devastating neurological disorders and are the third leading cause of death in developing countries. The non-availability of data about stroke and cerebral palsy has limited research output and both are underestimated by government and health ministry in developing countries. In this study, the albino rat`s brain was subjected to ischaemic injury (oxygen & glucose deprivation (OGD) using optic nerves. The brain of both adult and neonatal rats was subjected to ischaemia for a period of time (2 hrs.). The function of the brain was monitored before, during and after ischaemic insult where the drugs are administered by using electronmicroscopy (recording the compound action potential (CAP)). Optic nerves lost their function significantly following OGD in-compare to control in both adult and neonate. Administration of 1µM LT-12 and 100 µM TM-03 each of them alone has very high protection against ischaemic injury in both adult and neonatal rats. Combination of 1µM LT-12 and 100 µM TM-03 lead to highly significant and nearly complete protection against brain ischaemia in both adult and neonate. The last result was confirmed by using electron microscopy for structural changes before and after administration of 1µM LT-12 and 100 µM TM-03 together in albino rats. The brain structures of albino rats were reversed to normal after administration of both drugs together. The result indicating that the combination of 1µM LT-12 and 100 µM TM-03 has a complete protection against brain ischaemia in both adult and neonates.

Keywords: Brain (RONs), ischaemia, adult and neonatal rats (albino species), LT-12 & TM-03.

Introduction:

Stroke is the third leading cause of death and long term disability worldwide (1). The universal burden of stroke is large, yet there are still gaps in our knowledge (2). Stroke is characterized by fast appearance of clinical symptoms and signs from local to total loss of cerebral function lasting more than 24 hours or leading to death, with apparent cause of vascular origin (2). Stroke can occur at any age, but commonly occur in older people (3). There is two types of stroke, ischaemic stroke (most common) and haemorrhagic stroke. Mortality rate from haemorrhagic stroke declined consistently over the 20th century in many countries, but ischaemic stroke showed a fluctuation, reflecting vascular insufficiency (4). These differences indicate that the risk factors for the two subtypes of stroke might differ. Ischaemic stroke is a disease of multifactorial, but most commonly due to loss of oxygen and glucose in the brain that cause over production of different neurotransmitters such as glutamate and other neurotransmitters than glutamate that lead to over activation of their receptors and initiation of excitotoxicity (5). Two main neurotransmitters in the brain, excitatory and inhibitory (glutamate and gammaaminobutyric acid (GABA), respectively) are involved in the pathogenesis of ischaemic injury. Several experimental studies have revealed that the GABA concentration increases during ischaemic conditions in correlation with an increase in the concentration of glutamate; this indicates that brain ischaemia is associated with an increase in both excitatory and inhibitory amino acids (6). The increase in GABA concentration might be due to an increase in glutamate that leads to neuronal damage and consequently to an elevation in GABA, which plays a role in the pathogenesis of ischaemic injury. The receptors of these neurotransmitters have recently been demonstrated experimentally in both adult and neonatal brain (7). Activation of glutamate and GABA receptors during ischaemia aggravate excitotoxic injury in the brain of both adult and neonate. On the other hand, blocking of glutamate and GABA receptors significantly produces

neuroprotection against ischaemic injury in the brain (7). Recent advances in the treatment of acute ischaemic stroke have focused largely on drug treatments, and yet the number of effective and widely practicable treatments remains restricted.

Cerebral palsy

Cerebral palsy is a form of brain damage that involves the neonatal brain and is the leading cause of permanent neurological disability and death in neonates (8). The pathogenesis of cerebral palsy involves several interacting factors that can include maternal infection with the overproduction of cytokines, and inflammation (9), (10). Perinatal ischaemia is thought to be the primary cause of neurological disability and death in most cases (11). During ischaemia, different neurotransmitters level is increased in the neonatal brain, which leads to over activation of their receptors. The over-activation of these receptors causes excitotoxicity inside the cells, leading to the damage of brain structures and even death (10). However, the most efficacious neuroprotective strategies in treating cerebral palsy remain a matter of debate, while understanding the pathophysiology in neonatal brain will progressively lead scientists toward the further development of effective therapeutic interventions in the future (12).

Aim of the study

Stroke is increasingly becoming a challenging public health issue in developing countries, and it becoming one of the major causes of death and disability-adjusted life years. The higher number of death and disabilities due to stroke in developing countries suggests that the burden of stroke in those countries is still increasing and the treatment strategies are declining dramatically. As the prevention of stroke is a duty facing scientists and researchers, we reached to a good result concerning protection against stroke and cerebral palsy in experimental animals. In view of that, we are keen and enthusiastic in translating our results under clinical trials in Libya.

Methodology:

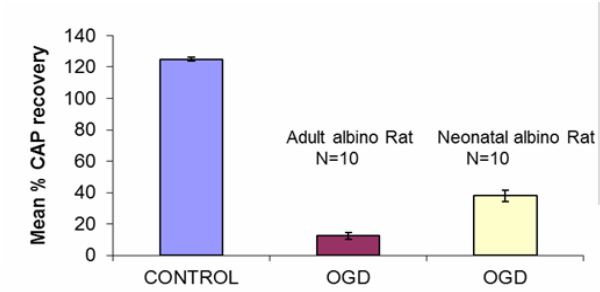
The study was done at research centre-faculty of medicine, Benghazi University. Albino rats (adult & neonate) were used in this study. Electrophysiology technique was used for measuring the function of optic nerves by recording the action potential during nourishment with normal condition [normal artificial cerebrospinal fluid (aCSF) and 95% O_2/ 5% CO_2], during ischaemia (95% N2 / 5% CO_2 mixture from which glucose was absent) for (2hrs) in adult neonatal rats, and during administration of 1µM LT-12 and 100 µM TM-03 to solution for (2hrs) either alone or in combination. Ultra structural changes of the brain were monitored by using electronemicroscopy during

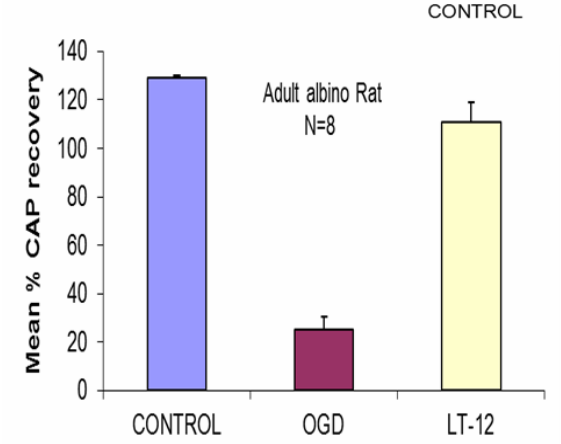
administration of 1µM LT-12 and 100 µM TM-03 together. Data are expressed as mean ± SEM, significance determined by *t*- Test or ANOVA as appropriate.

Result

Axonal function was measured in both adult and neonatal albino rats by recording the action potentials that remain stable for more than 2 hrs (116.79 ± 2.5%, n= 10 and 122.9

± 1.5%, n= 10) respectively. During the period of OGD, the axonal function lost in both adult and neonatal albino rats and reached to (12 ± 5.7%, n= 10 & 38 ± 7.8%, n= 10, respectively). Administration of LT-12 (1µM) during OGD time significantly protects the axonal function of both adult and neonate (108 ± 4.6%, n= 8 ((P<0.001 vs OGD)) & 56.02 ± 5.5%, n= 8 ((P<0.01 vs OGD)), respectively). Administration of TM-03 (100Mm) during the time of OGD has a significant protection against axonal injury in both adult and neonate (40.5 ± 5%, n= 8 ((P<0.01 vs OGD)) & 82.2 ± 11.3%, n= 8 ((P<0.001 vs OGD)), respectively). Combined administration of LT-12 (1µM) & TM-03 (100Mm) during the time of OGD has highly significant protection of axonal function in adult and neonatal albino rats ((P<0.0001 vs OGD)); (100 ± 5.6%, n= 8 & 120.1 ± 4.5%, n= 8, respectively); as shown in (*figure 1)*. Under electronmicroscopy, the axons were identified by the presence of neurofilaments and microtubules. Glial cells contain observable mitochondria, Golgi apparatus, endoplasmic reticulum and glycogen granules. The viability score" 3'' correlates to no pathology in both axons and glial cells. Axonal viability scores that measured blindly were significantly low during OGD (mean= 1.5 ± 0.4; P<0.0001 vs control) and glial cells viability scoring were low during OGD (mean= 1.0 ± .010; P<0.001 vs. control). Combination of both LT-12 (1µM) & TM-03 (100Mm) during the time of OGD significantly preserves the normal structures of axons and glial cells (mean = 2.8 ± 0.06; P<0.0001 vs. OGD) and (mean= 3 ± 0.02 P<0.0001 vs. OGD) respectively; as illustrated in *(figure 2).*


Mean % CAP recovery
140
120
100
80
60
40
20
0
Adult albino Rat
N=10
Neonatal albino Rat
N=10
CONTROL
OGD
OGD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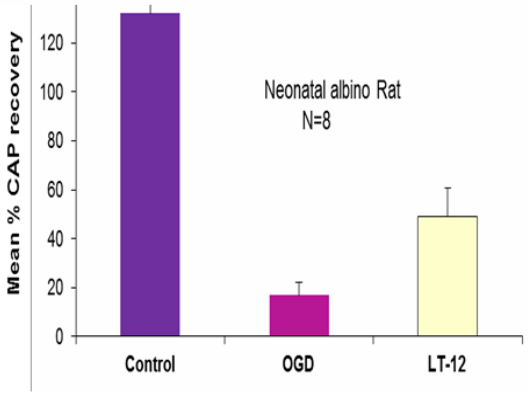
120
100
80
60
40
20
0
Adult albino Rat
N=8
CONTROL
OGD
LT-12


Mean % CAP recovery
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60
40
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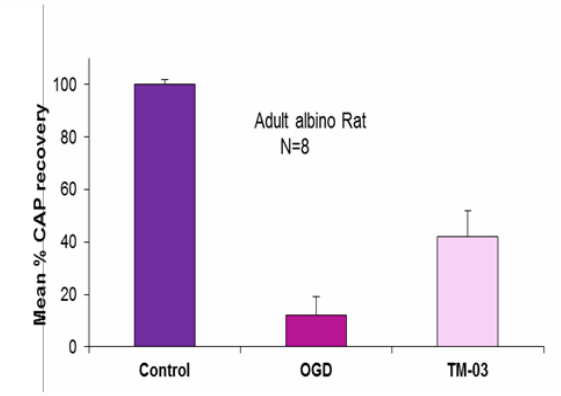
Neonatal albino Rat
N=8
Control
OGD
LT-12


Mean % CAP recovery
100
80
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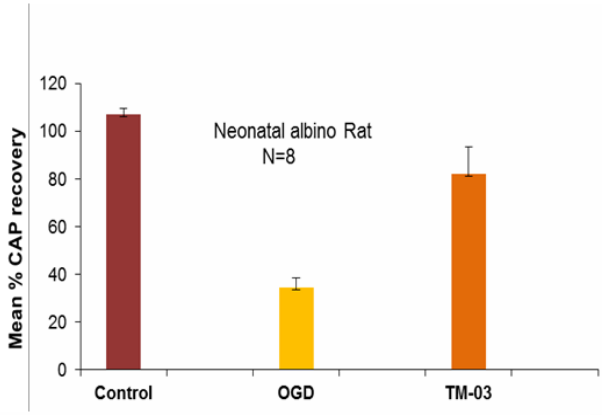
Adult albino Rat
N=8
Control
OGD
TM-03


Mean % CAP recovery
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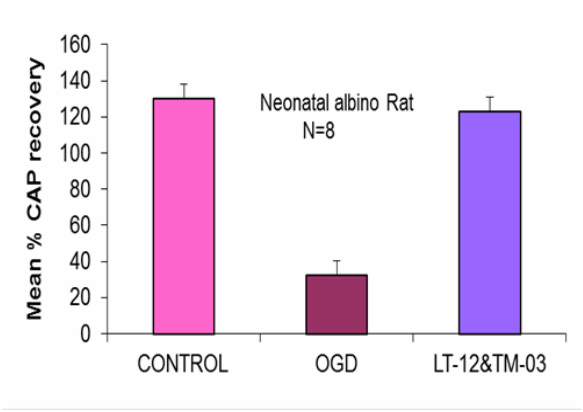
80
60
40
20
0
Neonatal albino Rat
N=8
Control
OGD
TM-03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120
100
80
60
40
20
0
Neonatal albino Rat
N=8
CONTROL
OGD
LT-12&TM-03

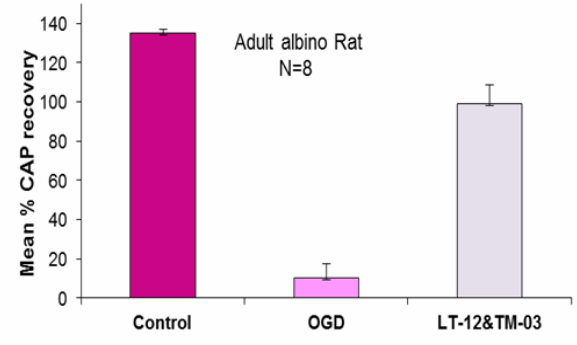
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Figure-1- Illustrated the mean% compound action potentials in both adult and neonatal albino rats during control, OGD & treatment with TL-12& TM-03 during time of OGD. Error bars are SEM.

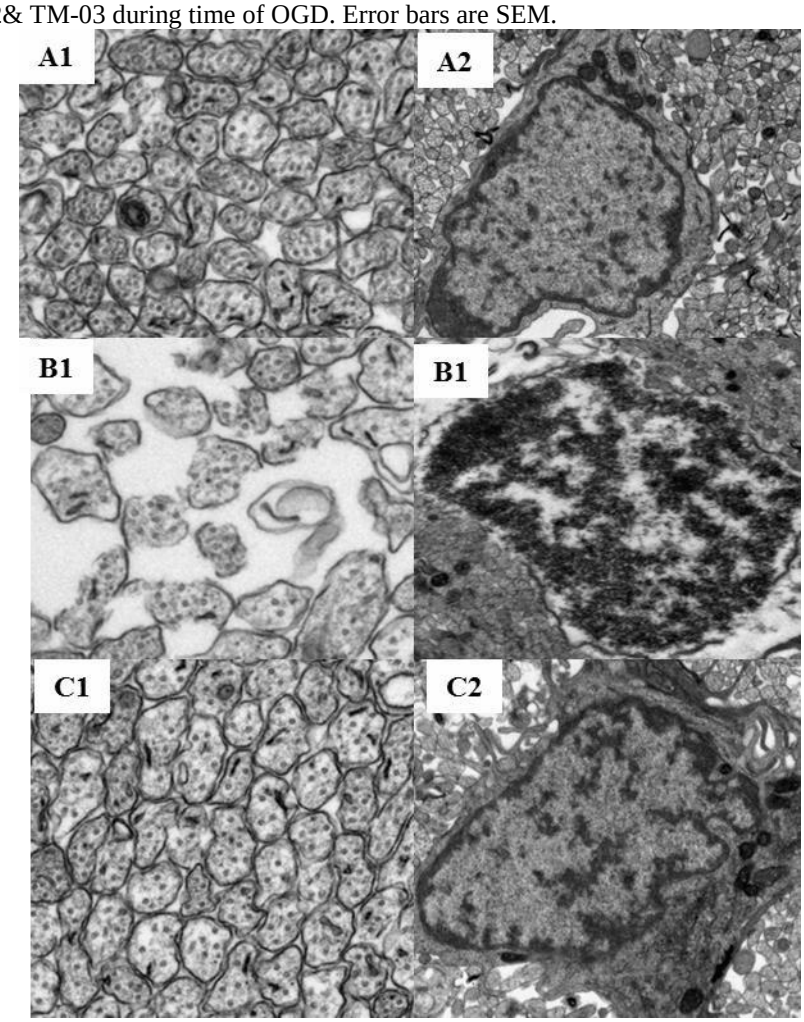

Figure 2- Illustrated ultra-structural changes of the brain. A1, B1 &C1: Normal axons; axonal swelling, degeneration with loss of axons during OGD; & axons were significant protected by co- administration of TL-12& TM-03 during OGD, respectively. **A2, B2 &C2**: Normal glial cells; glial cells swelling with damage of membrane integrity; & glial cells were significantly protected by co-administration of TL-12& TM-03 during OGD, respectively.

Discussion

The pathogenesis of brain damage by ischaemia in both adult and neonate has become a challenge to scientists and researchers nowadays. Axonal and glial cells damage that induced by withdrawal of oxygen and glucose indicating that brain structures are more vulnerable to ischaemia. This is consistent with the findings of previous studies that hypoxia-ischaemia causes injury to axons as well as glia *in-vivo* and *in-vitro* (13), (14). Axonal and glial injury during energy deprivation is not solely mediated by glutamate and its receptors; it is clear therefore that other neurotransmitters and

their receptors participate in brain injury during ischaemia. The pathophysiology of brain ischaemia was investigated electrophysiologically in this study using albino rats. Correspondingly; the same result has been confirmed by using electronmicroscopy. It was found that administration of both TL-12 and TM-03 has a protective effect, while combination of TL-12 and TM-03 significantly restoring the brain function and structures. Both produced a large protection against ischaemic injury which is exhibited by significant recovery in the CAP. They also reversed the lower of axons and glial cells viability scoring during ischaemia. The results of both electrophysiology and microscopy are consistent with a potential role of different neurotransmitters mediated injury in the brain. Constantinou and Fern, 2009, stimulated some types of neurotransmitter receptors initially and then exposed these receptors to the neurotransmitter blockade in neonates and they found that different types of neurotransmitters other than glutamate are participating in brain ischaemia. The current study was investigating the role of neurotransmitters and their receptors in brain injury in adult and neonates by administration of TL-12 and TM-03 during the time of ischaemia. **In conclusion**, brain structures are vulnerable to ischaemic insult in both adult and neonate. Brain injury and excitotoxicity during ischaemia is mediated by different types of neurotransmitters. In addition a specific antagonist of each receptor is highly protective. Therefore, a deeper knowledge of the mechanisms leading to brain injury and excitotoxicity will facilitate new pharmacological strategies in the treatment of brain disorders in adult and neonate. Likewise, these findings are consisting with various studies in experimental animals. **Future experiments:** we are in the process of using these drugs in controlled clinical trials at Benghazi Medical Centre, in Benghazi-Libya.

Volunteers from adult and old stroke patients already signed their consent to enrol in the study. Also, parents of children with cerebral palsy are interested in such clinical trial to test TL-12 and TM-03.

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