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Potential Protective effect of Vitamin C on Cerebral Ischaemia Reperfusion Injury in Rats

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ABSTRACT

Cerebral Ischaemia/reperfusion injury(I/RI)is a common cause of brain damage. The use of antioxidants has beneficial effects in I/RI, as they protect against brain damage by inhibiting reperfusion injury. The present study was conducted to investigate the role of vitamin C in the protection against cerebral I/RI, induced in rats and its effect on biomarkers of oxidative and nitrosative stress, and on markers of inflammation. Male rats were divided into six groups: The first was sham operated group, the second group was I/RI and the last four received intravenous (IV) vitamin C in doses 50 and 100mg/kg before and after I/RI. Reduced glutathione, malondialdehyde, nitric oxide, tumour necrosis factor alpha and interleukin one beta were measured in brain homogenates. Results showed that both doses of vitamin C given before and after I/RI induction had protective effect on brain tissue against I/RI

Keywords: Ischaemia, Reperfusion, Vitamin C, Brain, Oxidative, Nitrosative, Inflammatory.

INTRODUCTION

Stroke is the most common cause of chronic disability among adults. Cerebral Ischaemia/reperfusion (I/R) injury can lead to brain damage, leading to sensory and motor impairment [1]. I/R is the restoration of blood flow to the tissue that was previously deprived of blood flow [2]. Thrombolysis with recombinant tissue plasminogen activator (rtPA) is the only approved treatment for stroke for in Europe and North America ,also reperfusion is currently considered as a method of protection to the brain against ischaemic damage[3].However, reperfusion is risky, as it may cause hemorrhagic transformation and fatal edema [4]. Tissue damage after I/R is known as I/R injury (I/RI). Early detection of post-ischaemic damage associated with reperfusion injury in the brain is elusive[2].

I/RI is a complex disorder that produces molecular and cellular damage such as oxidative stress damage and inflammatory dysfunction. Cerebral I/R induces oxidative stress due to the generation of free radicals. After I/R process, during restoration of oxygen and glucose supply to tissues, the oxidative phosphorylation that takes place to neutralize energy requirements, leads to destructive biochemical processes that antagonizes the beneficial effect of reperfusion. That's why the use of antioxidants has beneficial effects in I/RI, as they may protect against brain damage by inhibiting reperfusion injury [5].

Vitamin C, or ascorbic acid (AA), is an important antioxidant which inspite of circulating in plasma in micromolar concentrations, reaches millimolar concentrations in most tissues. The central nervous system (CNS) contains the highest ascorbic acid concentration in mammalian tissues. L-ascorbic acid is an essential antioxidant for scavenging free radicals in brain tissues. Moreover intracellular ascorbate exerts several functions in the CNS which include peptide amidation, myelin formation and synaptic potentiation. AA participates both in sustaining the normal function of the CNS and ameliorating the damage caused by pathological conditions that increase the production of reactive oxygen species (ROS)[6].

The present study was conducted to investigate the role of vitamin C in the protection against cerebral ischaemia-repefusion injury (I/RI), induced in rats and its effect on biomarkers of oxidative and nitrosative stress, and on markers of inflammation .

MATERIALS AND METHODS

I.1Materials:

II.1.a)Animals:

Male Wister albino rats, weighing 250-280g were used throughout the experiments. The animals were obtained from the animal house colony of the National research centre, Dokki, Giza, Egypt. The animals were housed in standard metal cages in an air conditioned room at 22 ± 3˚C, 55 ± 5% humidity and provided with standard laboratory diet and water ad libitum. Experiments were performed between 9:00 and 15:00 o'clock. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and the animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory animals.

II.1.b)Chemicals:

Vitamin Campoules were purchased from *Memphis Pharmaceutical & Chemical Industry, Egypt*, and each ampoule of 5 ml contains ascorbic acid (Vitamin C) 500 mg . Kits For measurement of reduced glutathione (R-GSH), malondialdehyde (MDA), nitric oxide (NO) were purchased from *Bidiagnostic company , Egypt.* Elisa Kits for measurement of Tumour necrosis factor alpha (TNF-α) and Interleukin one beta (IL1β) were purchased from *Leader Trade company, Egypt.*

II.2.Methods

II.2.a)Animal grouping:

Rats were divided into 6 groups: First group was sham-operated rats , second group didn't receive vitamin C where left common carotid artery occlusion (CCAO) was done followed by removal of ligature to induce cerebral ischaemia /reperfusion injury, third and fifth groups received Vitamin C (50 and 100mg/kg) by intravenous injection route (IV) half an hour before occlusion, fourth and sixth groups received Vitamin C (50 and 100mg/kg) IV just after removing the ligature. Vitamin C was injected slowly.

II.2.b) Induction of Cerebral Ischaemia:

Animals were kept fasting for 12 hours before surgery. All animals were anesthetized with thiopental (50mg/kg). A longitudinal cervical incision (2cm) was made lateral to the midline and the common carotid artery (CCA) was carefully dissected. Ischaemia was induced by placing non traumatic micro vascular clip on the left CCA just prior to its bifurcation[7]. During Ischaemia rats were monitored for body temperature which was constant at 36.5±0.5˚C using heating pad and respiration pattern. The vascular occlusion was maintained for 30 minutes, and then the clips were removed to resume blood flow to the ischaemic region to induce I/RI[8]. Finally, the incisions were sutured, the animal was allowed to recover from anesthesia, and returned to a warm cage for recuperation during reperfusion period for twenty four hours before being sacrificed[9].

II.2.c)Measurement of Biochemical Parameters:

After twenty four hours, the rats were sacrificed. Brains were rapidly removed, then 0.5gm of affected hemisphere was homogenized, the homogenate was centrifuged; the supernatant was taken for the determination of brain level of MDA, estimation level of the NO_x metabolites, R-GSH, TNF-αand IL1β according to the methods adopted by [10-15]respectively.

RESULTS AND DISCUSSION

IV.1)Effect of vitamin C on oxidative and nitrosative stress:

Free radical oxygen has potential role in nerve cell damage in cases of I/RI. Brain tissue is susceptible to oxidative stress due to high level of free radicals and unsaturated fatty acids, as well as low protective antioxidant capacity [16]. Also reperfusion causes apoptosis and delayed death of cells through oxidative damage to lipids, proteins, and DNA in the ischemic region [17]. Also previous research demonstrated that nitric oxide synthase (NOS) utilizes L-arginine as the substrate during cerebral I/R to produce nitric oxide (NO) and thus leads to neuronal injury [18].

The previous reports provide an explanation to the results of our study, as our results revealed a significant decrease in R-GSH and increase in MDA and NO_x levels in ischaemia/reperfusion injury (I/RI) rats' brains (induced by CCAO), when compared to sham operated rats' brains.

Also the effect of vitamin C in our study is in agreement with the results obtained in the study done by Iwata et al (2014) [6]. Where IV injection of vitamin C in our study in dose of 50mg/kg before and after I/RI induction and 100mg/kg only before I/RI inductionsignificantly increased the R-GSH level in brain when compared to theI/RI group. This is because Vitamin C reacts with free radicals and deactivates them before they cause damage to proteins or lipids, it acts as a hydrogen donor leading to reversible oxidation and reduction [19].Yet in our study, the R-GSH level in all treated groups, was significantly less and the level of NO_x in the brains of group treated with vitamin C (50mg/kg) before and after I/RI induction was significantly higher than the sham operated group, but there were significant reductions in MDA and NO_x levels in brain homogenates when compared to the I/RI group.(Table 1).

Table 1: Effect of vitamin C on R-GSH, MDA and NO_x levels in brain tissue in cerebral schaemia/reperfusion injury model in rats.

Groups	R-GSH (ug/g tissue)	MDA (nmol/g tissue)	NO_x (ug/g tissue)
Sham operated	45.65 ± 0.36 *	19.48 ± 0.87 *	0.28 ± 0.02 *
Ischaemia/reperfusion injury(I/RI)	42.05 ± 0.14 #	64.6 ± 4.08 #	0.67 ± 0.05 #
50 mg/kg before	43.81 ± 0.37 *#	20.83 ± 0.44 *	0.47 ± 0.04 *#
50 mg/kg after	43.17 ± 0.40 *#	22.07 ± 1.55 *	0.50 ± 0.05 *#
100 mg/kg before	44.00 ± 0.30 *#	13.52 ± 0.61 *	0.27 ± 0.02 *
100 mg/kg after	42.98 ± 0.20 #	19.34 ± 0.43 *	0.43 ± 0.03 *

*Results are expressed as mean levels of GSH,MDA and NOx in brain homogenate ± SE.,N=8., p < 0.05.*Significantly different from Ischaemia/ reperfusion injury(I/RI) group, # Significantly different from sham operated rats.*

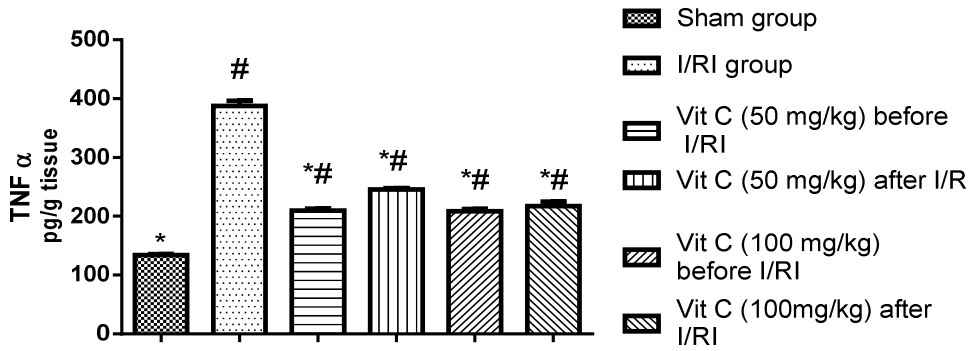

Figure 1. Effect of vitamin C on TNFα level in brain tissue in ischaemia/reperfusion injury (I/RI) rats.
Results are expressed as mean levels of TNFα in brain homogenate ± SE,N=8,
*p< 0.05, *Significantly different from I/RI group, # Significantly different from sham operatedgroup.*

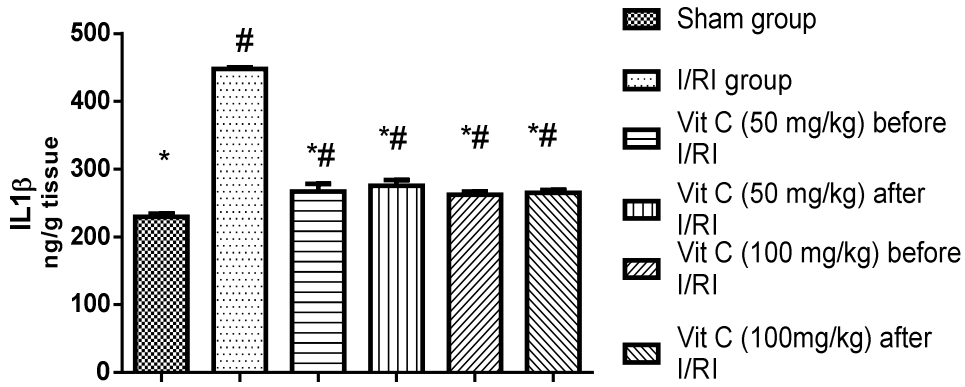

Figure 2. Effect of vitamin C on IL-1β level in brain tissue in ischaemia/reperfusion injury (I/RI) rats.
Results are expressed as mean levels of IL1β in brain homogenate ± SE,N=8,
$p< 0.05$, Significantly different from I/RI group,# Significantly different from sham operated group.*

IV.2)Effect of vitamin C on inflammatory biomarkers:

Ischaemia/reperfusion leads to inflammatory neuro-degeneration leading to increase of TNF-α and IL-1β levels which contributes to cerebral damage after I/R [20]. This was evident in the present study, asI/RI resulted in a significant increase in the inflammatory markers where brain TNF-α level in brains of I/RI group when compared to the sham operated group became (387.6 ± 8.9 *vs.* 133.8 ± 1.8 pg/g tissue)and IL-1β level in brains of I/RI group when compared to the sham operated group brains became (447.9 ± 2.8 *vs.* 229.7 ± 4.4 ng/g tissue). All treatment groups that received vitamin C in doses 50 & 100 mg/kg before and after I/RI, showed a significant decrease in the TNFαlevel (209.6 ± 3.6, 245.6 ± 2.1, 208.5 ± 3.7 and 217.5 ± 7.3pg/g tissue)respectively *vs.*I/RI group (387.6 ± 8.9 pg/g tissue).All Vitamin C treated groups showed a significant decrease in the IL-1β level (267.3 ± 11.35, 275.6 ± 8.5, 262.2 ± 5.1 and 265.2 ± 3.9 ng/g tissue)respectively *vs.*I/RI group (447.9 ± 2.8 ng/g tissue). (Figures 1-2).Our results are in agreement with the results of Abdel-Daim et al(2015) and Yavuz et al (2004) [21-22], who proved that Vitamin C decreased the elevated IL-1β and TNF-α levels and due to its anti-inflammatory and anti-apoptotic activities.

CONCLUSION

Ischaemia /reperfusion injury produces oxidative stress, inhibits protective antioxidant enzymes and may also increase inflammation .On the other hand Vitamin C has a protective effect against I/RI which may be attributed to its antioxidant and anti-inflammatory activities.

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