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Exenatideas Neuroprotectivevia Reducing Inflammation and Oxidative stress in Ischemic/Reperfusion of Adult Animal Rats

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Abstract

Background: Stroke is considered a major cause of death and disability worldwide. The most important mechanisms that lead to stroke are thrombotic occlusion, embolic occlusion, and vascular rupture (hemorrhage). Unfortunately, there has been only one pharmacological agent approved to treats stroke, recombine antalteplase agent(rtPA), and should be used within 4-5 h from onset of stroke with accurate diagnosis. Due to these difficulties, more than 10% of stroke patients were not received rtPA.Exenatide is incretin mimetic agent which mimics the action of the endogenous glucagon like peptide-1(GLP-1). Exenatide is approved as adjunctive therapy for patient with type2 diabetes [1-2].it is injected subcutaneously and the peak concentration reaches approximately within 2 hours with the duration of action 10 hours. Apart from its antidiabetic action, it's playing an important role in the GLP-1 receptors distributed on neuronal tissues. GLP-1 and its receptors had a beneficial role as neuronal protective, anti-inflammatory,and anti-oxidant agent as well as in neuronal learning & memory**,**so, the present study aimed the explore the neuroprotective effect of Exenatide.**Method:** Adult twenty-four Sprague-Dawley rats has been divided randomly into four equal groups. Sham group just undergone anesthesia at same time and condition of other groups. Control which undergone induction of ischemia 30 mints then reperfusion 60 mints. Vehicle group the same control group but differs by injected intraperitoneally the vehicle (1ml/kg of 10% of DMSO) of treatment before 120 mints from reperfusion. Treatment groups the same control group but differs from them by treated intraperitoneallywith (2µg/kg) of Exenatide before 120 mints.**Results:**the induction of ischemia/reperfusion in rats (control group)significantly ($P \leq 0.05$) increased the levels of IL-1β, MMP-9, 8-iso-PGF2α, as well asICAM-1. Exenatide at dose (2µg/kg) significantly ($P \leq 0.05$) loweringthe levels ofIL-1β, MMP-9, 18-iso-PGF2α, as well asICAM-.**Conclusions:**decrease in pro inflammatory agent IL-1β, MMP-9, ICAM-1, and oxidative stress 8-iso-PGF2α in grouptreated with Exenatide drug consider as neuroprotective for cerebral ischemia/reperfusion in male rat model.

Keywords: Exenatide, cerebral I/R, IL-1β, MMP-9, 8-iso-PGF2α, ICAM-1.

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Background

Stroke is considered a major cause of death and disability worldwide. The most important mechanisms that lead to stroke are thrombotic occlusion, embolic occlusion, and vascular rupture (hemorrhage) [1].Worldwide, stroke affects 13.7 million people per year with 5.5 million deaths per year [2].Unfortunately, there has been only one pharmacological agent approved to treats stroke,rtPA, and should be used within 4-5 hoursfrom onset of stroke with accurate diagnosis [3]. Due to these difficulties, more than 10% of stroke patients werenot receivedrtPA [4-5]**.**The pathophysiology of cerebral ischemia-reperfusion is complex and lead to initiate a cascade of events including a reduction in blood flow leading to decreased in glucose and oxygen mismatch the requiredof neurons, glial,and endothelial cells [6]. The inflammatory response triggered after the ischemic event plays an important role in the progression of stroke. Brain injury following stroke is driven by local inflammation, production of reactive oxygen species, and the infiltration of circulating immune cells [7]. Acute inflammation can exacerbate brain injury after ischemic stroke [8]. Thus, there is a need toanew treatmentto ameliorated ischemic stroke or act as protective for susceptible patient to stroke throughout the targetingof these inflammatory agents. Exenatide is incretin mimetic agentwhich mimics the action of the endogenous GLP-1 [9]**.**Exenatide is approved as adjunctive therapy for patient with type2 diabetes [10-12], it is injected subcutaneously and the peak concentration reaches approximately within 2 hourswith the duration of action 10 hours [12]. Apart from its antidiabetic action, it's playing an important role in the GLP-1 receptors distributed on neuronal tissues [13-15]. GLP-1 and its receptors had a beneficial role as neuronal protective, anti-inflammatory,and anti-oxidant agent as well as in neuronal learning & memory [10-11]**,** so, the present study aimedto explore the neuroprotective effect of Exenatide.

Methods

Animals

Twenty-four Sprague-Dawleyadult rats weighing (250-350g), they were purchased from the national center for drug control and research/Baghdad/Iraq. All animals were subjected to same condition and housed in the animal house of Kufa College of Science with controlled temperature(25°±1C) and room humidity (60–65%) as well as alternating 12-hr light/12-hr dark cycles and allowed free accesses to water and chow diet. Finally, the rats distributed randomly into four groups each group has 6 experimental rats as follow [16]:

Group-1:Sham group

This group subjected to same surgical procedure (anesthesia and identicalperiod of time) but excluded the bilateral common carotid artery occlusion (BCCAO) [17].

Group-2: Control group

This group subjected to anesthesia andBCCAO for half hour and one hour for reperfusion without treatment [17].

Group-3: Control-vehiclegroup (C-vehicle)

This group subject to same procedure of the control group in addition tointraperitoneal injection **1 ml/kg**of the vehicle (10 % DMSO) 2 hrs prior to ischemic/reperfusion induction (I/R) [18].

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Group 4: Exenatidetreatedgroup

This group subject to same procedure of the control group in addition tointraperitoneal injection**2 µg/kg**of body weightfrom Exenatide2 hrs prior to ischemic/reperfusion induction [18].

Preparation of Drugand administration

Exenatide(PURE POWDER OF EXENATIDE MEDCHEMEXPRESS, USA)has been prepared immediately via dissolving in a known volume of 10% DMSO(ABU DHABI MEDICAL, UAE)as a vehicle to prepared a stock volume of drug[18].

Induction of brain ischemia

Firstly all animals subjected to anesthesia with ketamine and xylazine (80mg/KG & 5mg/KG) respectively [19]. The procedureof induction of global brain ischemia has been done at 37 °C under light bulb.The anaesthetizes rat fixedin supine position on plat on their back, then we made an incision in its neck and the two common carotid arterieswere carefully isolated and clamped via vascular clamps to induce ischemia. The time ofischemiawas 30 mints and thenwent to reperfusion phase by removal the clamps and leftfor 60 mints and finally decapitation all of experimental rats [17].

Preparation of Sampling Tissue

Isolation of Brain Tissue

After decapitation, the brain has been extracted carefully from skull and washing with ice cold NaCl0.9% solution and putted on ice to facilitated the experimental processes and then divided coronally into 2parts (One-part for ELISA and the other for IHCstains).

Preparation of sampling Tissue for ELISA Technique

The brain tissue was homogenized tomeasuring the following parameters Rat IL-1β (BIOASSAY TECHNOLOGY LAB, CHINA), MMP-9 (RAT MMP-9 ELIZA KITBIOASSAY TECHNOLOGY LAB, CHINA), and8-iso-PGF2α (Rat 8-iso-PGF2 ELIZA Kit, BIOASSAY TECHNOLOGY LAB, CHINA).The homogenization process started by weighinga proper amount of brain and added to a mixture of ice-cold phosphate buffer saline (P^H 7.4 at 1:10 (w/v)), a protease inhibitor cocktail, and 0.2% Triton X-100 [17]. Thismixtures was grindedcarefully and homogenized by Sonicator devise (HIGH INTENSITY ULTRASONIC LIQUID PROCESSOR SONICS & MATERIALS, INC, USA)and then centrifuged by cooling ultra-centrifugation at 14000 Xg for 20 mints/4°C [20]. The supernatant was extracted and stored at -80°C° in deep freeze until it used.

Preparation of Samples for IHC

The isolated part of brain for IHC purposeshas been fixed in 10% formalin for **5 to10** mints and after many processing of tissue dehydration by different graded concentration of alcohol and finally with xylene to perfect cleared from alcohol andthenimmersed in paraffin wax. This way has been takentimes about 20 hr. by automated tissue processor (LEICA, CHINA).Sections at 5µ-thickness were taken, stain according to kit procedure (LEICA BIOSYSTEM FOR STAING KIT, CHINA) and examined under a light microscope by a pathologist unaware of the treatment protocol. The intensityscore ranged from (0-3), where 0 score expressed no-staining, 1score mean weak stain, 2 score mean moderate stain, and 3 score means strong stain, and proportional scorethat ranged as <10% =0 score, 10-25%=1 score, 26-50%=2 score, 51-75%=3 score and >75%=4 score)of each staining slide and then

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obtained the H-score, the result of multiplied the intensity of stain by proportional of cell staining, which ranged from (0-300) [21-22].

Statistical analysis

Statistical analysis has been done by using SPSS version 23. The data were express asmean±standard error of mean. One-way ANOVAfollowed by Turkey's test was used to explore the difference between groups. *P* value ≤0.05 was representing a significant.

Results

Effect of Global cerebral I/R on cerebral IL-1β, MMP-9, 8-iso-PGF2α, and ICAM-1 levels.

In the present study, the levels of cerebral parametersIL-1β, MMP-9, 8-iso-PGF2α, and ICAM-1 in both control and C-vehicle groupswere significantly (P≤0.05)increased compared with sham group,while there was insignificant difference(P>0.05) between controland C-vehicle groups.

Effect of Exenatide onCerebral IL-1βLevel

The pre-treatment with Exenatide to significant lowering(*P*≤0.05) inthecerebralIL-1βlevel compared withC-vehicle group. In addition, there was insignificant difference(*P*>0.05) inthis parameterbetween Exenatidetreated groupandsham group. The changes in cerebral IL-1β level were summarized infigures(1).

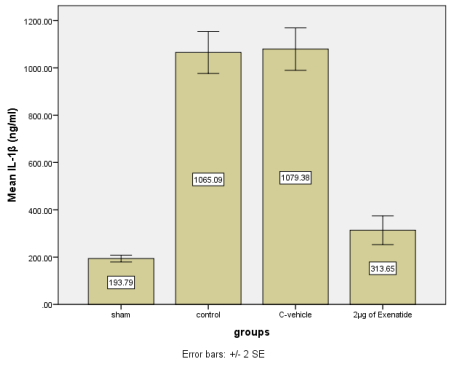

Figure 1: Mean Level of Cerebral IL-1β (ng/ml) of all four groups (N0. = 6): Data are expressed as mean ±SEM, *P <0.05 versus sham, **P <0.05 versus control and vehicle groups

Effect of Exenatide on Cerebral MMP-9Level

The Exenatide treated groupsignificantlylowers(P≤0.05) thecerebralMMP-9 level compared with C-vehicle group. While there was insignificant difference (P>0.05) in this parameterbetween Exenatide treated group and sham group. The changes in cerebral MMP-9 level were summarized infigures(2).

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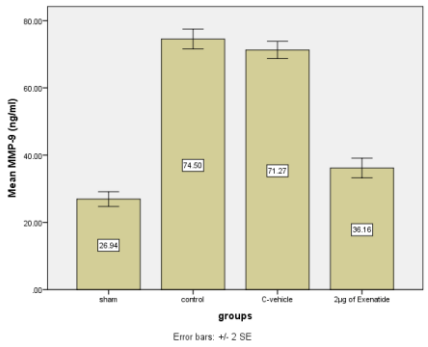

Figure 2: Mean Level of Cerebral MMP-9 (ng/ml) of all four groups (N0. = 6): Data are expressed as mean ±SEM, *P <0.05 versus sham, **P <0.05 versus control and vehicle groups

Effect of Exenatide on Cerebral 8-iso-PGF2α Level

The pre-treatment with Exenatide to a significant lowering (P≤0.05) effect onthecerebral8-iso-PGF2α level compared with C-vehicle group. In addition, there was insignificant difference (P> 0.05) in this parameterbetween Exenatide treated group and sham group. The changes in cerebral 8-iso-PGF2α level were summarized infigure(3).

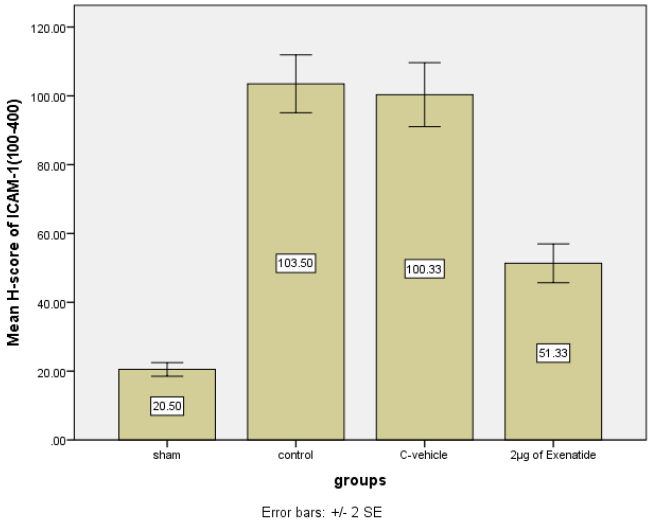

Figure 3: Mean Level of Cerebral 8-iso-PGF2α (ng/ml) of all four groups (N0. = 6): Data are expressed as mean ±SEM, *P <0.05 versus sham, **P <0.05 versus control and vehicle groups

Immunohistochemistry Finding

Effect of Exenatide on CerebralICAM-1 Level

In comparison to C-vehicle group, the cerebralICAM-1 level of Exenatide treated groupwas significantlydecreased(*P*≤0.05). In addition, there was insignificant difference (*P*>0.05) in this parameterbetweenExenatide treated group and sham group. The changes in cerebral ICAM-1 level were summarized infigures(4)and (5).

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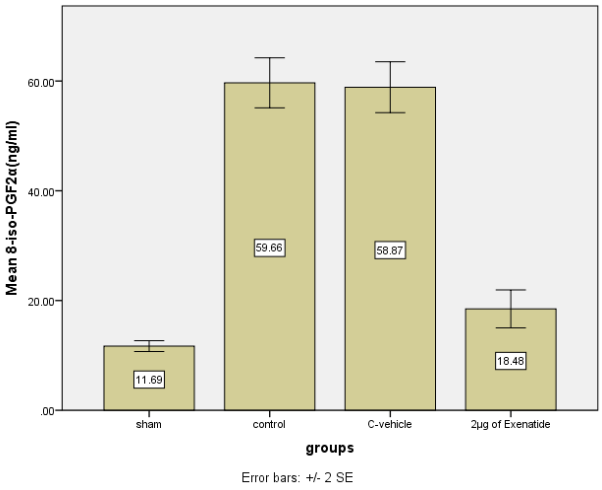

Figure 4: Mean Level of Cerebral ICAM-1 (ng/ml) of all four groups (N0. = 6): Data are expressed as mean ±SEM, *P <0.05 versus sham, **P <0.05 versus control and vehicle groups

Discussion

In present study,the levels ofeach ofcerebral parametersIL-1β, MMP-9, 8-iso-PGF2α, and ICAM-1 were significantly increased($P \leq 0.05$)inboth control and C-vehicle groupscompared with sham group,while there was insignificant difference ($P > 0.05$) between control and C-vehicle groups.These results were in agreement with several previous studies [23-25] that concluded an increase in these markers after cerebral ischemia. The increases in theseparametersIL-1β, MMP-9, ICAM-1, and 8-iso-PGF2α had a role in the deterioration of CNS after stroke.Kany S. *et al*, explained the role of proinflammatory IL-1β in early time of stroke and how to derive the damaging inflammatory process in the brain tissues [26]. Several different studies showed the role of MMP-9 during ischemia and the how could exacerbate the condition to edema and hemorrhage [27-30]. The expression of ICAM-1 mRNA significantlyincreased in the ischemic stroke and leadto increases in ICAM-1 expression on cerebral endothelial cells which had a deteriorated effect on infarct size[3132].The present study showed that the levels of cerebral IL-1βwas significantlylowered($P \leq 0.05$)in Exenatidetreated group when compared with C-vehicle group.Several studies showed that the deterioration effects of IL-1βin the cerebral ischemic stroke as well as the beneficial effects obtained when deletion or antagonize of the IL-1receptor(IL-1R) in the neuronal cells [23-25]. Lambertsen KL*et al*, 2019, explainedthat the pro-inflammatory cytokines (e.g., IL-1) represent one of themost important sites that could be targeted as neuroprotectivetherapy [33]. So, all thesestudies supportthe results of our study thatconcluded CNS neuro-protection after ischemic stroke required the loweringan important factor and one of them was IL-1β.The pre-treatment with Exenatide to a significantdecrement($P \leq 0.05$)in the levels of cerebralMMP-9 when compared with C-vehicle group.Decreased production of MMP-9 represent an approach that has a possible neuroprotective and maintained intact BBB. This finding was in agreement with the resultsconducted by different studies [34-36]**.**The levels of cerebral8-iso-PGF2α was significantlydecreased($P \leq 0.05$)when Exenatide had used prior to

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induction of cerebral reperfusion stat.Li J, *et al* and Xie K, *et al*,they revealed in an experimental studies the neuroprotective were associated with decreased in the level of 8-iso-PGF2α and this findingwere in accordance with the present study [37-38],finally,the pre-treatment with Exenatide to a significantlowering ($P \leq 0.05$)in the level of cerebralICAM-1 compared with C-vehicle group.This result agreed with Al-Hassani ZK, 2014,who found a decrease in neuronal damage when experimental groups pretreated with amlodipine and or can desertingand this improvement attributed to a decreases in many factors and one of the them was ICAM-1 [39].Moreover,several studies revealedthatthe role of ICAM-1 in adhesion and transendothelial leukocytes to site of injury and a deterioration of insult region.They alsoasserted when inhibit the up regulation of the ICAM-1, aneuroprotective effect was obtained [35-36].

Conclusion

Decrease in pro inflammatory agent IL-1β, MMP-9, ICAM-1, and oxidative stress 8-iso-PGF2α in grouptreated with Exenatide drug consider as neuroprotective for cerebral ischemia/reperfusion in male rat model.

Abbreviations

ANOVA: The Analysis of Variance; rtpA: recombinantalteplase agent; ELISA: enzyme-linked immunosorbent assay; Fig:figure; H&E: hematoxylin and eosin; I.P.: intraperitoneal; I/R: ischemia/reperfusion; IL-1β:interleukin-1β; GLP-1:glucagon like peptidase-1; GLP-1RA: glucagon like peptidase-1 receptor agonist; NaCl: normal saline;IHC: Immunehistochemistry;8-iso-PGF2α: 8-iso Prostaglandin F2 Alpha; SEM: standard error means; ICAM-1: intracellular adhesion molecule-1; MMP-9: matrix metaloproteanase-9.

Declarations

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Ethical approval

The current study has been conducted according to the national research guidelines for the care and use of laboratory animals. All protocols have been approved by the High Committee for Review and Approval of Research Proposals of the Faculty ofMedicine, University of Kufa(Ref.#767, date:13/01/2020).

Availability of data and materials

The datasets used and/or analyzed for the present study are accessible from the corresponding author on reasonable request

Consent to publish: Not applicable

Competing interests: The authors confirm that there is no conflict of interest.

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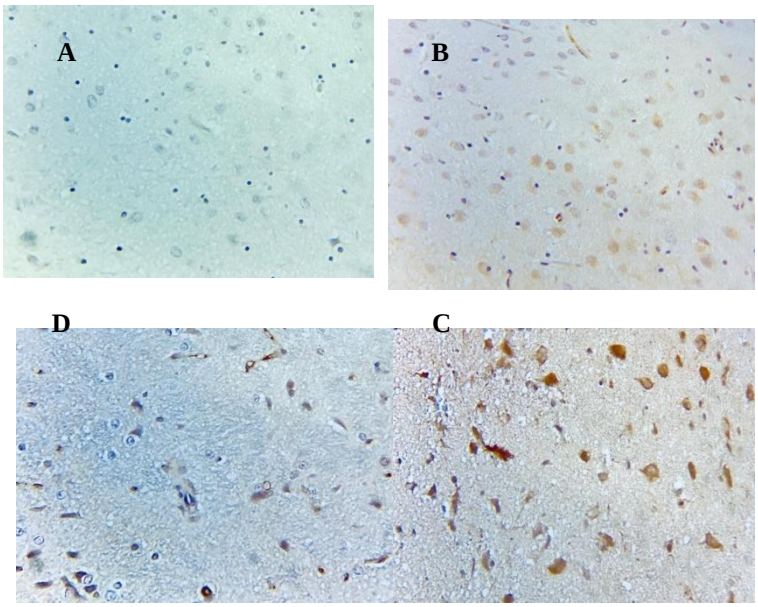

Figure 5: Explain the different levels of ICAM-1 appearing in different groups which expressed as brown color.

(A) Sham group showing normal state (no stain).

(B) Control group showing strong positive.

(C) Vehicle group showing positive.

(D) The treatment group that appears a slight positive