

Histological Structure of Dentate Gyrus in Global Cerebral Ischemia/Reperfusion Injury and Role of Nitraetes in Protection

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ABSTRACT

Background: Ischemia-reperfusion injury is a hell mark of stroke that happens if blood supply is returned back after a period of ischemia. However, the benefit from restored blood supply by reperfusion can cause bad effect compared to permanent occlusion.

Objectives: This study aimed to analyze morphological alterations in dentate gyrus tissue during ischemia and its effects on histology of cells and to understand mechanisms of brain ischemia-reperfusion injury to develop effective therapy. **Results:** Many experiments proved that the mechanisms of ischemia-reperfusion injury include complement activation, leukocyte infiltration, oxidative stress, platelet adhesion and aggregation and mitochondrial-mediated mechanisms leading to blood-brain-barrier (BBB) disruption, which leads to brain edema.

Conclusion: In summary, over the past few years there have been significant advancement in our understanding of molecular and cellular mechanisms of ischemia-reperfusion injury in the brain. The major mechanisms of reperfusion injury are clear but are still far from being clinically successful. Thus, further investigations on brain reperfusion injury mechanisms are wanted. Also, role of nitrates in protection for ischemia-reperfusion brain injury.

Keywords: Blood-brain-barrier, Complement, Dentate gyrus, Ischemia, Reperfusion, Mitochondria.

INTRODUCTION

In this review, we discuss pathology, molecular and cellular mechanisms of brain ischemia-reperfusion injury, and possible protection of nitrates strategy. According to the World Health Organization (WHO), 15 million suffer from stroke in the world every year⁽¹⁾. Of these, 5 million die and other 5 million are disable. High blood pressure causes more than 12. 7 million strokes injury all over the world. Approximately European averages 650,000 die every year from stroke⁽²⁾. Stroke is the second comes after ischemic heart disease in causes of death, and over a third of these deaths occur in developing countries. Populations in Arab countries have a similar diet and lifestyle that may increase stroke risk, and affect survival after stroke, as well as other characteristics in comparison with Oriental and Western populations⁽³⁾.

The causes of brain ischemia vary from blood clots, sickle cell anemia, congenital heart defect that have a higher predisposition to brain ischemia, plaques in the arteries, ventricular tachycardia and extremely low blood pressure. This cause impairment in body movement, vision and speaking, which lead to irreversible damage of brain and unconsciousness⁽⁴⁾.

In spite of stroke being a serious life-threatening risk and causing disability, the only effective accepted treatment is tissue plasminogen activators. However, the duration limitations of this treatment makes it can only be given to a small number of patients, 1–2%⁽⁵⁾. Despite decades of intense research, the beneficial treatment of stroke remains limited⁽⁶⁾. In light of this, we search for effective methods ameliorating cerebral ischemia-reperfusion injury, which is one of the major problems of experimental medicine and biology⁽⁷⁾.

The goals of this review were to analyze morphological alterations in dentate gyrus tissue during ischemia and its effects on histology of cells. This review also summarized and discussed the underlying mechanisms responsible for the preventive effects of vasodilators against brain ischemia.

General appearance and microscopic structure of brain dentate gyrus:

The brain dentate gyrus is a cortical area that is a part of the larger functional cerebral system called the hippocampal formation, which is a portion of the limbic system⁽⁸⁾. The dentate gyrus forms new memory episodic. The limbic system includes structures that lie in the border zone between the cortex and diencephalon⁽⁹⁾. The limbic structures include limbic lobe (cingulate, isthmus and parahippocampal gyrus), hippocampal formation, amygdaloid nucleus, septal areas, mammillary bodies of the hypothalamus, some thalamic nuclei, habenular nucleus of the epithalamus and brainstem structures e.g. autonomic nuclei of the brainstem, connecting pathways as the fornix, stria terminalis and others. The hippocampal formation consists of hippocampus proper, dentate gyrus, subiculum and indusium griseum⁽⁹⁾. The hippocampus is an important component of humans' brains.

It came from the Greek two words for horse (hippo) and sea monster (campus) and has a C- shaped appearance in its coronal section like a sea horse. The hippocampus proper is divided into four distinct fields including CA1, CA2, CA3 and CA4 on the basis of their cellular variances (density, branches of axons, dendrites and size)⁽¹⁰⁾.



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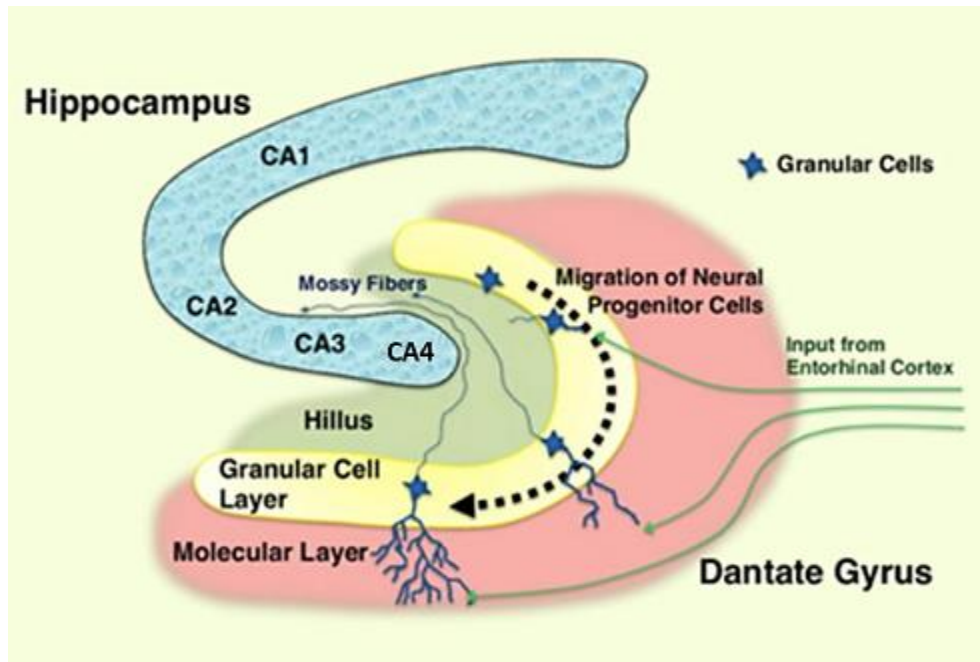


Figure (1): Hippocampus anatomy with migration of young neurons ⁽¹¹⁾

Histological aspect of the human dentate gyrus:

Histologically, the human dentate gyrus has polymorphic, granular cells and molecular layers. The molecular layer is cell-free. It is occupied by the dendrites of the granule cells and fibers of the perforant path that originate in the entorhinal cortex. There are a variety of extrinsic inputs terminate there and a small number of interneurons ⁽¹²⁾. The polymorphic cell layer constitutes the third layer of the dentate gyrus (Fig. 2 & 3). Many cell types are founded in the polymorphic layer but the most prominent is the mossy cell ⁽¹³⁾.

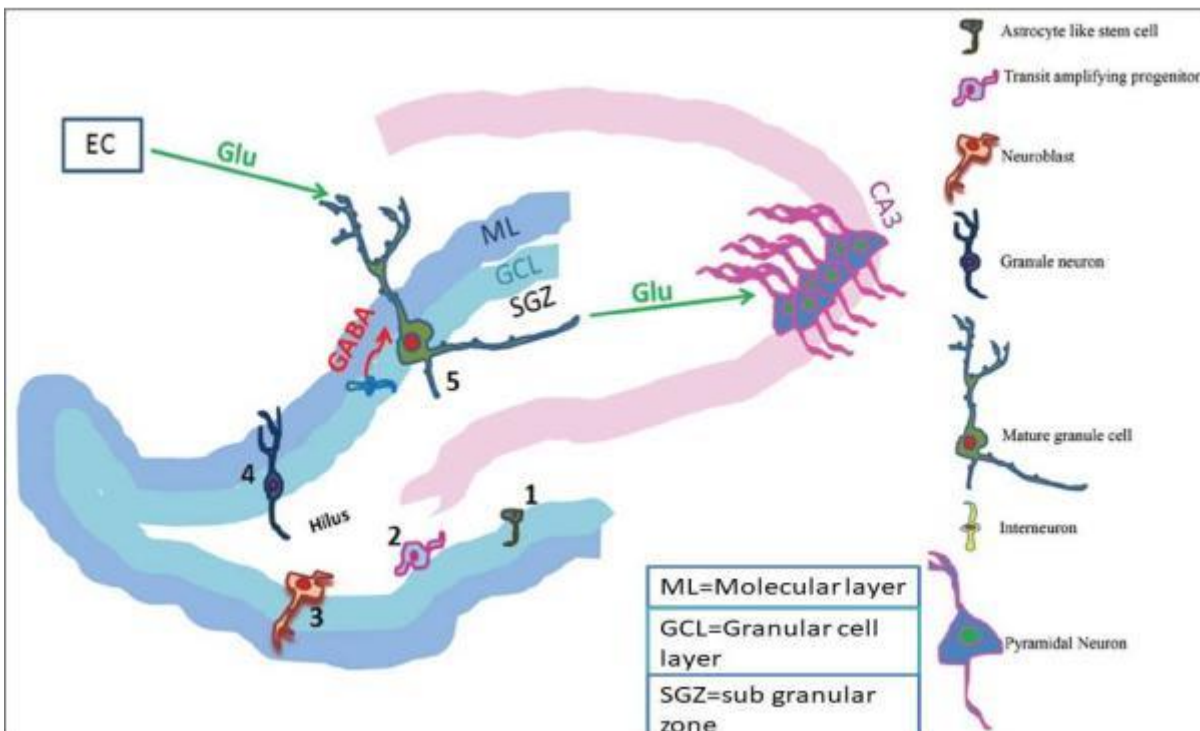


Figure (2): Cell types of the human dentate gyrus and their connection ⁽¹⁴⁾

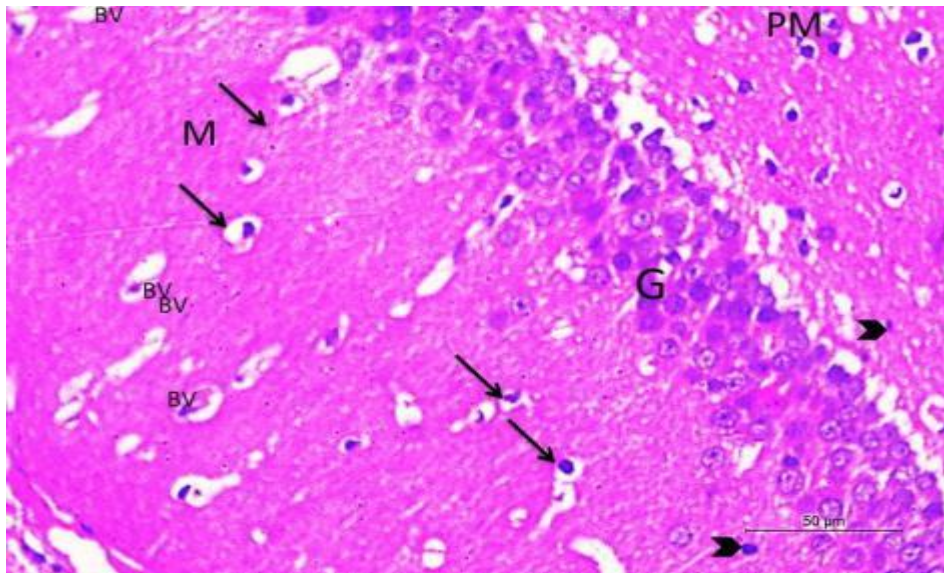


Figure (3): A photomicrograph of a coronal section in the brain dentate gyrus of a control rat showing the granular cells (G) appeared as an elliptical cell body and basophilic cytoplasm the granular cells are packed tightly in the granular layer of the dentate gyrus. Notice: Oligodendroglia cells with dark small nuclei with perinuclear halos (black arrows), astrocytes with oval vesicular nuclei (head arrows), nerve fibers (F) and blood vessels (BV). (**H & E X 400**).

The subgranular zone (SGZ) in the dentate gyrus shelters the astrocyte like stem cells (1), which generate progenitor cells (2). These cells mature into neuroblasts (3), which migrate (4) into the granule layer and differentiate into granule neurons (5) as shown in figure (2). These newly granule neurons interconnect by axons that extend along the mossy fiber towards the CA3, and other dendritic branches into the molecular layer (ML). They receive inputs of glutamate (Glu) from the entorhinal cortex when they mature (EC) and inputs of GABA from the interneurons and provide inputs of glutamate to the CA3 neurons ⁽¹⁴⁾. (Fig. 2, 3).

The granule cells form the principal layer in the dentate gyrus (Fig 4).

The granule cell in dentate has an elliptical body cell with approximately 10 µm width and 18 µm height. The granule cells are packed tightly together and, there is no glial sheath between cells. The granule cell has apical dendrites of a cone-shaped tree of spiny. The branches of the dendritic tree extend through molecular layer and their distal tips end at the fissure of hippocampus. The length of the dendrites of granule cells in the suprapyramidal blade are on average, larger than those in the infrapyramidal blade ⁽¹⁵⁾.

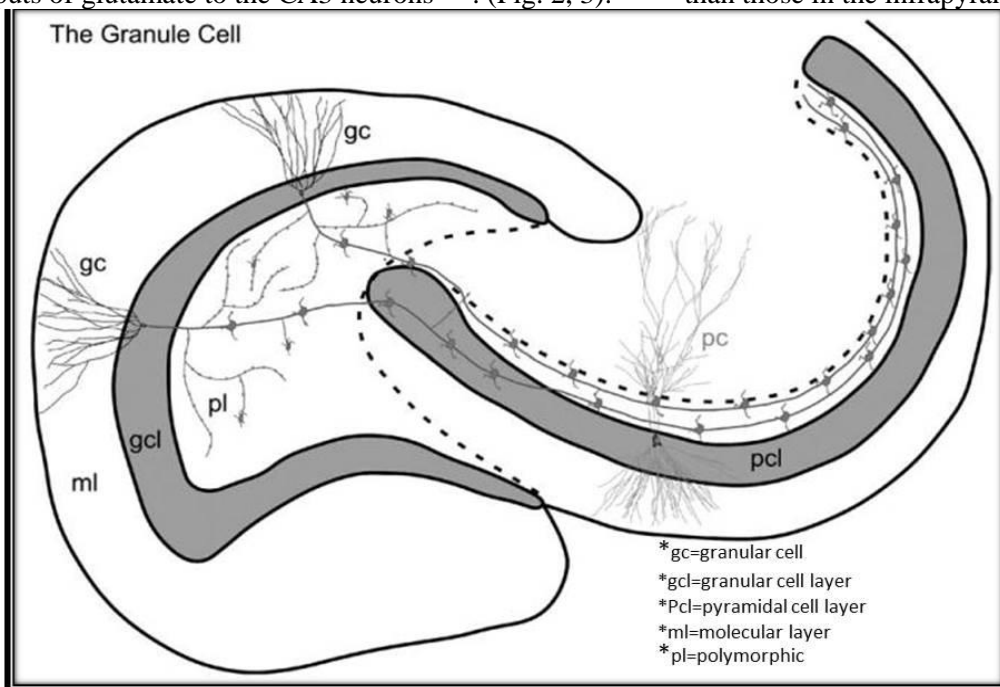


Figure (4): The dentate granule cell. The characteristic features of the human dentate granule cells are illustrated including its axonal arbor ⁽¹⁰⁾

Pathophysiology of brain Ischemia-reperfusion Injury:

The main mechanisms of reperfusion injury include leukocyte infiltration, mitochondrial mechanisms, platelet aggregation and activation, complement activation, oxidative stress and blood-brain-barrier (BBB) disruption, which lead to brain edema or hemorrhagic transformation and causing severe neural death and neurological dysfunctions⁽¹⁶⁾.

Oxidative stress in ischemia-reperfusion injury:

It occurs when the reactive oxygen species (ROS), mainly peroxides and free radicals such as superoxide anion, exceeds over than antioxidant capacity. ROS is a main mechanism in the pathology of many diseases including brain injuries. Overproduction of ROS damages directly cellular components including DNA, RNA, proteins and lipids. Increasing superoxide has been detected in a brain ischemia and reperfusion model. Furthermore, oxidative stress-mediated BBB dysfunction was detected in a brain reperfusion model of superoxide dismutase (SOD)-deficient mice⁽¹⁷⁾. The main source of oxygen radicals production after reperfusion is mitochondria. Ischemia-reperfusion induces modification of proteins by oxidative phosphorylation, which increases potential of mitochondria membrane. This condition leads to more generation of oxygen radicals. Another important source of oxygen radical's production is NADPH oxidase (NOX), which transport electrons of the plasma membrane chain that causes free radicals by transferring an electron to molecular oxygen. The NOX contributes in the pathology of reperfusion injury, and the potential of NOX targeting as a strategy in therapy⁽¹⁸⁾.

Mitochondrial mechanisms in ischemia-reperfusion injury:

Mitochondrial mechanisms in reperfusion injury are not only generation of ROS, but also mediate other processes such as necrosis and apoptosis. It has been demonstrated that reperfusion after a long period of ischemia can open permeability pore of mitochondria, a non-specific pore in its inner membrane. This leads to Ca²⁺ flux into mitochondria, and also mitochondria swelling, uncoupling, ATP deprivation and cell necrosis⁽¹⁹⁾. Division of mitochondria and fusion is reflected by the dynamic combination of fragmented and tubular morphology of it. Fission is mainly regulated by guanosine triphosphatases (GTPases), which is dynamin-like protein (Dnm1) in yeast. Mitochondria fusion begins with the contact and merge of mitochondrial outer membranes, which is mediated by (Mfn1) mitofusin 1 and (Mfn2) mitofusin 2 in humans⁽²⁰⁾. Many studies have suggested that division and fusion are critical regulators for its homeostasis, protection and exchange of materials. More important evidence suggests that

morphological changes of mitochondria are involved in apoptosis and survival of cell. These findings strongly support that division and fusion of mitochondria is involved in reperfusion cell injury and apoptosis. Many studies have reported that ischemia and reperfusion is accompanied by fragmentation of mitochondria^(21, 22).

Leucocyte infiltration in ischemia-reperfusion injury:

Leucocyte infiltration is a sequent of processes include leukocytes migration directed by chemotactic signals, leukocytes "rolling" on the endothelium, adhesion of leucocyte to the surface of microvascular endothelium through receptor/ligands interaction, matrix metalloproteinase production for breakdown of BBB, leucocyte extravasation into brain tissue and finally the brain inflammation response triggered by cytokines release⁽²²⁾. Animal studies have demonstrated the effect of infiltration of leucocytes in reperfusion injury. Like, in a rat model of MCAO, **Zhang et al.**⁽²²⁾ found that accumulation of neutrophil at the site of neuronal injury occurred in the reperfused tissue greater than in tissue of occlusion. Furthermore, the infiltration of leukocytes in reperfusion injury is also supported by the benefit from depletion of neutrophil, in which many animals after ischemia showed smaller size of infarction when given anti-neutrophil antiserum⁽²³⁾.

Platelet-mediated ischemia-reperfusion injury

Platelets are activated then accumulate in vascular beds in ischemia-reperfusion at early time. When platelets are active they release pro-inflammatory factors such as platelet-derived growth factor, thromboxane A₂, metabolites of arachidonic acid, serotonin, and platelet factor 4 (PF4) and generate free radicals. Many studies have shown that the activated platelets accumulate on cells of endothelium and their interaction can be mediated by P-selectin⁽²⁴⁾. Another important factor in platelet activation after reperfusion is platelet-activating factor (PAF), which has been suggested to play important roles in different models of brain ischemia, evidenced by the beneficial effect of PAF antagonists in brain function. Platelet activation is also involved in leukocyte infiltration, as activated platelets adhere to microvascular endothelial cells, causing the latter to release mediators that results in chemotaxis and migration of leukocytes, thus exacerbating the inflammatory cascade⁽²⁵⁾.

Complement-mediated ischemia-reperfusion injury:

During reperfusion, the complement system is activated through many pathways, including the antibody-dependent classic pathway, the alternate pathway, or the lectin pathway involving MBL/MASP (mannan-binding lectin/mannan-binding lectin-associated serine proteases)⁽²⁶⁾. The classic, alternate

and also lectin pathways are initiated by C1q, MBL/ficolins/Collectin-11 and C3b, respectively. As a result, multiple inflammation mediators will be induced including anaphylatoxins C5a, and C5b-9, which form a complex called membrane complex attack (MCA) with another four complex proteins, C6, C7, C8, C9. C5a can stimulate leucocytes infiltration into damaged tissue, and may also induce the release of pro-inflammatory factor such as interleukin1-6 and TNFa (27).

BBB dysfunction in ischemia-reperfusion injury:

BBB is a selective permeable barrier that separates blood circulation from the brain tissue. It is mainly composed of pericytes, endothelial cells and astrocytes. BBB disturbance is a common pathophysiological feature in brain ischemia and reperfusion injury, and can be considered an outcome of all other reperfusion injury mechanisms. It has been proved that there are 3 stages in its permeability changes after injury. Stage one is reactive hyperemia,

which increases permeability. Stage two is hypoperfusion caused by microvascular obstruction via swollen endothelial cells and end-feet of astrocyte, and formation of endothelial microvilli.

This leads to nutritional deficiency in the brain tissue and enhances infiltration and inflammation response. Stage three is the increase of paracellular permeability, which occurs in two phases response. The first one occurs 3-8 hours post-reperfusion caused by enhanced inflammation and oxidative stress on the endothelial cells.

The second one occurs 18-96 hours after reperfusion and this increase vasogenic edema and angiogenesis (28). Stage 1, hyperemia, is associated with cytotoxic edema, while the biphasic stage 3 is characterized by edema and altered BBB tight junctions, which ultimately lead to increased permeability for macromolecules across BBB. Figure (5) shows the sequence of BBB changes after ischemia-reperfusion (28).

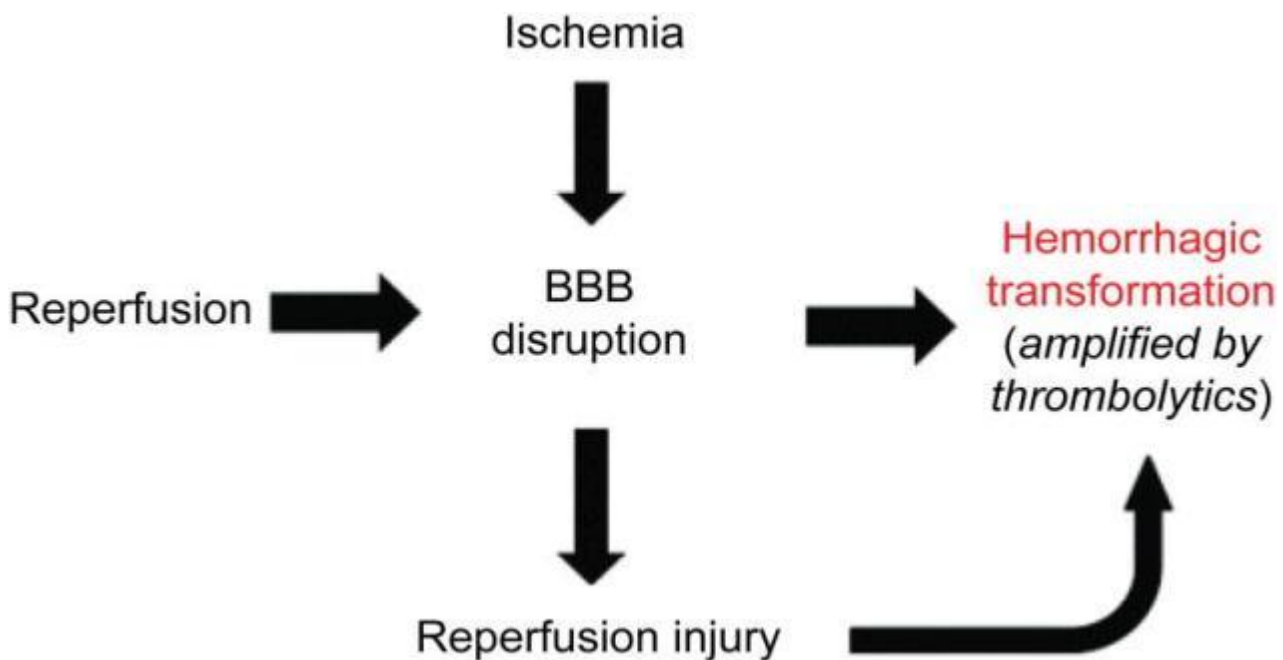


Figure (5): Representation of blood-brain barrier (BBB) changes in acute ischemic stroke (28)

Effects of Ischemia-reperfusion Injury on the dentate gyrus

The granular cell layer showed apparent decrease in thickness and the cell number with disturbed arrangement. Most of the granular cells appeared degenerated with shrunken hyperchromatic nuclei, perinuclear halos and lost nuclear details. Some granular cells showed swelling and others appeared as ghost like cells (Fig. 6). The neuropil of both molecular and polymorphic layers showed many vacuolations, less blood vessels and apparent increase in number of astrocytes. Sub granular zone (SGZ) revealed numerous spindle shaped cells (Fig. 6).

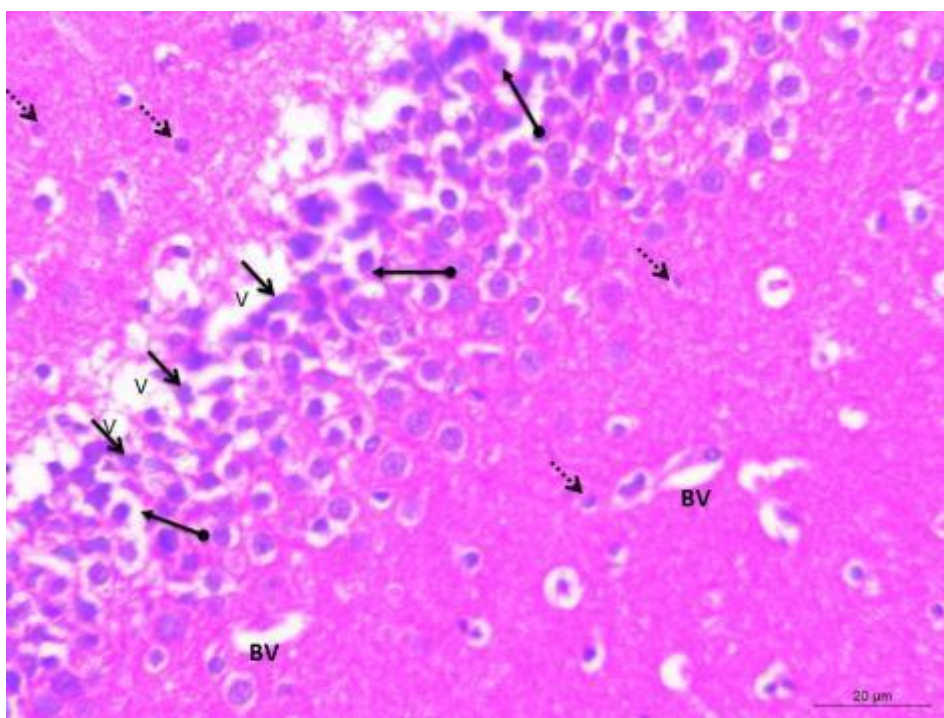


Figure (6): A cross section in dentate gyrus showing degenerated granular cells with hyperchromatic shrunken nuclei with perinuclear halos (round ended arrows), numerous spindle shaped cells (thin arrows) in subgranular zone (SGZ), Vacuolations (V), congested blood vessels (Bv), apparent increase in number of astrocytes (dot arrows) are observed in the neuropil of both molecular (M) and the polymorphic (Pm) layers. (Hx & E x 400)

Effect of nitrate in protection from brain ischemia:

Effects of nitrate (NO₃) on the dynamics of neurological disorders and the lethality rate after brain ischemia induced by simultaneous bilateral occlusion of common carotid arteries were investigated in rats. (NO₃) administered in doses of 5 mg/1000 g and 50 mg/1000 g 60 min before and 1–2 s after the occlusion of both common carotid arteries statistically reduced the severity of neurological impairment and the death rate of rats ^(29, 30).

Natural sources of nitrates: Despite vital roles of nitrate and nitrite in immune and vascular function, consideration of nitrates and nitrites sources in food has received little attention. Approximately 80% of nitrates in diet are taken from consumption of vegetables, fruits and processed meats, which also contain nitrites. The oxidation of nitric oxide produces nitrites endogenously and through nitrate reduction by commensal bacteria of the mouth cavity and GIT tract. As such, the dietary provision of nitrates and nitrites from vegetables and fruit may contribute to the blood pressure-lowering effects of the Dietary Approaches to Stop Hypertension (DASH) ^(31, 32). Nitrates are naturally found in vegetables such as: Beets, Celery, Lettuce, Radishes and Spinach. Adverse effect of the presence of nitrates and nitrites in food is associated with an increased risk of gastrointestinal cancer and, in infants, methemoglobinemia. We can avoid this by taking medical doses ⁽³²⁾.

CONCLUSION

In summary, over the past few years there have been significant advancement in our understanding of molecular and cellular mechanisms of ischemia-reperfusion injury in the brain. The major mechanisms of reperfusion injury are clear but are still far from being clinically successful. Thus, further investigations on brain reperfusion injury mechanisms are wanted. Also, role of nitrates in protection for ischemia-reperfusion brain injury.

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