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Role of oxidative stress on acute ischaemic stroke

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ABSTRACT

After the onset of a stroke, blood flows disrupted in areas affected by vascular occlusion limit the delivery of oxygen and metabolic substrates to neurons causing ATP reduction and energy depletion. The glucose and oxygen deficit that occurs after severe vascular occlusion is the origin of the mechanisms that lead to cell death and cerebral injury caused of oxidative stress. Oxidative stress constitutes mechanism of injury of many types of disease processes. On oxidative stress occurs on the increase in ROS and RNS. This paper will discuss about cerebral ischemia that causes activation of ROS and RNS, also the mechanisms that play a role in cell death after cerebral ischemia, for example the role of phospholipase, Haber-Weiss reaction, and lipid peroxidation. It is also described about anti-oxidants to fight free radicals, for examples glutathione peroxidase, catalase and superoxide dismutase.

Setelah onset stroke, terjadi gangguan aliran darah di daerah yang mengalami oklusi vaskuler, sehingga terjadi gangguan pengiriman oksigen dan metabolisme substrat pada neuron, hal ini menyebabkan pengurangan ATP dan energi. Terjadinya defisit glukosa dan oksigen yang terjadi setelah oklusi vaskular merupakan mekanisme utama yang menyebabkan kematian sel dan cedera otak oleh karena stres oksidatif. Pada oksidatif stres terjadi peningkatan ROS dan RNS. Tulisan ini membahas tentang iskemia serebral yang menyebabkan aktivasi ROS dan RNS, mekanisme yang berperan dalam kematian sel setelah iskemia serebral, misalnya peran fosfolipase, reaction Haber-Weiss, dan peroksidasi lipid, serta beberapa anti-oksidan untuk melawan radikal bebas, misalnya glutation peroksidase, katalase dan superoksida dismutase.

BACKGROUND

Cerebral ischaemia is a condition in which the blood flow to the brain decreased, either due to ischaemic stroke with acute focal or global neurological deficits.[1] Brain ischaemia increases the levels of ions Na^+ intracellular (Na^+i) and Cl^- intracellular (Cl^-i), as well as K^+ extracellular (K^+e) causing neuronal depolarization, release of glutamat, and opening of Ca^{++} channel.[2,3,4] The activation of glutamat receptors, especially N-methyl-D-aspartate (NMDA), would be followed by the entrance of Ca^{++} into the cells (Calcium influx).[5,6] In addition to that, an increase of intraneuronal Ca^{++} concentration would occur due to failure of active $Ca^{++}i$ elimination to extracellular through Sodium pump, as well as the release of Ca^{++} from mitochondria and endoplasmic reticulum to cytoplasma. The increase of $Ca^{++}i$ would generate the activation of enzymes phospholipase A2, nitrate oxide sintase (NOS), guanilat cyclase, calsineurin, endonuclease, protease and protein kinase.[7,8,9] The increase of $Ca^{++}i$ would activate phospholipase A2 which hydrolizes phospholipid membrane that consist of linoleic acid and arachidonic acid. Hydrolized arachidonic acid

would form leucotrient, prostaglandin, and free radical anion superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxil (OH-), by the assistance of lipoxygenase and cyclooxygenase (COX_1 dan COX_2).[10,11,12]

Free radicals anion superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxil (OH-) are the main pathway of the formation of Reactive Oxygen Species/ ROS through the reduction of molecular oxygen into H_2O. The presence of transitional minerals like Fe^{2+}, would cause hydrogen peroxide (H_2O_2) to go through fenton reaction, and increase the concentration of radical hydroxil (OH^-). This radical hydroxil is highly reactive and is a major cause of oxidative stress. As a defense mechanism against free radicals, there are anti-oxidant enzymes like yaitu glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD). Superoxide dismutase transforms O_2– into H_2O_2, and afterward glutathione peroxidase and catalase transforms H_2O_2 into H_2O. These enzymes are able to bind antioxidant like ascorbic acis, and α-tocopherol, resulting in decreased ROS concentration. During this process either an increase of free radicals concentration or the decrease of antioxidant concentration would cause oxidative stress and evoke damage to lipid, protein, or DNA, resulting into cell death through either apoptosis or necrosis.[12]

Oxidative stress as a result of increase ROS concentration,[13] might occur due to oxygen metabolism, oxygen reperfussion injury from hypoxic state, as well as hemoglobin and myoglobin oxidation.[14] Normally, ROS plays many roles in physiological processes, such as : the body's defense system, hormones biosyntethic, fertilization, and celullar signaling.[13] ROS also take part in the immune system by combating antigen during phagocytosis.[14] Nevertheless, the increase of ROS production have implications in many diseases, for instance hipertension, aterosklerosis, diabetes, heart failure, and stroke.[13]

ISSUES PROJECTION

A lot of ischaemic stroke patients were seen to still become worsen even with appropriate care in the Stroke Unit of RSUP Dr. Sardjito Yogyakarta. Even after standard management had been provided, risk factors had been controlled, and no complications were found.

Many research had reported the occurence of complications post-stroke. There were even one research that reported 96% of all stroke patients who were hospitalized had more than one medical or neurological complications.[15,16,17] A thorough study is needed to determine its patophysiology, therefore the possibility of oxidative stress as the cause of worsening in acute ischaemic stroke patients need to be examined.

Published research about oxidative stress and worsening of ischaemic stroke patients are still controversial due to the complexity of its mechanism. A research by Tsai et al.,[18] on 120 non-cardioembolic acute ischaemic stroke patients at Chang Gung Memorial Hospital's Neurological ward in Kaohsiung Taiwan, concluded that oxidative stress contribute to progressive neurological damage on certain cases of acute ischaemic stroke patients, which were shown on bad NIHSS score and stroke subtipe.

Another research by İçme et al.,[19] on 68 subjects, which consist of 34 ischaemic stroke subjects and 34 non-stroke control subjects, at Atatürk Training and Research Hospital Ankara Turki from January 2012 until January 2013, concluded that the increase of oxidants in oxidative stress contribute on the pathogenesis of ischaemic stroke, but there were no significant correlation between Total Anti-oxidant Status (TAS), Total Oxidant Status (TOS), Oxidative Stress Index (OSI), infarction volume, NIHSS score, and oxidative stress with the severity of stroke.

İçme et al.,[20] also reported based on their research on 92 subjects which consist of 74 acute ischaemic stroke subjects and 18 hemmorhagic stroke subjects at Atatürk Training and Research Hospital Ankara Turki from January 2013 until November 2013, oxidative stress contribute in the pathogenesis of both ischaemic and hemmorhagic stroke, however oxidative stress had uncertain correlation with the severity ofboth types of stroke.

Based on these datas, the mechanism of

ischaemic stroke which might contribute to the worsening of patients condition needs to be studied. The objective of this study is to determine the role of oxidative stress on acute ischaemic stroke.

REACTIVE OXYGEN SPESIES

Free radicals consist of several species that contain one or more unconjugated electron. Free radicals are reactive chemical spesies, such as reactive oxygen spesies and reactive nitrogen spesies. ROS consist of anion superoxide (O^2–), hydrogen peroxide (H_2O_2) and hydroxil (OH-), while RNS consist of radical nitrit oxide (NO-) and peroxynitrate ($ONOO^-$).[12,21,22]

Figure 1 showed the role of phospholipase in the formation of ROS and lipid peroxide on brain ischaemia.

Excess Ca^{++} intra cells, Ca^{++}i could be eliminated by the activation of Calcium adenosine triphosphatase plasma membrane .

In ischaemic state where the concentration of ATP (adenosine triphosphate) is very low, this mechanism would become inactive resulting in uncontrolled increase of Ca^{++} concentration intra cells. Increase Ca^{++} inside the cells would activate various enzymes, such as nitrate oxide sintase (NOS), phospholipase A2, and phospholipase C, protease and endonuclease.

NOS activation would result in the formation of nitrNOS activation would result in the formation of nitrit oxide, while the activation of phospholipase A2 would result in the hydrolization of phospholipid membrane which then disintegrate arachidonic acid into prostaglandin, prostacycline, and tromboxane A2, as well as the formation of free radicals superoxide, hidrogen peroxide, and hydroxil as ROS.[9,23,24]

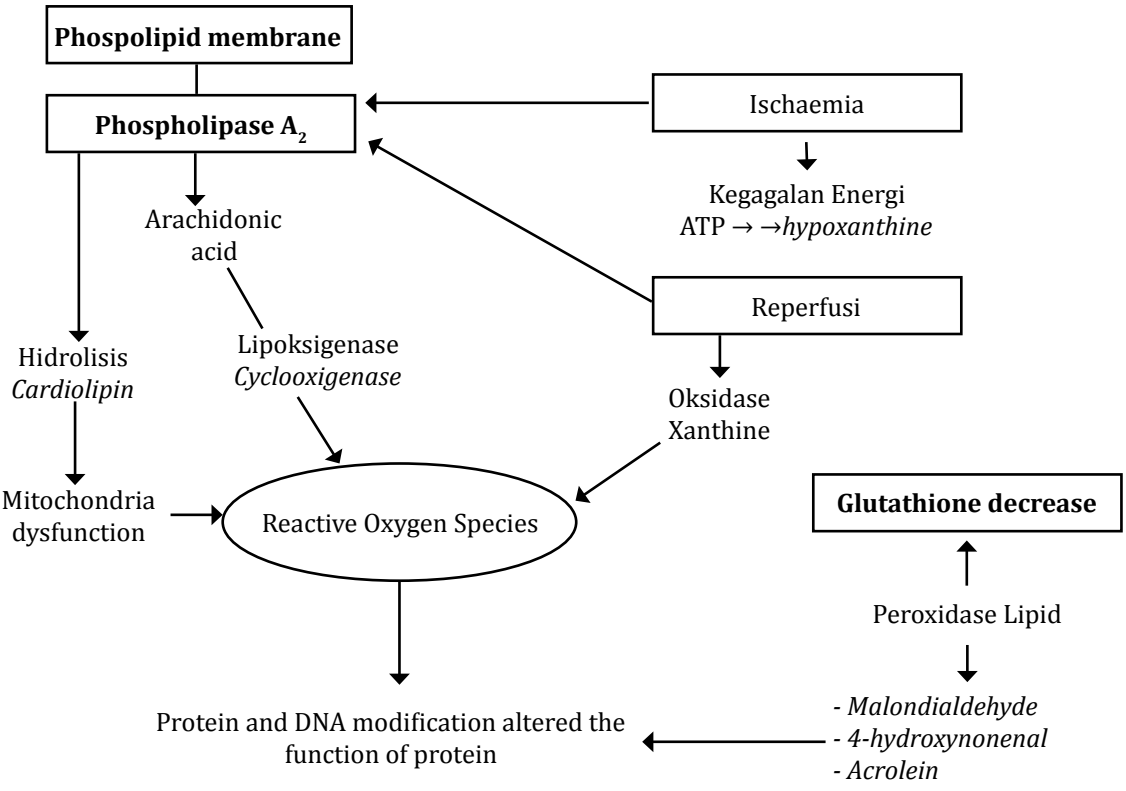

Figure 1. The role of phospholipase in the formation of reactive oxygen species

During ischaemia, ATP would decreased causing the increase of AMP concentration and subsequently supply hypoxanthine substrats into xanthine oxidase.[25] Afterward, reperfusion would also supply oxygens which would act as a secondary substat that is needed by xanthine oxidase to produce free radicals.[26,27] Hidrogen peroxide could also be formed from other mechanism which is oxidoreductase in cellular peroxisome organelles.[25,28] Hidrogen peroxide is a free radical that does not have electron pairs. Whilst not reactive, hidrogen peroxide could easily difuse through biological membrane,[29,30] and the presence of ferro (Fe^{2+}) or cupro (Cu^{+}), would cause hidrogen peroxide to transform into radical hydroxil (reaction 1). Oxidized iron (Fe^{3+}) would be reduced by superoxide anion (rection 2). Reaction 1 and 2 are called Haber-Weiss reaction.[22,31]

Hydroxil is a very strong oxidant of the biological system. Hydroxil would take one electron from the thiol of an enzyme, for instance glutathion reductase and glutathion peroxidase, DNA and polyunsaturated lipid, or other hydroxilated molecules. Hydroxil also could alter the function of mitochondria, inactivate electron-carrying-protein and mithocondrial ATPase, as well as peroxidized lipid membrane.[25,32]

Haber-Weiss Reaction

$Fe^{2+} + HOOH \rightarrow Fe^{3+} + OH^{-} + OH^{-}$ (reaction 1)
$Fe^{3+} + O_2^{-} \rightarrow Fe^{2+} + O_2$ (reaction 2)

Single oxygens are formed from the interaction of superoxide anion with another superoxide anion, peroxinitrate with hidrogen peroxide, or superoxide anion with hidrogen peroxide. Single oxygens are able to inactivate Calcium ATPase, denatured protein, inactivate superoxide dismutase and catalase, and oxidized polyunsaturated lipid into lipid hidroperoxide and endoperoxide. With the help of ions, these peroxides could trigger the development of lipid peroxidized chains or increase the production of single oxygens.[25,33]

REACTIVE NITROGEN SPECIES

Nitrit oxide as well as its enzyme, which is nitrate oxide sintase (NOS), contribute in brain ischaemia. Normally, nitrit oxide is a benign molecular signal. Nitrit oxide is synthesized from arginine by NOS, and it also has three isoforms which are endothelial NOS (eNOS) that is produced by endothelial cells, inducible NOS (iNOS) that is produce by macrophage, and neuronal NOS (nNOS) that is produced by neuron.[8,34] *Neuronal* NOS (nNOS) and endothelial NOS (eNOS) are highly dependant on Calcium and Calmodulin. *Inducible* NOS (iNOS) is produced by cytokines activation through second messenger pathway and does not require the increase of cytosolic Calcium.[35] *Inducible* NOS produce nitrit oxide that is toxic in brain ischaemia.[8,36] Nitrit oxide is able to interact with anion superoxide to produce peroxynitrate which will transform into nitrogen dioxide (an oxide reaction similar to radical hydroxil), oxidized protein that contain thiol, DNA base and polyunsaturated lipid, and inhibit mitochondrial electron transport hence causing a decrease of ATP production and an increase of ROS production.[32,37] In brain injury, polymorphonuclear leukocyte could produce nitrit oxide and superoxide. Neuron and oligodendrocyte are highly vulnerable to the effect of nitrit oxide and peroxynitrate.[31,38]

Nitrit oxide has high difusion velocity so that it can bind with hemoglobin. In the presence of free radical superoxide, nitrit oxide molecules would bind with oxygen and form peroxynitrate which is cytotoxic to neuron.[4,8,36] The cytotoxic effect of nitrit oxide occurs in two ways : (1) nitrit oxide would decrease the amount of growth factor, and (2) increase the concentration of enzymes that disintegrate protein, both resulting in DNA damage. Nitrit oxida that is mediated by the reaction with superoxide would produce peroxynitrate, a neurotoxic free radical. Peroxynitrate would trigger protein nitrosylation, which lead to DNA damage and induce brain cell death, as well as altering brain function.[11]

LIPID PEROXIDATION

When brain contains a lot of polyunsaturated fatty acids, ROS could easily increase lipid peroxidation.[39] Anion superoxide would cause deesterification of phospholipid membrane to release fatty acids. Radical hidroxyl extract is a

hidrogen atom that originated from methylene carbon of unsaturated fatty acids and produce radical lipid carbon-centred (L•, reaction 3). Radical lipid would react with oxygen molecules to form peroxy radicals (reaction 4), which transform into lipid hidroperoxide (LOO•) by separating hidrogen atom from methylene carbon that contiguous with unsaturated fatty acids (reaction 5).[29,32,40,41]

$LH + OH^{\bullet} \rightarrow L^{\bullet} + HOH$ (reaction 3)
$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (reaction 4)
$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH$ (reaction 5)

Lipid peroxidation could also be triggered by non-radical ROS, for instance single oxygen and hipochloride. As the presence of iron or iron complex, lipid hidroperoxide could form alkoxy radicals (reaction 6) or peroxy (reaction 7) and then generate new peroxidation chains (reaction 8 and 9), especially in acidic condition, for instance during ischaemia.[22,31,42,43,44]

$LOOH + Fe^{2+} \rightarrow Fe^{3+} + LO^{\bullet} + OH^{-}$ (reaction 6)
$LOOH + Fe^{3+} \rightarrow Fe^{2+} + LOO^{\bullet} + H^{+}$ (reaction 7)
$LO^{\bullet} + LH \rightarrow LOH + L^{\bullet}$ (reaction 8)
$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (reaction 9)

Lipid peroxidation could alter membrane instability and permeability, as well as the function of ion pumps on the membrane border. This condition would endangered ion homeostasis, and could result in the loss of membrane integrity and cell damage.[22,31,45] Lipid peroxidation could also trigger disintegration of pro-inflammatorik isoprostanoid mediator which is highly dangerous and release strong oxidant like 4-hydroxynonenal (4-HNE).[31,46]

ACUTE ISCHAEMIC STROKE AND OXIDATIVE STRESS

Ischaemia would be followed by reperfusion that can generate ROS and NOS that are toxic to brain tissues. Reperfusion cause an increase of oxidative stress due to the restoration of oxygen into brain tissues.[22,42,47] Oxidative stress contributes in the pathophysiology of ischaemic stroke, and brain is the organ most sensitive to oxidative stress. The presence of free radical activities would cause the brain to consume more lipid, increase oxygen demand, and trigger the oxidation of dopamine and glutamate. Furthermore, the concentration of catalase enzyme in the neurons would decrease, causing deterioration of gluthation peroxidase ability to eliminate hidrogen peroxide. ROS is a dangerous end-product of oxidative phosphorylation and reperfussion injury, because it could cause cellular redox which will influence the activity of protein-protein bond and DNA-protein bond of certain enzymes and transcription factors.[48]

Main pathway of the formation of ROS is through molecular oxygen reduction into H_2O. This reduction would generate dioxygen molecules from superoxide anion (O_2–) and hidrogen peroxide (H_2O_2).The presence of Fe^{2+} as transitional metal might cause hidrogen peroxide to undergo fenton reaction, which will increase the concentration of radical hydroxyl (OH–). Radical hidroxyl is highly reactive and is a major cause of oxidative damages. There are many instances of antioxidant enzymes, such as glutathion peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD).[41,49] Superoxide dismutase would change O_2– into H_2O_2, and glutathion peroxidase and catalase would change H_2O_2 into H_2O. These enzymes would coupled with antioxidant like ascorbic acid and α-tocopherol, resulting in the decrease of ROS concentration.

The increase of free radicals concentration or decrease of antioxidant would cause oxidative stress, resulting in lipid, protein, or DNA damage, which all will lead to cell death.[12]

NUEROPROTECTIVE DRUG THAT TARGETS OXIDATIVE STRESS

The objective of therapy in stroke patients is to increase clinical outcome by minimalizing the amount of damaged brain cells, treating complications, and accelerating the recovery of neurological function.[50] Drugs that are able to prevent or decrease the effect of oxidative stress and minimalize the amount of cell damage is called neuroprotective drugs, such as : antioxidant, agonis GABA, as well as antagonis AMPA.

Until now, there are more than 100

neuroprotective drugs had been studied and showed positive impact on ischaemic stroke in preclinical stage, however none has been clinically proven. Nevertheless, the result of these researches would be able to enhance our understanding on the biological basic of ischaemic brain injury and could become a strong keystone to enhance the management of stroke in the future.[51] Below are the description of these neuroprotective drugs

1. Ebselen

Ebselen showed antioxidant effect by inhibiting lipid peroxidation and the expression of inducible nitric oxide synthase (iNOS) in the cerebral cortex of mice that suffered frome stroke due to spontan hypertension.[52]

2. Tirilazad

Tirilazad is a non-glucocorticoid drugs that is composed by 21-aminosteroid which prevent lipid peroxidation. A systematic review on tirilazad showed efficacy on animal models who suffered from focal ischaemia, which was shown by a decrease of infarction volume and increase of neurobehavior score.[53] None the less, tirilazad was not significant in regards of infarction volume in patient with acute ischaemic stroke.[54]

3. Edaravone

Edaravone is an anti free radicals which work by preventing endothelial injury that would cause neuron damage in brain ischaemia. Edaravone will increase eNOS (NOS that is able to improve endothelial function, which would be beneficial in ischaemic stroke), as well as decreasing nNOS and iNOS (non-beneficial NOS).[55] Antioxidant edaravone significantly reduce infarction volume and increase neurological deficits score in mice,[56] reduce cell death due to oxidative in mice,[57] repair the size of lession in ischaemic stroke and neurological deficits in patients with small vascular oclusion, however there had not been any significant difference on clinical outcome.[58]

4. Clomethiazole

Clomethiazole work as neuroprotectant by being GABA agonis, in ischaemia model is well tolerated.[59] Nevertheless, it was reported to not have significant effects on sequele or the volume of infarction in ischaemic stroke patients.[60]

5. Aptiganel (CNS-1102)

Aptiganel is a neuroprotectant that works as non-competitive NMDA antagonist which is effective in focal ischaemia in rat models.[61] Phase II/III of the research was stopped due to lack of efficacy and mortality risk.[62]

6. YM872

YM872 is a neuroprotectant agent that works as AMPA antagonist, which significantly reduce infarct volume and improve neurological deficits in rat models with embolic stroke.[63] Results of phase II research have not been published.[64]

7. Magnesium Sulfat

Ion Magnesium could affect various enzymes that contribute in a lot of celullar function, such as : cell membrane permeability, mitochondrial function, and ion membrane for cell conduction, which in hypoxia would prevent cell death.[65] Magnesium showed neuroprotective effects on animal models,[66] as well as phase II research in stroke patients.[67] Namun demikian Magnesium failed to show significant effect on phase III research.[68]

8. Dapsone

Dapsone as neuroprotectant works by inhibiting glutamat exitotoxicity and inflammatory respond after brain ischaemia.[69] Dapsone was declared safe and effective on animal models,[70] and research on stroke patients had been done.[69] Phase III research had also been done but the results have not been published.[71]

9. Cromolyn

Cromolyn works by inhibitting cell mast migration and degranulation, activates glial cells and prevent neural cell death. Cromolyn is also effective in reducing brain oedem and repairing blood-brain barrier permeability in animal model with stroke.[72] Currently clinical research phase III on acute ischaemic stroke patient is ongoing.[73]

According to Cheng et al.,[74] there are a lot of reasons that affect the success of neuroprotectant medicines in phase I and II research, none the less, it failed on clinical research, such as : (1) preclinical research use very short window periods in drug administration, while clinical research has longer window periods, (2) the target of preclinical research is ischaemic penumbra, while clinical research

targets its outcome, (3) optimal duration for neuroprotective drug administration is unknown, (4) preclinical research uses different outcome measuring, for instance the size of infarct to determine the success of therapy, while clinical research uses itc clinical outcome, like Rakin scale or Barthel index modification, (5) preclinical research depend on early outcome, while clinical research depend on its end outcome, (6) stroke pthological variation, most preclinical research uses the occlusion of medial cerebral artery as ischaemic stroke model, hence there are no heterogenicity of stroke pathophysiology that would affect the duration and severity of ischaemic stroke. In contrast, research in human has a wide range of heterogenicity of stroke pathophysiology, and (7) comorbidity differences, most experimental model uses young healthy rats that was not exposed to other medicines, while real stroke patients usually has various severe secondary diseases (history of ischaemic attack, cardiovascular diseases, etc) and history of other medicine usage.

CONCLUSION

Cerebral ischaemic is the most common cause of acute ischaemic stroke. The pathophysiology of ischaemic stroke is very complex and involve various mechanism including the formation of free radicals. Imbalance of cellular production in the form of free radicals and the ability of cells to combat against it is called oxidative stress. Eventhough the mechanism is unclear, oxidative stress is one of, if not the most, important event in ischaemic stroke and contributes to the worsening of stroke event.

There are already a lot of medicines to combat against oxidative stress that are known as neuroprotective agent which have been studied and showed positive effect on ischaemic stroke in preclinical research, however none of them have shown significant clinical effect. Nevertheless, the results of these research could improve our understanding about the mechanism of ischaemic brain injury and become a strong basis to develop better stroke management in the future.

There are many potential medicines that might be able to prevent or reduce the effect of oxidative stroke, hence minimizing the amount of cell damage. These medicines are called neuroprotective, for instance : antioxidant, GABA agonist, as well as AMPA antagonist.

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