Oxidative Stress for the Stroke: A Review of Mechanisms

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Abstract: The aim of this study is to provide fundamental knowledge for the mechanism of oxidative stress in stroke. The collection of literatures in this study was performed through a biomedical database, Pubmed, Korean research information service system, Riss4U, a science and technical database, ScienceDirect and Internet. This study investigated the known mechanisms of oxidative stress in stroke through published literature reviews. Stroke is associated with the production of reactive oxygen species (ROS) after ischemia-reperfusion in prehospital emergency system, and the use of many antioxidants is being considered in order to reduce the pathological damage caused by ROS. To enhance the quality of prehospital practice, the emergency medical technicians need to understand the accurate mechanism of oxidative stress, identify the risk factors affecting the process of ROS production, and monitor the cellular balance in oxidation-reduction. Providing effective prehospital care reducing oxidative stress will reduce cerebral damage and improve the status of health. Very few studies related to molecular mechanisms on the development of CVA were conducted in health care service field including nursing, therefore, it is important to encourage and continue related researches in the fields.

Key words: Reactive oxygen species (ROS), Stroke, Literature reviews, Prehospital care, Oxidative Stress

1. Introduction

1.1. Background and Justification of the Study

Stroke or cerebrovascular accident (CVA) refers to an abnormal condition of the brain that shows characteristics such as embolism, thrombosis, cerebrovascular bleeding, or occlusion due to vascular spasm, whereby the damaged blood vessels can lead to ischemia in the brain tissue (Anderson, Novak & Elliot, 2002). CVA is the primary cause of common and fatal neurological disabilities and one of the major causes of adult disabilities in general. The early clinical symptoms of CVA include sudden weakness or paralysis of the face and limbs, sudden disorientation, loss of vision in one eye, sudden speech impediment or difficulty in speech cognition, sudden severe headaches without a known cause, unexplained vertigo, and abnormal or sudden falls (Goodman, Fuller, & Boissonnault, 2003).

Approximately 20% of CVA patients require long-term inpatient care, lasting longer than 3 months, and 15 - 30% patients suffer permanent disabilities (American Heart Association, 2003). Although the mortality rate is showing a downward trend with the advancement of technology, the CVA-associated mortality rate is 75.5 per 100,000 persons (American Heart Association, 2003). According to the 2012 Annual Report of the National Emergency Medical Center, brain disorders including CVA account for 15.3% of emergency transport cases. The three most statistically significant emergency cases are severe trauma cases with less than fair chance of survival, CVA, and acute myocardial infarction—i.e., the frequency of CVA patients receiving emergency care is second only to severe trauma patients (Yoon, Yoon,
Jung, Lee, & Kang, 2013). Reportedly, it takes 6 hours to transport a CVA patient to hospital through the emergency transport system and the average hospital stay is 6 hours (Yoon, Yoon, Jung, Lee, & Kang, 2013). The primary cause of CVA is associated with pathological processes related to blood vessels in the brain and ischemic or hemorrhagic strokes account for 83% of all CVA cases (American Heart Association, 2003). Given that the aftereffects of CVA depend on the location and the severity of ischemia, ischemic stroke has a heavy cost burden for the national healthcare system as well as individuals (American Heart Association, 2003).

In this review, different studies were analyzed in an attempt to enhance the on-site treatment capabilities of the emergency medical technicians, who are the first to treat CVA patients in the emergency transport system, by providing them with a deeper understanding of the cytophysiological mechanisms underlying CVA. We focused on the oxidative stress mechanism, one of the common pathophysiological mechanisms involved in ischemic CVA. Therefore, health providers need to understand about the process for free radicals to develop diseases through damage to the human body and the effects of antioxidants, which respond to the damage, in order to provide an intervention for people who are exposed to various diseases. This article is written not only to understand cellular damage caused by reactive oxygen species (ROS) and antioxidant defense mechanism but also to suggest the effects of antioxidants in nursing interventions by identifying an extensive range of antioxidant application, and develop a systemic and evidence-based health provider research based on this.

1.2. Study purpose
The purpose of this study is to provide fundamental knowledge for the efficient prehospital care provider by identifying cellular damage by ROS.

2. Study Method
2.1. Study subjects and investigational method
This study investigated the known oxidative stress mechanisms of stroke through internet searching as well as literature review. The collection of literatures in this study was performed through a biomedical database, Pubmed, ScienceDirect and Internet. In order to identify the state of stroke research using oxidative stress, web searching was performed using keyword, “stroke” and “oxidative stress” or “reactive oxygen species”

2.2. The state of research using stroke and oxidative stress
One investigator, H Lee majored in molecular biology and research topic is antioxidant enzyme and critical care. The other investigator, Y. Kim majored in neuroanatomy and research topic is stroke, oxidative stress and antioxidant enzyme. Two investigators independently searched the literature restricted to Korean and English language published in Pubmed, CINHAL, Korean research information service system, Riss4U, a science and technical database, ScienceDirect from 1993 up to December 2013. Keywords for searching included “stroke”, “oxidative stress” or “reactive oxygen species”, and “mechanisms” (Table 1). In the national research, 21 journal articles and 71 theses were identified, and they were total of 92 articles. There is no redundancy between journal articles and theses. Stroke and oxidative stress-related research in Korea was being performed in various studies such as medicine, athletics, nutrition, food science and technology, oriental medicine, oriental pharmacy, pharmacognosy and biological sciences. However, there is no mechanism of “stroke” and “oxidative stress” and one review article are identified. One review article is about diabetes mellitus and coronary artery diseases, as the most common research were related to antioxidant effects. Most of them were to verify the effects after the intake of vitamins or polyphenols. In terms of study subjects, 28 studies were from Sprague Dawley rats, 10 studies were from human subjects and 38 studies were from cells.

International research, total 825 articles were identified on Pubmed. Further search was limited to review articles because our research was focused on
the molecular mechanisms of oxidative stress in CVA. Total 171 review articles were identified, and after review of titles and abstracts to remove the irrelevant articles, as the results, 70 articles were identified. Only 1 article was related to nursing (Wadas, 2009). In CINHAL, total 38 titles returned in the initial search and review articles were 7. There was no nursing review for mechanisms including oxidative stress in CINAHL. Together, total 167 national and international review articles were included in full paper screening, and our literature review was conducted based on these articles.

3. Literature Review

3.1. Reactive oxygen species (ROS)

ROS is a generic term used for oxygen free radicals and their redox derivatives containing oxygen molecules, including superoxide anion radical (O$_2^-$), which plays a key role in oxidative toxicity, hydroxyl-radical (HO), hydrogen peroxide (H$_2$O$_2$), and singlet oxygen (1O$_2$) (Halliwell & Gulteridge, 1989). ROS are formed by intrinsic factors such as intramitochondrial respiration, enzymatic reactions performed by nicotin amideadenine dinucleotide phosphate-oxidase (NADPH), bacterial activities, and emotional stress (Muqbil, Azmi, & Banu, 2006). ROS are also formed by extrinsic factors such as polluted air, increased metabolism, smoking, carcinogens, sunlight, heat, and radiation (Bar-Shai, Carmeli, Ljubuncic, & Reznick, 2008). As long as an organism is alive, their intracellular production of ROS continues.

The chemical composition of an oxygen free radical, a type of ROS, is characterized by an unpaired electron—i.e., a free ion or molecule—in the outermost orbit and high reactivity with surrounding molecules in order to lose or gain an electron and return to a stable state (Halliwell & Gulteridge, 1989). The presence of a large amount of ROS within the body activates a switch protein called OxyR, which segregates altered proteins from normal proteins (Halliwell & Gulteridge, 1989). The radicals not removed by

Table 1. Flow Chart of Retrieval Process of the Systematic Review
defensive mechanisms react quickly with biomolecules, leading to protein denaturation or lipid peroxidation in the membrane, thus inducing oxidative stress (Eliasson, et al., 1999). When lipid peroxidation is induced, it can result in damage to cell membranes, loss of organelle function, inactivation of membrane receptors, and problems with the cell transport system (Halliwell & Gulteridge, 1989). The lipid peroxide radicals that spread inside the cell or move through the bloodstream can create new radical reactions that trigger various chronic diseases such as cancer, Alzheimer’s disease, or atherosclerosis, as well as aging. In addition, ROS stimulates inflammatory cells and activates NF-κB (nuclear factor kappa B) through intracellular signaling pathways, enabling production of inflammatory mediators (Bar-Shai, Carmeli, Ljubuncic, & Reznick, 2008). As described above, ROS react readily with neighboring molecules, such as lipids, proteins, and carbohydrates, to induce cell damage and promote radical reactions, subjecting the body to aging as well as acute and chronic diseases.

3.2. Ischemic CVA

A potential pathway for cellular damage in ischemic CVA may be oxidative stress that is induced by ROS elevated to above-normal physiological levels (Kleinschnitz, et al., 2010). Oxidative stress results in tissue damage and death of nerve cells (Kleinschnitz, et al., 2010). ROS are known as radicals that can cause damage to the cell membrane by peroxidation of unsaturated fatty acids in membrane phospholipids (Halliwell & Gulteridge, 1989). Furthermore, after the cell membrane is damaged, ROS can cause changes in nucleic acid sequences, gene damage, and gene repair activities, which can then cause damage to cellular components via cell necrosis or apoptosis (Liu, 2003). In oxidative stress, nitric oxide (NO) and superoxide radical (O₂⁻) are the major contributing ROS factors (Nakka, Gusain, Mehta, & Raghubir, 2008). The toxicity of ROS is precipitated by interactions between peroxynitrite (ONOO⁻)-mediated protein oxidation and tyrosine residue nitration (Eliasson, et al., 1999). Other important oxygen species include hydrogen peroxide (H₂O₂) and hydroxyl radicals (•OH) (Image retrieved, 2011, from http://journals.prous.com/journals/dot/20033901/html/dt390019/images/Kulkarni_f4.gif) (Figure 1).

Under normal physiological conditions, ROS are produced in low concentrations and controlled by an endogenous antioxidant system involving superoxide dismutase, glutathione peroxidase, catalase, and antioxidant vitamins (Nakka, Gusain, Mehta, & Raghubir, 2008). However, when ROS production overwhelms the endogenous antioxidant system in the brain, harmful effects occur (Chong, Li, & Maiese, 2005). For example, deleterious effects induced by excessive ROS production after ischemia and reperfusion may result in lipid membrane damage, docosahexaenoic acid peroxidation, cytosine methylation, and cutting of DNA during guanine hydration (Nakka, Gusain, Mehta, & Raghubir, 2008). ROS also suppress the complex enzymes involved in the electron transport chain, blocking mitochondrial respiration (Yamamoto et al., 2002). Oxidative stress after ischemia and reperfusion precipitates the formation of holes in
mitochondria, which increases the release of inner and outer mitochondrial membrane components, including proteins related to apoptosis (Kim, Kondo, Noshita & Chan, 2002). Endoplasmic reticulum is also sensitive to oxidative stress related to post-ischemic neuronal death (Hayashi et al., 2005).

3.3. Oxidative Stress

ROS activate a number of transcription factors and various signal pathways related to regulating cell survival and death (Chan, 2001). Cell death-related signal pathways include neuronal hyperexcitability mediated by activated N-methyl-d-aspartate (NMDA) receptors and excessive flux of calcium, ROS formation, oxidative damage, energy depletion, and cell structure damage. In particular, targeting oxidative stress proteins can be a useful therapeutic intervention against ischemia and reperfusion (Mehta, Manhas, & Raghubir, 2007). Examples of potential intervening measures include intravenous administration of an antioxidative fusion protein to counteract apoptosis and the use of melatonin, as shown in an ischemic experimental model in which it was used to reduce the neuronal loss (Pei, Pang, & Cheung, 2002). However, because oxidative damage does not occur by a single process, but by complex interactions between several processes, such as CVA-related excitotoxicity, apoptosis, and inflammation, a sufficient neuroprotective effect is difficult to achieve by blocking a single process (McCulloch & Dewar, 2001). Owing to the complex interactions that induce apoptosis, effective strategies for brain injury prevention and/or treatment require interventions at various levels of the mechanism (Nakka, Gusain, Mehta, & Raghubir, 2008). Of the agents developed to eliminate free radicals to date, NXY-059 has demonstrated distinctive effects in small-scale clinical trials with small animals and primates over the past decade, but showed no significant effects in large-scale clinical trials (Jung, 2011; Shuaib et al, 2007).

3.4. Nitric Oxide (NO)

Nitric oxide (NO) is a chemical that is very important for survival of vascular cells and maintenance of the blood-brain barrier. In addition, it can improve survival of nerve cells by increasing blood flow, inhibiting platelet aggregation, interfering with inflammatory responses, and removing free radicals (Kim, 2009). The factors that produce NO in vivo are intracellular NO synthase (NOS) and blood nitrite. Ischemic damage-induced superoxide can promote NO to generate peroxynitrite, which has adverse effects on cell survival (Warner, Sheng, & Batinich-Haberle, 2004). Ischemia-induced NO overproduction is partially ascribable to glutamate-mediated increases in intracellular calcium concentration, which lead to increases in calmodulin-dependent NOS. The NOS family of enzymes includes NOS1–3. Ischemia is believed to be involved in increasing NOS1 activity in neurons and probably glial cells, as well as in reversal of the secondary glutamate reabsorption by the synapse, along with NMDA receptor-mediated increases in intracellular calcium levels (Nakka, Gusain, Mehta, & Raghubir, 2008). On the other hand, increased NOS3 activity in vascular endothelial cells and NOS2 activity in ischemic brain tissue are caused by infiltration of neutrophils, macrophages, activated microglial cells, and astrocytes (Nakka, Gusain, Mehta, & Raghubir, 2008). Three types of increased NOS activation after ischemia and reperfusion have been reported thus far (Nakka, Gusain, Mehta, & Raghubir, 2008). The activities of NOS1 and NOS2 after ischemia and reperfusion can be harmful, whereas inactivation of NOS1 and NOS2 can have neuroprotective effects (Love, 1999). A recently published report indicates that in an ischemic brain injury model, nitrite therapy can reduce the size of cerebral infarction, increase cerebral blood flow, and inhibit oxidative damage (Jung et al, 2006).

3.4.1 NADPH Oxidase Enzyme

NADPH oxidase is a ROS-producing enzyme (Opitz et al., 2007). Ischemic damage causes oxidative toxicity that is produced by the intercellular mitochondrial respiratory chain and NADPH oxidase, as well as by various cytosolic oxidases (Lee, 2012). Four rodent genes, encoding NOX catalytic subunits, have been identified (Nox1–4), of which Nox1,
Nox2, and Nox4 are expressed in the blood vessels. Nox4 is the most abundant isoform in the bloodstream, and is known to be induced at a higher rate in brain blood vessels of CVA patients than in peripheral blood vessels (McCann, Dusting, & Roulston, 2008). In particular, Nox4 was shown to be induced by ischemic CVA in both mice and humans (Kleinschnitz et al., 2010). Nox4-mediated oxidative stress induces neuronal damage by causing leakage in the blood-brain barrier and induces apoptosis (Kleinschnitz et al., 2010).

3.4.2 Hydrogen Gas

Oxidative toxicity is the primary mechanism that causes damage to the blood-brain barrier and nerve cells. Therefore, therapeutic interventions that inhibit oxidative toxicity are very important and needs to be applied to patients using the emergency medical transport system, without any delay after the onset of ischemic injury. Two major limitations of antioxidant therapeutic interventions are difficult diffusion of medications into cells and indiscriminate removal of substances, including superoxide and NO that play important roles in cell homeostasis, instead of selective removal of highly toxic hydroxyl radicals (Jung, 2011). Hydrogen gas can easily diffuse into cells and has the ability to selectively eliminate hydroxyl radical and peroxynitrite (Wood & Gladwin, 2007). If a method can be developed to allow easy clinical application of hydrogen gas, especially in the context of the emergency medical transport system, it could become the most powerful method to decrease cell damage in acute CVA patients.

3.5. CVA Animal Model

Ischemic CVA models are divided largely into focal and global ischemia models, where clinical cerebral infarction would fall under the former model (Kim, Koh, & Kim, 2011). The most commonly used focal ischemia animal models are the intraluminal filament and Trauma models. Thromboembolic, endothelin, and photochemical models are also used (Kim, Koh, & Kim, 2011). The photochemical model is especially well suited for studying neuroprotective effects in specific areas of the brain or studying materials for ROS removal. In this model, test animals are injected with a photosensitive dye that can pass through the blood-brain barrier, and then a laser or other light source is projected on a specific blood vessel to generate singlet oxygen and activated oxygen free radicals that can damage the vascular endothelium and create fine clots to cause ischemic damage to a specific brain areas (Kim, Koh, & Kim, 2011).

4. Conclusion and Suggestion

CVA is the most devastating neurological disorder affecting adults today. Studies are being conducted in various fields to examine the causes of CVA and to determine their underlying mechanisms. A multi-disciplinary team approach combining emergency care, medicine, microbiology, nursing, and rehabilitation is needed. Urgent and appropriate initial treatments are critical to minimize damage and save the patients from CVA, therefore, understanding the cell biological kinetics that occur after CVA is of paramount importance in reducing subsequent excitotoxicity-mediated damage to the brain and the body. A clear understanding of oxidative stress mechanisms by emergency medical technicians, those who provide initial medical care for patients, will greatly contribute to effective patient care and reduction of damage. Very few studies related to molecular mechanisms on the development of CVA were conducted in health care service field including nursing, therefore, it is important to encourage and continue related researches in the fields.

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