Process, Effects, Detection Methods and Possible Solutions for Reduction of Radicals, and ROS after Post-Ischemic Reperfusion Damage

Author:	Marith E. den Otter – S4127161
Date:	June 2022
Department Author:	Bachelor Life Sciences & Technology, Major Biomedical Engineering
Supervisor:	Dr. R. (Romana) Schirhagl
Department Supervisor:	UMCG Department of Biomedical Engineering
Co-Supervisor:	Yue Zhang

Foreword

This thesis was written for my bachelor's degree Life Sciences and Technology with specialisation in Biomedical Engineering at the University of Groningen. The subject of this thesis is about reperfusion damage that occurs in tissues or organs after an ischemic period. More specifically, the generation of radicals and reactive oxygen species. A very interesting topic that spoke to me, since it connects with organ donation, which is a vital topic in our society. Helping doing research on this topic, and possibly finding something useful, has been very interesting and has taught me a lot.

Summary

Post-ischemic reperfusion damage is an often occurring problem after limited or stopped blood flow. There are a multitude of factors that play a role, one of which is oxidative stress caused by generation of radicals and reactive oxygen species (ROS). Knowledge about generation and detection of these radicals and ROS is plenty. However, few solutions have been found for this problem so far, and none that are working perfectly. In order to help patients that suffer from post-ischemic reperfusion damage, the aim of this thesis was to research the process, effects, detection methods and possible solutions for reduction of radicals, and ROS. Electron spin resonance spectroscopy, certain fluorescent techniques, certain spectrophotometric methods, and fingerprinting are discussed as detection methods. From these detection methods fingerprinting was determined to be the most promising and effective detection method as it assesses the damage that is done by the radicals instead of the radicals themselves. A post-ischemic enriched environment, radical trapping, manganese-porphyrin (MnP) complexes, and antioxidant systems are discussed as possible solutions, with a combination of antioxidant systems and the MnP complexes being the most promising. This because the body's own way to fight oxidative stress is by using its antioxidant systems. Since it can take some time to alter these defence systems MnP complexes can be used to reduce any damage caused by the oxidative stress up until that point. The combination of antioxidants and MnP complexes would regain lost homeostasis and decrease damage that has already been done.

Table of contents

Introduction 5
Generation of Reactive Oxygen Species 6
Detection of Reactive Oxygen Species in Cells 6
Electron Spin Resonance Spectroscopy 6
Fluorescent Techniques 6
Spectrophotometric Methods 7
Fingerprinting 7
Detection of Reactive Oxygen Species in Mitochondria 8
Promising Solutions 8
Post-Ischemic Enriched Environment 8
Radical Trapping 9
Manganese-Porphyrin Complexes 9
Antioxidant Systems 9
Discussion 10
References 11
Afterword 12

Introduction

Organ donor shortage is a worldwide problem. Every 10 minutes, someone new is added to the list in need of a new organ, but even when a recipient receives the organ there is the risk for ischemia and reperfusion damage (Flescher, 2018). Ischemia is the oxygen shortage in a body part or organ, usually caused by limited or stopped blood flow (Roberto et al., 2017). During reperfusion damage a surge in free radical generation occurs when the blood flow returns after being undersupplied, causing cell damage, and in turn tissue damage. In fact, post-ischemic reperfusion damage is the number one cause of myocardial infarction after replacement of an aortic valve. The susceptibility of a tissue to ischemia differs and depend on tissue type and age of the cells.

The general process of post-ischemic reperfusion damage is shown in figure 1. In the early phase, anaerobic respiration takes place due to a decrease in oxygen supply, which causes lactate accumulation. This process causes chemical mediators and enzymes to be activated which convert cell membrane phospholipids into inflammatory mediators, causing cell damage. When the cell goes into necrosis, there is nothing that can be done to save it anymore, however before cell necrosis takes place, oxygen replenishing can occur through reperfusion (Khalil et al., 2006). This, however, comes with its own problems.

The adhesion of leukocytes to the vascular endothelium is increased by the previously produced chemical mediators, which increase the permeability of the postcapillary venules. The increased leukocyte-endothelium interactions generates radicals, which are molecules with unpaired electrons. Because of their unpaired electrons, which causes their instability, they are capable of oxidizing many biological molecules, such as the cell membrane, which has effects on platelets and leukocytes, and creating reactive oxygen species (ROS). ROS are highly unstable oxygen containing molecules. These ROS then release chemotactic agents which recruit more leukocytes. This is a problem, because leukocytes release factors which can lead to vasoconstriction and platelet aggregation, decreasing tissue perfusion. Another process that can happen simultaneously, is complement activation. This causes formation of complexes which create pores in the cell membranes, and thereby damaging the cell (Khalil et al., 2006).

Post-ischemic reperfusion damage is a serious problem, and one without many solutions yet. In order to increase the amount of successful organ donations, and thereby possibly saving lives, the effects of reperfusion damage should be reduced. The damage is caused by various connected factors, though in this article the process, effects, detection methods and possible solutions for reduction of radicals, and ROS will be discussed.

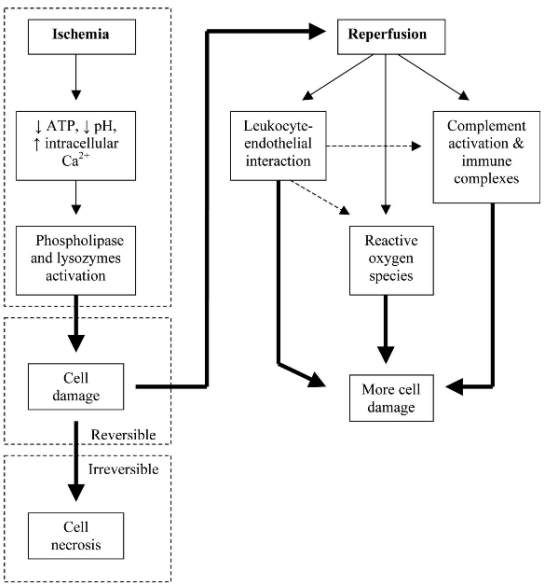

Figure 1: The process of post-ischemic reperfusion damage in cells, in general (Khalil et al., 2006).

Generation of Reactive Oxygen Species

As discussed before, ROS are highly unstable oxygen containing molecules. They are generated during aerobic respiration. An example of a very reactive radical is O_2•-. This radical can react with water to form the ROS H_2O_2. The most common source of ROS production are the mitochondria. Enzymes, such as cytochrome P450, lipoxygenases, cyclooxygenases, xanthine oxidase, and NADPH oxidase (Armstrong & Whiteman, 2007), as well as dysregulated glucose or lipid metabolism can also be important cellular sources of ROS production, however less so than mitochondria (Oxidative Stress, 2021). Specifically, about 95% of the intracellular ROS production come from the electron transport chain (ETC) on the inner lining of the mitochondria.

The ROS are formed during mitochondrial oxidative phosphorylation, where an electron leaks out of the mitochondrial electron transport chain, and passes directly to oxygen (Oxidative Stress, 2021). This will generate the radical O_2•-. The most important sources of mitochondrial O_2•- production are the mitochondrial respiratory complex I, the respiratory complex III, and the coenzyme Q radical. The enzyme manganese superoxide dismutase (MnSOD) is an enzyme that is produced in the mitochondrial matrix and makes sure that the O_2•- levels are kept low enough that it does not damage proteins of the matrix that are important in metabolic regulation. This enzyme also catalyses the breakdown of O_2•- into H_2O_2. The sources for ROS production and their breakdown are shown schematically in figure 2.

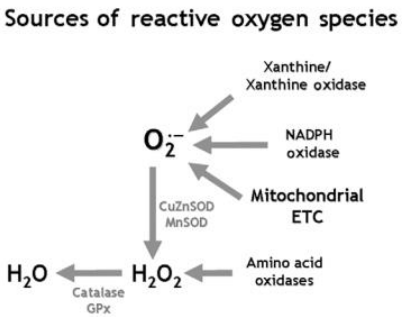

Figure 2: Cellular and mitochondrial sources of ROS production and breakdown (Armstrong & Whiteman, 2007).

When produced in excess, H_2O_2 can also be toxic and react with ferrous iron to form OH• which in turn can react with molecules such as DNA (Halliwell and Gutteridge, 1999). Normally the combination of MnSOD and the glutathione/gluthatione peroxidase (GSH/GSHPx) system is enough to keep the ROS generation at a nontoxic level. If this is not the case the cell also possesses an antioxidant defence system which includes the cytotoxicity antioxidant (CAT), singlet oxygen detection (SOD), GSH and GSH/GSHPx systems (Armstrong & Whiteman, 2007). If somehow the ROS also beat the antioxidant defence system, the balance between production of oxidants and their detoxification by antioxidants is lost. The loss of this cellular redox homeostasis within the cell leads to oxidative stress which can lead to cell damage and cell death (Oxidative Stress, 2021).

Detection of Reactive Oxygen Species in Cells

In order to gain more knowledge about ways to reduce the damaging effects of ROS, it is important to be able to detect radicals and ROS in cells, and mitochondria. Since there are multiple ways to detect ROS, the most promising detection methods will be discussed here.

Electron Spin Resonance Spectroscopy

Electron spin resonance (ESR) spectroscopy is a technique that can detect free radicals, because of its capability to detect the presence of unpaired electrons. A problem that occurs with this technique is that unpaired electrons usually do not get produced in detectable levels. In order to solve this problem, spin traps or probes can be used. These cause the reactive radicals to form more stable, and longer-lasting radicals which have their own characteristic ESR signatures. There are many spin traps available for both whole animal studies as well as cell culture studies, such as 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). 1,1,3-trimethylisoindole N-oxide (TMINO), N-2-(2-ethoxycarbonyl-propyl)-α-phenylnitrone (EPPN), and 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) are newer compounds that are more O_2•- specific (Armstrong & Whiteman, 2007). Some problems concerning ESR spectroscopy are that the detectible products, formed during the process, are removed fast, which makes detecting them difficult. Additionally, a very good control needs to be set up, since there are a lot of reactions that can take place between the different compounds and molecules (Armstrong & Whiteman, 2007).

Fluorescent Techniques

Fluorescent techniques are techniques that use certain compounds that react well with specific target molecules, thereby generating a fluorescent molecule. The fluorescence of the fluorescent molecule can then be detected and analysed. An example of such a fluorescent technique for detecting H_2O_2 is by use of the compound 2',7'-dichlorofluorescin diacetate (DCFH-DA). The compound is taken up by

the cell and is hydrolysed into 2',7'-dichlorofluorescin (DCFH). The DCFH gets trapped inside the cells where a reaction with ROS takes place, forming the fluorescent compound dichlorofluorescin (DCF). The fluorescence that is emitted from this compound can be detected. Since DCF gets produced through reactions with various ROS, specific scavengers of ROS are needed to know how the signal came to be (Armstrong & Whiteman, 2007).

A problem for measuring H_2O_2 using DCFH is that the oxidation of DCFH by H_2O_2 happens slowly when no ferrous iron is present or can be completely stopped by desferroxamine. Additionally, DCFH is also somewhat fluorescent by itself, which can auto-oxidize into hydrogen peroxide. Another problem is that various other compounds are able to induce DCF formation directly when there is no H_2O_2 present, such as peroxidases, $ONOO^-$, and HOCl. Because of these problems, the fluorescent dye is better for determining cellular oxidant stress than to quantify formation of H_2O_2 (Armstrong & Whiteman, 2007).

O_2•- levels can also be determined using fluorescent dyes. In this case for example, the compound hydroethidine (HE) is used which can be taken up by the cell and react with O_2•- to form ethidium. The ethidium is inserted into DNA which in turn results in nuclear fluorescence. This nuclear fluorescence happens at an excitation wavelength of 520 nm and an emission wavelength of 610 nm. In comparison to using DCFH for H_2O_2 detection, oxidation of HE by O_2•- is specific, because the same reaction does not happen in presence of other ROS. This makes the fluorescent dye technique ideal for detection of O_2•- (Armstrong & Whiteman, 2007).

There are some general problems for fluorescent techniques. The first problem is that the fluorescent dye can diffuse, which decreases the spatial resolution of the measurement. The second problem is that the measurement is not done in real time, which means that the measurements are not in full a visualisation of the processes happening at that moment. Lastly, the photobleaching effect plays an important role when using fluorescent molecules. Photobleaching is the irreversible process of destroying fluorophores stimulated by radiation within the excitation spectrum (Nathalie et al., 2007). This causes potential useful information to be eliminated.

Spectrophotometric Methods

Spectrophotometric methods are broadly used and accepted techniques to measure extracellular O_2•- production. For instance by isolated enzymes, cell homogenates, and neutrophils. The idea behind spectrophotometric methods, is that a compound is used which will be oxidised. This reduction of the compound can be measured, which can indicate the levels of O_2•- present (Armstrong & Whiteman, 2007). The different methods are shown in table 1.

The first method uses nitro blue tetrazolium (NBT). Similar to dihydroethidium (DHE), NBT can detect intracellular O_2•-. NBT is an aromatic compound that can be transformed into monoformazan by O_2•-. The reduction of NBT, and thereby the formation of monoformazan, can be detected using a spectrophotometer and a wavelength of 550-560 nm. A downside is that a NBT radical intermediate can generate O_2•- by itself, thereby reducing NBT. Also, NBT is not only reduced by O_2•-, but also other tissue reductases which makes the results difficult to interpret. Using the NBT therefore should be pared with an additional measure of O_2•-.

The second method uses cytochrome c which is transformed into its reduced form by O_2•-. The reduced cytochrome-c levels are measured using a spectrophotometer. The increase in absorbance is measured at a wavelength of 550 nm. However, cytochrome c reduction is not specific for O_2•-, and can be the cause of various compounds. Compounds such as CN-, ROS scavengers, and urate can prevent certain compounds from reacting with the cytochrome c. Additionally, the specificity of this method can be increased by measuring SOD-inhibitable cytochrome c, or by using acetylated or succinoylated forms of cytochrome c (Armstrong & Whiteman, 2007).

The last spectrophotometric method uses aconitase. This is a citric acid cycle enzyme which can catalyse the reaction where citrate is turned into isocitrate. Aconitase is inactivated by O_2•-, which means that a decreased activity of aconitase is a result from high levels of O_2•-. The aconitase activity can be measured by analysing the cis-aconitate levels that are formed from isocitrate at a wavelength of 240 nm (Miller & Griendling, 2002). The downside of this method is that aconitase is not O_2•- specific, because it can be inactivated by various ROS, and NO. Also, the process of aconitase-inactivation takes several hours up to a few days, which makes it difficult to increase its specificity (Armstrong & Whiteman, 2007).

Fingerprinting

A different approach to analyse the ROS levels, is by measuring the damage or cell response caused by ROS, instead of measuring the radicals themselves. This can be done using an alternative to trapping, which is called fingerprinting. This method can detect a unique 'fingerprint' left after a ROS has attacked a biological molecule. The amount of damage is then an indicator to the levels of ROS. This method can also be used to analyse the effects of antioxidants or other potential agents, since less damage would mean the treatment is more effective.

Lipid peroxidation is such an example of damage caused by ROS. Lipid peroxidation is the process where oxidants such as free radicals attack lipids that contain carbon-carbon double bonds. Polyunsaturated fatty acids (PUFAs) are especially attacked (Ayala et al., 2014). Detecting lipid peroxidation can be done using specific assays, such as the thiobarbituric acid reactive substances (TBARS) assay that can detect products of lipid peroxidation, lipid hydroperoxides, and aldehydes (Potter et al., 2010).

Table 1: Spectrophotometric methods for detecting O2•-.

Compound used	Nitro Blue Tetrazolium (NBT)	Cytochrome-c	Aconitase
Process	NBT transformed into monoformazan by O_2•-	Cytochrome-c Transformed into reduced cytochrome-c by O_2•-	Catalyses transformation of citrate into isocitrate.
Detection	Momonoformazan levels detected at a wavelength of 550-560nm	Reduced cytochrome-c levels detected at a wavelength of 550nm	Aconitase activity measured due to the formation of cis-aconitate from isocitrate at a wavelength of 240nm
Pros	• O_2•- activity can be measured through monoformazan	• O_2•- activity can be measured through reduced cytochrome c • CN-, ROS scavengers, and urate can prevent unwanted compounds to react with the cytochrome c • Specificity can be increased by measuring SOD-inhibitable cytochrome c, or by using acetylated or succinoylated forms of cytochrome c	• O_2•- activity can be measured through aconitase
Cons	• NBT radical intermediate can generate O_2•- by itself • NBT not only reduced by O_2•-	• Cytochrome c reduction is not specific for O_2•-	• Aconitase is not O_2•- specific • Process takes several hours up to a few days

DNA damage is another detectable form of damage done by ROS. Nitration of tyrosine residues of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments are examples of mechanisms of oxidative damage. Measuring such DNA damage can be done by measuring the metabolic rate since it is directly related to the rate of oxidative DNA damage (Hemnani and Parihar, 1998).

Lipids, DNA, and proteins are very useful as biomarkers since damage on those specific compounds can cause the most severe damage. With these biomarkers specific damaging sites can be marked and analysed (Halliwell and Gutteridge, 1999). This is useful since it is often more important to know what is damaged by ROS than it is to know how many ROS are in the cell, since a lot of the molecules will react in an unimportant matter (Halliwell & Whiteman, 2004).

Detection of Reactive Oxygen Species in Mitochondria

As discussed before, mitochondria are the most common source for ROS production. Therefore, methods to detect ROS specifically inside the mitochondria are essential to discuss. In order to detect H_2O_2 in mitochondria, the Amplex red reagent is used. This substance, in the presence of horseradish peroxidase (HRP), will react with H_2O_2 and create a fluorescent resorufin product. The fluorescence of this product can then be detected and analysed. In comparison to H_2O_2, the detection of O_2•- is done by using DHE. DHE will react with O_2•- to form a fluorescent substance that can be detected and analysed. The excitation wavelength at which the fluorescence can be detected is 520nm, and its emission wavelength is 610nm (Armstrong & Whiteman, 2007).

Even though there are multiple ways to detect ROS, there are some problems with detecting ROS that should be considered. Firstly, cell culture itself induces oxidative stress through aid of ROS generation and by limiting the adaptable upregulation of cellular antioxidants. Secondly, obtained results can be confound by free radical reactions taking place in the culture media. Knowing what reactions take place in the culture medium alone, before cells are added, is therefore very important (Armstrong & Whiteman, 2007).

To get around these problems, the aromatic compounds or spin traps to study ROS production in cells can be used. However, it is possible that rapid decrease of free radical-spin trap will produce electron spin resonance (ESR)-silent species. This is done by use of nonenzymatic antioxidants and cellular enzymatic reducing systems. Armstrong & Whiteman, 2007, found an interesting combination to be 5-[(2-carboxy)phenyl]-5-hydroxy-1-(2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)methyl-3-phenyl-2-pyrrolin-4-one sodium salt. This is a nonfluorescent nitroxide. However, when it combines with ROS, the nitroxide is removed which will lead to ESR signal loss and restoration of fluorescence (Armstrong & Whiteman, 2007).

Promising Solutions

Post-Ischemic Enriched Environment

In order to reduce post-ischemic reperfusion damage, the damaging processes that happen during the reperfusion need to be limited. Dorfman et al. (2013) studied the effect of a post-ischemic enriched environment (EE) compared to a standard environment (SE) on the

retina of adult rats. An EE refers to environmental conditions that facilitate sensory, cognitive, motor, and social stimulation compared to standard conditions. The study was done by inducing ischemia, through elevating the intraocular pressure, and by intravitreal injection of supraphysiological levels of glutamate. This partially reproduced retinal alterations normally induced by post-ischemic reperfusion damage. Glutamate was used, since it is the main excitatory neurotransmitter in the retina, and is toxic in excessive amounts. The study showed that EE housing significantly protected retinal function and histology from post-ischemic reperfusion damage, which likely occurred through a glutamate-dependent mechanism. This method could be promising, however it has to be altered for different tissues, since the main excitatory neurotransmitters differ for different tissues.

Radical Trapping

Another method that could decrease the effects of ROS is by trapping the radicals. Spin trapping, as discussed earlier, is a way to form a more stable radical that can be detected. However, there are various other forms of trapping that can be done which can restrain the ROS molecules and thereby inhibiting oxidative damage in cells (Halliwell and Gutteridge, 1999). The idea behind this is to use trapping molecules specific to the radical that needs to be trapped. It would have to react fast with the radical and have no negative effect on surrounding mechanisms. This would cause the radical to be immobile and therefore cause no damage. A study from Abe et al. (2015), showed [(3)H]hydromethidine to be a useful scavenger for detecting ROS generation such as hydroxyl radical. Melatonin might also be a possible scavenger for detecting ROS, although research results vary. For example, research done by Zang et al. (1998) showed that melatonin inhibited DMPO-O formation depending on the dose, and that it did not show a significant effect on OH•. However, a study done by Reiter et al. (1994) shows promising results for melatonin as a free radical scavenger. Therefore, research on specific potential scavengers should be done before executing this method. Once tailored to the specific radicals that need to be trapped, this method shows a lot of promise in reducing or limiting post-ischemic reperfusion damage.

Manganese-Porphyrin Complexes

Wu et al. (2007) studied the effect of manganese-porphyrin complexes on reperfusion damage in isolated perfused rat livers. Since metalloporphyrins (MP) are stable catalytic antioxidants with the ability to scavenge superoxide, hydrogen peroxide, peroxynitrite and lipid peroxyl radicals, their potentially reducing effect on post-ischemic reperfusion damage is interesting to research. The team used three different manganese-porphyrin (MnP) complexes that each had a different catalytic activity. The three MnP complexes the authors used were manganese tetrakis-(N-ethyl-2 pyridyl) porphyrin (MnTE-2-PyP), manganese tetrakis-(N-methyl-2 pyridyl) porphyrin (MnTM-2-PyP), and manganese tetrakis-(ethoxycarbonyl) porphyrin (MnTECP). Their research showed that the MnP complexes can attenuate hepatocellular damage, lipid peroxidation and protein nitration from post-ischemic reperfusion damage in isolated perfused mouse livers, with MnTe-2-PyP being the most effective MnP complex. This, in combination with the various antioxidant properties of MP's make them a very promising therapy for injuries caused by post-ischemic reperfusion damage, as it occurs after organ transplantation.

Antioxidant Systems

As discussed earlier, oxidative stress is caused by a disbalance between the production of oxidants and antioxidants, where there is too much of the first, and too little of the second. Antioxidant defence systems are in place to prevent this. The antioxidant systems consist of nonenzymatic and enzymatic parts. The nonenzymatic components glutathione (GSH), ascorbic acid, vitamin E, uric acid, and ubiquinol could reduce the reactivity of ROS through donation of electrons, thereby transforming into less reactive free radicals themselves (Oxidative Stress, 2021). GSH hereby being the most crucial nonenzymatic antioxidant. The antioxidant enzymes include catalases (CATs), superoxide dismutases (SODs), glutathione peroxidases (GPxs), glutathione reductases (GRs), peroxiredoxins (Prxs), glutaredoxins (Grxs), and thioredoxins (Trxs). These enzymes reduce harmful ROS into less harmful components. For example, catalase, GPxs, and Prxs break various SOD down into H_2O (Oxidative Stress, 2021). Within the body, modulation of transcription of specific genes modulates the antioxidant defence system against oxidative stress. Since the problem during post-ischemic reperfusion damage is the fact that cellular redox homeostasis is lost, a promising therapy would be to modulate the antioxidant defence systems in such a way that more antioxidants would be produced after post-ischemic reperfusion damage, thereby regaining homeostasis and thus limiting oxidative damage. Another way would be to insert antioxidants into the body, which would be taken up by the cell, and that way regain homeostasis.

Discussion

There are different ways to detect the effects of ROS during post-ischemic reperfusion damage. Each method having its own positives and negatives. ROS can be detected though ESR quite well, however the detectable products disappear fast, and various other unwanted reactions can take place inside the cell, which make the results difficult to interpret and requires a very good control. Techniques based on fluorescent dyes are useful in detecting oxidative stress, and O_2•-, but are not specific enough to detect H_2O_2. Spectrophotometric methods are broadly used and accepted techniques to measure extracellular O_2•- production, however the compound used for detection has to be very specific, since it will otherwise not give the right results. Cytochrome c, in this case, might be the most promising compound, because its specificity can be improved using compounds such as CN-, ROS scavengers, and urate. The compounds Amplex red reagent, and DHE used for detection of ROS inside the mitochondria might be a promising method, since they are H_2O_2 and O_2•- specific respectively. Fingerprinting, which assesses the damage done instead of the radicals themselves, might also be a promising detection method. When combined with fluorescent biomarkers, the specific damage sites and the levels of ROS can be analysed simultaneously which will give useful information. Beside detecting ROS, the effectiveness of a treatment can also be analysed using fingerprinting. When less damage is analysed after using a specific treatment, this would lead to the interpretation that the treatment might be effective. Whichever method is used, it is important to think about, and understand, how the method works exactly, what its limitations are and what possible result confounders can be. Understanding of these aspects allows better interpretations of the results and errors can be minimized.

Beside different methods of ROS detection, different possible solutions have also been discussed. A post-ischemic enriched environment showed to be effective on the retina of adult rats. However, the process has to be altered for specific situations, and not enough research has been done on this method yet to know its effectiveness on other tissues. The reduction of the effects of ROS by radical trapping shows a lot of promise since, once more research has been done to specific scavenger molecules, the method can be tailored to the specific radicals that need to be trapped. Once trapped, and made immobile, the post-ischemic reperfusion damage would be minimized. The use of manganese-porphyrin complexes, specifically the MnTe-2-PyP MnP complex, has also been shown to be a promising solution since it showed an attenuation effect on hepatocellular damage, lipid peroxidation and protein nitration. The last possible solution discussed was using antioxidants, the body's own defence system against oxidative stress. The amount of antioxidants would be increased inside the cells, either by altering transcription of certain genes, or by externally adding it to the cells. This would make sure disbalance between the production of oxidants and antioxidants is restored, and damage is minimized.

Looking over all the detection methods and possible solutions, keeping in mind that the most promising way of decreasing post-ischemic reperfusion damage caused by ROS needs to be found, a combination of MnP Complexes and antioxidants seems to be the best way to reduce the damaging effects caused by ROS. Since antioxidants are the body's own way to fight oxidative stress, facilitating this defence system to function better might be the best tactic. Modulating the body's gene regulation to produce more antioxidants might take too long, causing ROS to create more damage. Therefore, externally inserting antioxidants into the cells might be the most optimal way. Additionally, MnTe-2-PyP MnP complex could then be used for potential damage that has already been done before homeostasis could be regained. This combination of antioxidants and MnP complexes would then regain lost homeostasis and decrease damage that has already been done.

References

Abe, K., Tonomura, M., Ito, M., Takai, N., Imamoto, N., Rokugawa, T., Momosaki, S., Fukumoto, K., Morimoto, K., & Inoue, O. (2015). Imaging of reactive oxygen species in focal ischemic mouse brain using a radical trapping tracer [(3)h]hydromethidine. Ejnmmi Research, 5(1), 115–115. https://doi.org/10.1186/s13550-015-0115-1

Armstrong, J. S., & Whiteman, M. (2007). Measurement of reactive oxygen species in cells and mitochondria. Mitochondria, 2nd Edition, 80, 355–377. https://doi.org/10.1016/S0091-679X(06)80018-X

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxidative Medicine and Cellular Longevity, 2014, 1–31. https://doi.org/10.1155/2014/360438

Dorfman D., Fernandez, D. C., Chianelli Mónica, Miranda, M., Aranda, M. L., & Rosenstein, R. E. (2013). Post-ischemic environmental enrichment protects the retina from ischemic damage in adult rats. Experimental Neurology, 240, 146–156. https://doi.org/10.1016/j.expneurol.2012.11.017

Flescher, A. M. (2018). The organ shortage crisis in america : incentives, civic duty, and closing the gap. Georgetown University Press.

Halliwell, B., and Gutteridge, J. M. (1999). ‘‘Free Radicals in Biology and Medicine.’’ Oxford University Press, UK.

Halliwell, B., & Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean?: measuring reactive species and oxidative damage. British Journal of Pharmacology, 142(2), 231–255. https://doi.org/10.1038/sj.bjp.0705776

Hemnani, T., & Parihar, M. S. (1998). Reactive oxygen species and oxidative DNA damage. Indian journal of physiology and pharmacology, 42(4), 440–452.

Huang, C., & Zhang, Y. (Eds.). (2021). Oxidative stress : human diseases and medicine. Springer. https://doi.org/10.1007/978-981-16-0522-2

Khalil, A. A., Aziz, F. A., & Hall, J. C. (2006). Reperfusion injury. Plastic and Reconstructive Surgery, 117(3), 1024–33.

Miller, F. J., & Griendling, K. K. (2002). Functional Evaluation of Nonphagocytic NAD(P)H Oxidases. Methods in Enzymology, 220-233. https://doi.org/10.1016/s0076-6879(02)53050-0

Nathalie, B. V., Javier, E. D. Z., Javier, F. A., Enrique, V. P., & Víctor H Casco. (2007). Photobleaching correction in fluorescence microscopy images. Journal of Physics: Conference Series, 90(1). https://doi.org/10.1088/1742-6596/90/1/012068

Potter, T. M., Neun, B. W., & Stern, S. T. (2010). Assay to Detect Lipid Peroxidation upon Exposure to Nanoparticles. Methods in Molecular Biology, 181–189. https://doi.org/10.1007/978-1-60327-198-1_19

Reiter, R. J., Tan, D.-X., Poeggeler, B., & Menendez-Pelaez, A. (1994). Melatonin as a free radical scavenger: implications for aging and age-related diseases. Annals- New York Academy of Sciences, 719, 1–1.

Roberto, F., Cristina, B., Michele, M., Gabriele, G., Giampaolo, M., Matteo, B., Simone, B., & Gianluca, C. (2017). Reperfusion damage : a story of success, failure, and hope. Circulation Journal : Official Journal of the Japanese Circulation Society, 81(2), 131–141.

Wu, T.-J., Khoo, N., Zhou, F., Day, B., & Parks, D. (2007). Decreased hepatic ischemia-reperfusion injury by manganese-porphyrin complexes. Free Radical Research, 41(2), 127–134.

Zang, L.-Y., Cosma, G., Gardner, H., & Vallyathan, V. (1998). Scavenging of reactive oxygen species by melatonin. Bba - General Subjects, 1425(3), 469–477. https://doi.org/10.1016/S0304-4165(98)00099-3

Afterword

I would like to thank my loving family and friends for their support during this period. Especially I would like to thank my supervisor (Dr. R. (Romana) Schirhagl) and co-supervisor (Yue Zhang) for their guidance, revision, and suggestions. They helped me get the best work out of the time we had, and were always there whenever I needed them. Working with them has helped me learn a lot and increase my research skills.

Marith Elise den Otter
June 2022