Redox Probing for Oxidative Stress in the Plasma Samples of Healthy vs. Schizophrenia Patients

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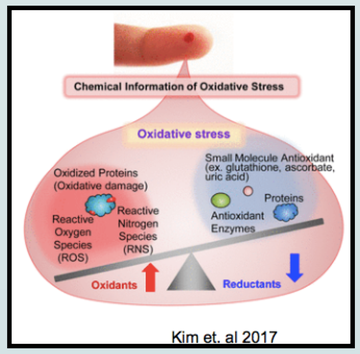
Background

Increasing evidence links oxidative stress to schizophrenia Oxidative stress is the imbalance between free radicals in the human body and antioxidants which neutralize the damage caused by free radicals (Figure 1).
Schizophrenia is a debilitating mental disorder which affects approximately 1% of the population. The hallmark symptom of schizophrenia is psychosis (hallucinations and delusions), and is not typically seen until a patient's late teens or early twenties. Recent studies hypothesize that the failure of antioxidant defenses to protect against free-radical generation damages cell membranes, with resulting dysfunction that might impact on neurotransmission and, ultimately, lead to symptomatology in schizophrenia.
Objective: The goal of this experiment is to develop an easy, clinical measure of oxidative stress through plasma probing. Because increasing evidence reveals schizophrenia is highly related to oxidative stress, this test would potentially provide a biometric of psychosis/schizophrenia through blood tests. The Ir-reducing assay could discriminate between healthy and schizophrenia patients and correlate to disease severity. The plasma samples that are being tested are from various patients with schizoaffective disorder and schizophrenia, and have been extensively phenotyped with cognitive and brain based biomarkers.

FIGURE 1: Oxidative Stress is an imbalance of free radicals such as reactive oxygen or nitrogen species, which naturally occur in the human body and antioxidants, which protect the body from free radical damage. Oxidative stress markers could potentially be detected in the plasma samples of patients.



Possible Outcomes & Significance

This plasma assay could potentially be used to diagnose patients with psychosis if the measure of oxidative stress can quantitatively differentiate between healthy and schizophrenia patients.

This discovery could be leveraged by pharmaceutical companies to develop a treatment that reverses oxidative stress through antioxidants, and predict if this diminishes the effects of psychosis.

The outcomes from this experiment could provide guidance on future experiments. One could observe the results on a cellular level and see if the strong antioxidant glutathione can reveal markers of oxidative stress in the cells of people with psychosis.

Methods

The basis of the experiment is to use an Iridium-based strong oxidant K_2IrCl_6 which can detect reducing species. As electrons are transferred from the reducing species or antioxidants in the plasma to Ir ox, and optical signal is generated (Figure 2). The strength of the signal is dependent on the strength of the reductant and also correlates to the amount of oxidative stress. We hypothesize that schizophrenia patients will have more oxidative stress, which could potentially be revealed by this simple analysis of a patient's plasma sample.
The assumption must be made that the chemical information on oxidative stress is present in plasma and can be accessed by appropriate measurements. Glutathione (GSH), a known strong antioxidant, was tested and acted as the positive control. K_3IrCl_6, which is the 100% reduced form of the iridium salt acted as the negative control.

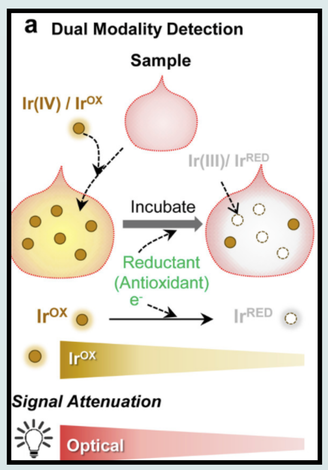


Figure 2: Plasma sample is mixed wth dilute iridium salt and incubated. Increased oxidative stress specific to each plasma sample is indicated by a greater color change from yellow to clear.

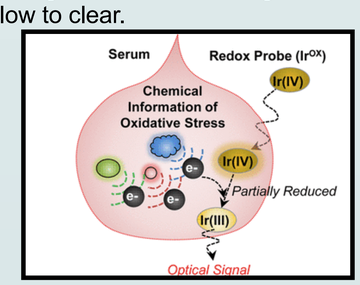


Figure 4: The electrons present in the plasma samples from the free radicals due to oxidative stress will be attracted to the redox probe (iridium salt). As the iridium salt accepts electrons and gets reduced it will change from yellow to clear. The amount of oxidative stress will correlate to the color change will be measured by an optical signal.

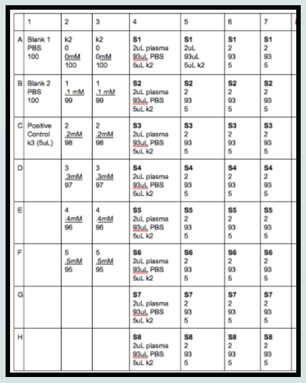


Figure 3: Sample Run sheet of 96 well plate.

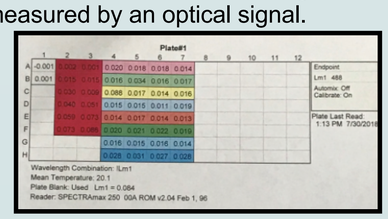


Figure 5: Sample Data obtained from microplate reader.

Ir-Reducing Capacity Assay in Plasma Protocol:
1.Prepare 96 well plate
2.2 blanks, Standard K2IrCl6 dilution curve (0, 0.1mM, 0.2mM, 0.3mM, 0.4mM, 0.5mM), plasma samples in quadruplicate
3.Each plasma well containing:
- i) 2 µL of diluted serum
- ii) 93 µL of 0.1M PBS
- iii) 5 µL of 10mM K2IrCl6

4. Mixing by pipetting
5.Incubate 30 minutes room temp.
6.Optical response: color change yellow→ clear
7.i) measure absorbance at 488 nm using microplate reader
Samples consisted of healthy controls (HC) (n=36), patients with schizoaffective disorder (SAD) (n=29), and patients with schizophrenia (SZ) (n=32).

Results

Figure 6: Average Redox values in each group: healthy controls, schizoaffective disorder and schizophrenia were compared. The psychosis groups revealed significantly more oxidative stress.

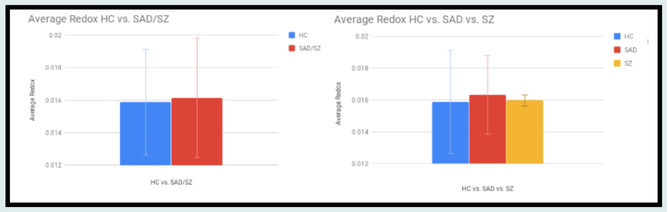
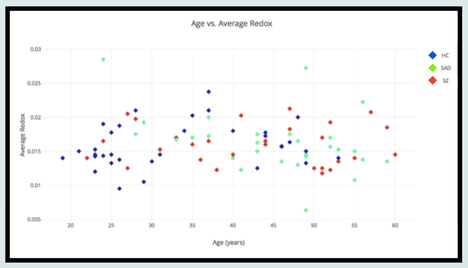


FIGURE 7: Average Redox values in healthy controls, and psychosis groups were sorted based on site of plasma collection. Results revealed great variability in redox values based on site: Dallas vs. Chicago.

Figure 8: A plot of age versus average redox was constructed to visualize results between healthy controls vs. schizoaffective disorder. vs. schizophrenia. As age increases oxidative stress tends to increase. Most healthy controls appeared to be young.



Analysis:
Univariate ANCOVA in SPSS
Covariates: Age, Site, Day of experiment
Fixed Factor: DX1 (HC vs SZ/SAD) or DX2 (HC vs SAD vs SZ)
Dependent Variable: Average Redox (x 4 reads/sample)
DX2: F=2.999 p=.055 DX1: F=4.917 p=.029

Conclusions & Future Directions

- Several factors were identified as likely confounds: iridium salt degradation, plasma degradation, collection site, age.
- These factors were included as covariates in analyses in order to account for variance not explained by diagnosis group.
- Plasma was tested instead of serum, which is different from the original protocol and may have an immeasurable effect on results. Future research should compare serum and plasma from the same individuals.
- **Once all factors are accounted for, the Healthy Control group demonstrated significantly more conversion of the K_2IrCl_6 than the SZ/SAD group, indicating more resilience to oxidative stressors.**

In the future, continue this initial study:
- Increase sample size
- Look at Biotypes
- Integrate with clinical data (psychosis symptoms, cognitive ability & sensorimotor tests)
- Observe oxidative stress at the cellular level *in vitro*

References

Kim, Eunkyoung, Winkler, Thomas E., Kitchen, Christopher, Mijeong Kang, George Banis, William E. Bentley, D Deanna L. Kelly, Reza Ghodssi, and Gregory F. Payne. Redox Probing for Chemical Information of Oxidative Stress Analytical Chemistry 2017 89 (3), 1583-1592 DOI: 10.1021/acs.analchem.6b03620

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Acknowledgments

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