

# EFFECT OF PROCAINAMIDE ON THE OXIDATION OF HYDROGEN PEROXIDE

Systemic lupus erythematosus (SLE) is an autoimmune disease whose causes are so far still unknown. One variation of this disease, drug-induced lupus (DIL), occurs after patients begin a regimen of drugs used to treat hypertension, thyroid conditions, or heart conditions. Only about 20% of SLE patients are afflicted with DIL and, after the medication is discontinued, DIL usually disappears. In this study, the effects of procainamide, an anti-arrhythmic drug, were examined. About 30% of all patients who take procainamide are afflicted with DIL.

This project tested the hypothesis that procainamide causes lupus by altering the reactivity of endogenous hydrogen peroxide, thus causing the denaturation of cellular proteins. No living organisms were used in this project; a chemical reaction was studied using either procainamide or a control (water). The chemicals used were: procainamide; hydrogen peroxide; a phosphate buffer, dithiobisnitrobenzoic acid (DTNB); ethylenediaminetetra acetic acid (EDTA); and cysteine. The absorbance levels of the two chemicals were measured on the spectrophotometer, with ranges from 1.0 to 0, 0 being 100% absorbance of light, or colorlessness. By observing the rate at which hydrogen peroxide decolorized thionitrobenzoic acid (TNB), we could examine the effect of procainamide on the hydrogen peroxide to determine how the chemicals in the cuvettes interacted to cause a state of DIL.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that occurs when antibodies designed to attack antigens attack the nuclei of healthy cells instead. There are many variations of SLE, including drug-induced lupus (DIL) which affects up to 20% of patients taking drugs meant to treat hypertension, heart conditions, and thyroid conditions.

It has been shown that more than 30% of patients who take procainamide, a drug used to treat arrhythmia, develop lupus-like symptoms after at least one year. Interestingly, when the medication that caused DIL to occur is discontinued, DIL almost always disappears. It is this fact that prompted investigations examining the changes the body undergoes after being exposed to the lupus-inducing drug.

## METHODS

No living organisms were used in this project; chemicals were used to replicate conditions in a human body. Phosphate buffer (pH 7.4) was used as a solvent because it has a pH similar to blood. Dithiobisnitroic acid (DTNB) is a reagent that detects sulfhydryl groups in proteins. Cysteine is a nonessential amino acid that contains sulfur. It was used to prepare thionitrobenzoic acid, which can be used to measure peroxide reactivity. Procainamide was added to help simulate a person on the drug. The hydrogen peroxide was used to replicate the body's production of a small, non-lethal amount of hydrogen peroxide.

It is thought that, when procainamide is taken by a patient, the combination of the medication and the chemical causes the procainamide to be

Student Researcher: Larhondé Sealey  
Mentor: Donald A. Gerber, MD

metabolized by hepatic microsomes in the liver, and changed into hydroxylamine (PAHA) and nitroso (nitroso-PA) metabolites. These metabolites then bind to histone protein (an antigen to which most auto-antibodies in procainamide-induced lupus bind) and are toxic to lymphocytes. As the experiment was modified, ethylenediaminetetra acetic acid (EDTA) was added to test if any of the previous tests that had been performed without it, were metal sensitive. EDTA removes traces of metal (catalysts) from the solutions in this project that potentially affect the outcome of the experiments. These chemicals, with the exception of the procainamide were combined, and then separated into two cuvettes (specially designed test tubes). A small amount of procainamide was then added to one cuvette, and to the other, an equal volume of distilled water. These tubes represented a patient on procainamide and one on no medication, respectively. A yellow hue, a result of the cysteine, was apparent after the chemicals were combined. The cuvettes were then inserted into a spectrophotometer to measure the absorbance levels of the solutions.

As time passed, the color of the chemical mixtures in the cuvettes lightened, from a dark yellow to a transparent liquid. As a result, the absorbance level numbers taken at each interval became smaller, eventually reaching zero. At this point the mixtures were completely colorless. In the tests, when numbers were high (eg, .40), it meant that the peroxide had not begun to work on the cysteine, and the solution was still very yellow. When zero or colorlessness was reached, the peroxide had eliminated the cysteine and had nothing else to work on. We analyzed the measurements taken during the experiments

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From the State University of New York Health Science Center at Brooklyn (Downstate Medical Center); Brooklyn, New York.

Avg. Absorbance Levels

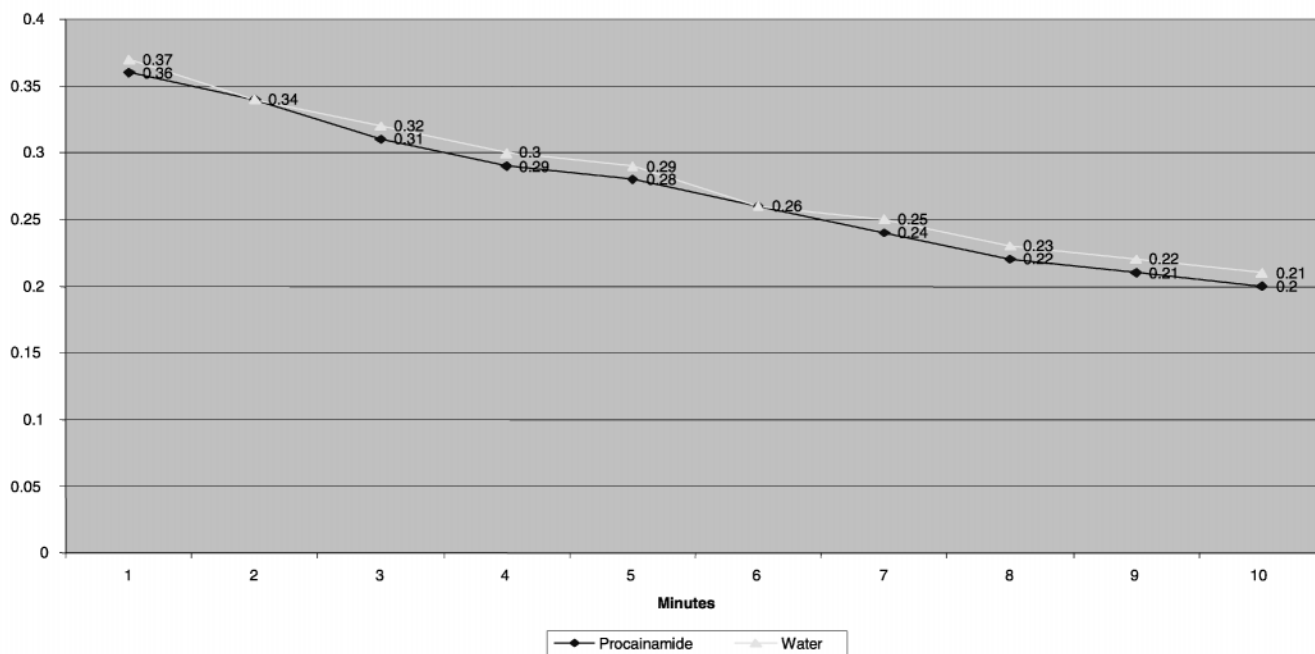


Fig 1. Average absorbance levels for all format 1 tests (min. 1–10)

to determine their significance and the differences between chemicals that mimicked a body in which no medication was present and one in which procainamide was present. These results helped us to determine what interactions took place between the hydrogen peroxide and the procainamide and how they might lead to the chemicals' simulation of a body afflicted with DIL.

### Data Analysis

Figure 1 illustrates the average absorbance levels for all 21 Format 1 tests in the first 10 minutes of all tests. Format 1 tests include: 6mL phosphate buffer, 200λ DTNB, 150λ cysteine, 50λ peroxide solution, mixed together, and split into two cuvettes. 50λ procainamide is then added to one cuvette and 50λ distilled water to the other. The solutions containing procainamide experienced, on average, a 44% decrease. The solutions containing distilled water experienced, on average, a 43% decrease.

Figure 2 illustrates the average absorbance levels for all 14 Format 2 tests

in the first 10 minutes of all the tests. Format 2 tests include: 6mL phosphate buffer, 200λ DTNB, 150λ cysteine, 50λ peroxide solution, mixed together, and split into two cuvettes. 150λ procainamide is then added to one cuvette and 150λ distilled water to the other. This test differs from Format 1 in that the amount of procainamide was tripled. The solutions containing procainamide experienced, on average, a 50% decrease. The solutions containing distilled water experienced, on average, a 51% decrease.

Figure 3 illustrates the average absorbance levels for all 10 Format 3 tests. Format 3 tests include: 6mL phosphate buffer, 200λ DTNB, 200λ EDTA, 150λ cysteine, 50λ peroxide solution, mixed together, and split into two cuvettes. 150λ procainamide is then added to one cuvette and 150λ distilled water to the other. This test differs from Format 2 in that EDTA was present. The solutions containing procainamide experienced, on average, a 38% decrease. The solutions containing distilled water

experienced, on average, a 36% decrease.

## RESULTS

In two out of the three analyses, the solutions containing procainamide had higher average absorbance levels than the solutions containing water. The higher levels seen in the procainamide graphs seem to suggest that in those reactions involving procainamide, hydrogen peroxide exists for a longer period of time, making it incapable of doing more damage to the body. Because peroxide lasts longer in procainamide, it can diffuse into the rest of the solution before being reduced.

Format 1 tests, however, is contrary to this theory in that the averages of both sets of solutions did not differ greatly from each other. This most likely occurred because of the small amount of procainamide and water (50μL) added to the respective solutions.

We found a more pronounced difference in the other two analyses for two

Absorbance Levels

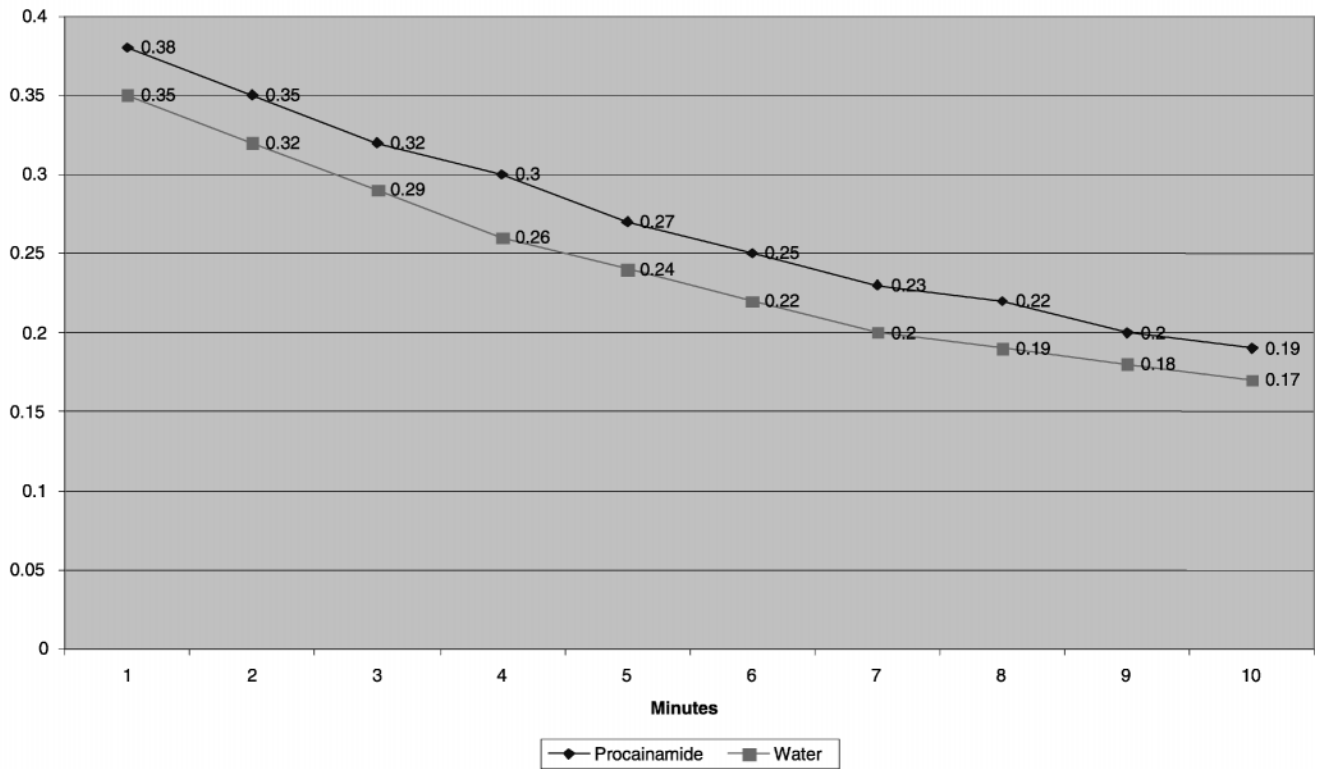


Fig 2. Average absorbance levels for all format 2 tests (Min. 1–10)

Absorbance Levels

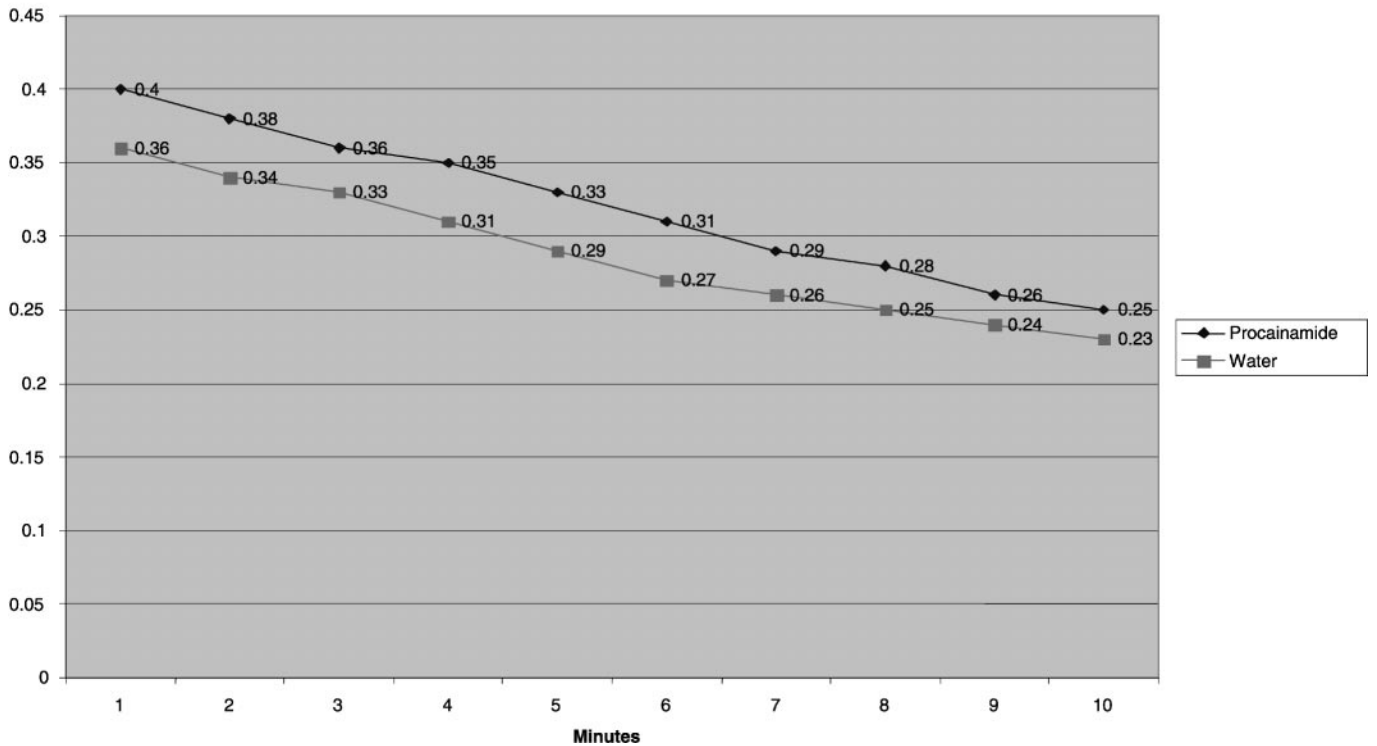


Fig 3. Average absorbance levels for all format 3 tests

main reasons. In the Format 2 tests, the amount of procainamide and water added to the respective solutions was tripled, from 50 $\mu$ L to 150 $\mu$ L. This increase led to more distinctive patterns in the absorbance levels for all the tests. The addition of EDTA (200 $\mu$ L) led to the creation of the Format 3 tests. The EDTA removed any trace metals that might have remained even after reactiv-

ity had ceased, and caused an alteration in the previous test results.

## CONCLUSION

These results, showing that procainamide has an effect on the existence on hydrogen peroxide, supported our hypothesis that procainamide causes lupus

by altering the reactivity of endogenous hydrogen peroxide, thus causing the denaturation of cellular proteins.

## ACKNOWLEDGMENTS

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