Investigation into the Toxicity of N-Acetylcysteine Amide on a T-lymphoblastic Cell Line

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Abstract

The diagnosis and treatment of schizophrenic patients are inadequate and patients suffer from a reduced life expectancy. The diagnostic methods of today are imprecise and the mental disorder itself results in e.g. secondary cardiovascular diseases that are only reinforced by the obligatory psychotropic medicine prescribed. In this study, the toxicity of N-Acetylcysteine Amide (NACA) was tested as well as its effect on cell proliferation. This was examined by the Bürker method regarding cell culture viability. NACA did not demonstrate any toxic properties, merely a slower cell proliferation. Therefore, NACA is a possible future medicine for the treatment of oxidative stress, a biological response to psychosis. Further, large scale testing of NACA is necessary. This needs to be done in order to reduce the risks of secondary diseases and side effects from medication, hence prolonging the life expectancy of the patients and reducing the intracellular oxidative stress.

Contents

Abbreviations

NACA	N-Acetylcysteine Amide
NAC	N-Acetylcysteine
ROS	Reactive Oxygen Species
CEM	Cell Line Human T-cells
DMSO	Dimethyl Sulfoxide
RPMI	Roswell Park Memorial Institute
FBS	Fetal Bovine Serum
PS	Penicillin-Streptomycin

1 Introduction

Individuals with major mental disorders live, on average, 20 to 30 years shorter than the general population. In schizophrenic and bipolar patients this is primarily due to cardiovascular diseases. The risk factors associated with cardiovascular diseases may also be induced or exacerbated in patients with schizophrenia or bipolar disorder due to psychotropic drugs. Patients with schizophrenia have a 40 % increased risk of death from various other medical causes compared to the general population, some related to antipsychotic treatment being hypertension, hyperglycaemia, diabetes and weight gain. [1]. Schizophrenia and bipolar disorder are also associated with increased oxidative and inflammatory stress [2]. In order to prolong the life expectancy of these patients it is vital to diagnose and treat the disorders early on in order to diminish the secondary diseases of the disorders and the side effects of medications obligatory for the treatment of these.

N-Acetylcysteine (NAC) is an antioxidant precursor to glutathione. Glutathione is a primary endogenous antioxidant. Glutathione neutralizes, for instance, reactive oxygen species (ROS) and is responsible for maintaining the cellular oxidative balance. Oxidative stress is, as previously mentioned, elevated in individuals suffering from schizophrenia and bipolar disorder. A study on the benefits of NAC as psychiatric medication concludes that schizophrenic patients treated with this medicine showed improvements in self-care, insight, motivation, social interaction, volition, stabilization of mood and psychomotor stability. Because of the similar chemical structures of NAC and NACA, the latter is believed to function analogously, as displayed in Figure 1 [3]. The purpose of this study is to establish whether NACA could operate as a medicine. The toxicity of NACA is measured as well as the influence of NACA on cellular proliferation. Toxicity and influence on cell proliferation are two important factors to test pre the development of a medicine.

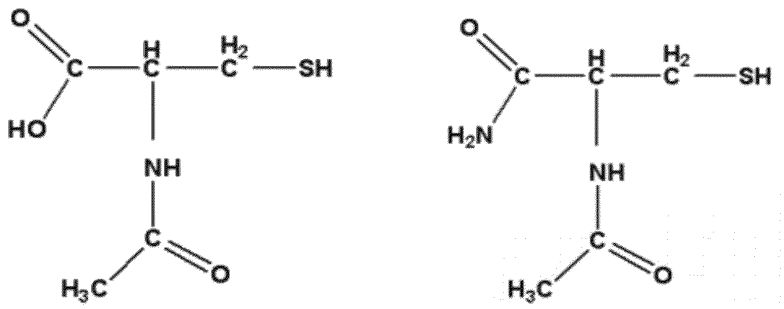

Figure 1: Chemical Structures of NACA and NAC [4].

2 Method

A cell culture consisting of CEM cells, specifically a T-lymphoblastic cell line, was grown in order to test the toxicity of the chemical NACA in human blood. Live cells possess intact cell membranes that exclude many chemicals, among these; trypan blue. Dead cells do not have these impermeable membranes which results in the blue dyeing of their cellular cytoplasma. Therefore, the toxicity of NACA can be derived from a cell viability test and use of trypan blue. Another factor that was studied, was the effect of NACA on cellular proliferation. These facts could later lead to new medicine and recommended values of ingestion of the compund. NACA was diluted in dimethyl sulfoxide (DMSO), which has proven to be cytotoxic in high concentrations.

The cellular proliferation method was used in a small scale experiment. The cells were grown in a solution consisting of RPMI 1640 medium with 10% FBS and 1% PS. The cells were stored in a cell incubator with a consistent humid environment with a temperature of 37°C and 5 % carbon dioxide. When the cells were cultured 300 000 cells/ml were grown in a total amount of liquid of 3 ml. When the cells were divided into six wells consisting of the aforementioned solution and number of cells, NACA compound was added. The following concentrations of NACA were added respectively into each well; 0 (control),

0.00001, 0.0001, 0.001, 0.01 and 0.1 M. The highest concentration of DMSO was in the 0.1 M well, reaching a level of 1 % DMSO. In the control well there was no addition of DMSO and in the other wells the concentration of DMSO was less than one promille. After two days of incubation, more NACA compound of the same amount and concentration was added to each well. After a total of four days of incubation, the cells were counted and dyed with trypan blue in order to perform an exclusion test of cell viability.

Hereafter, 50 µl of trypan blue was added to each well and the liquid was mixed homogeneously. Afterwards, the cells were added to a Bürker chamber. The cells were counted respectively post dyeing using the Bürker chamber and method. The Bürker chamber consists of a number of A-squares containing 16 B-squares. The Bürker method involves counting the cells inside three A-squares and hereafter calculating the mean value of cells in the known amount of solution. Ergo, the extent of proliferation could be derived from these facts. The cells were counted visually with the use of a microscope in two categories: dyed and non-dyed, in order to establish the cell viability.

3 Results

None of the cells were dyed blue after two respective four days. The number of cells increased to separate extent, this is visible in Figure 2. The well without NACA contained 540 cells post two days and 2193 cells post four days. The wells containing NACA compund in the range of $0.00001 - 0.01$ M contained a mean value of approximately 497 cells/µl post two days and 1629 cells/µl post four. The highest concentration of NACA compound, 0.1 M implied cellular concentrations of 107 cells/µl post two days and 233 cells/µl post four days.

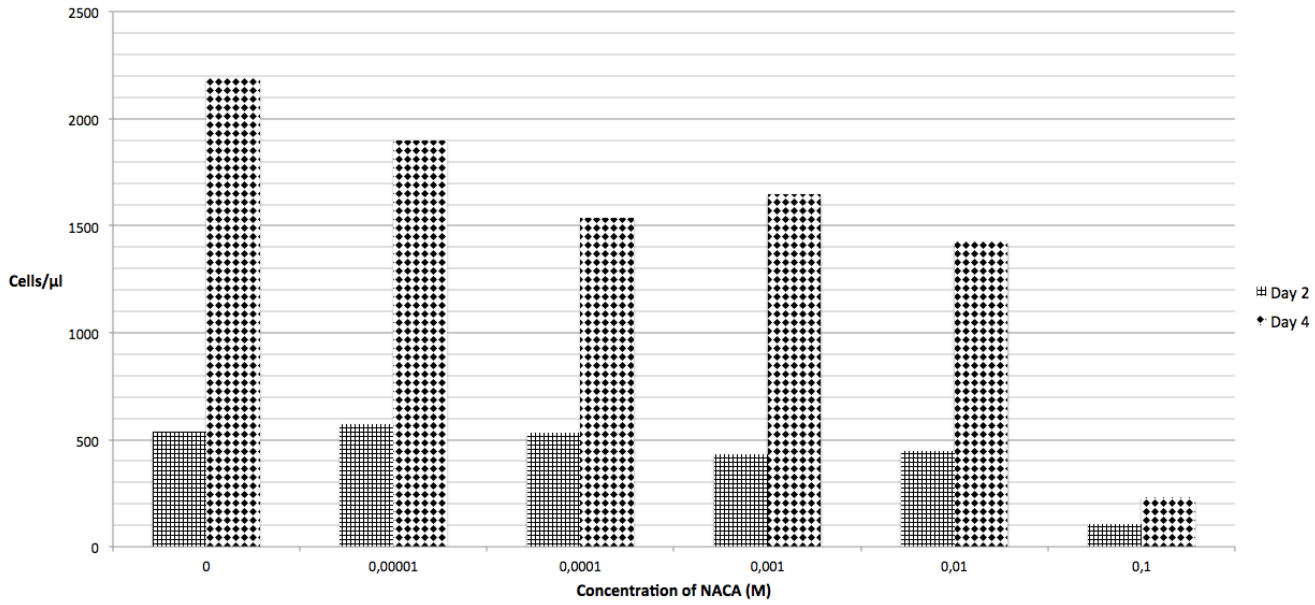

Figure 2: Bar Diagram Displaying Amount of Cells per Concentration and Day

4 Discussion

None of the cells were dyed blue which implies that the toxicity of NACA is neglectable. The control well, i.e. the untreated cells, serve as a comparison value for the cell proliferation. The control well contained 540 cells post two days and 2193 cells post four days. The wells containing NACA compund in the range of 0.00001-0.01 M contained a mean value of approximately 497 cells/µl post two days and 1629 cells/µl post four. This indicates that there is no significant difference in number of cells post two days but the difference in amount increases over time, as seen post four days. The highest concentration of NACA compound in the wells, 0.1 M, resulted in a very low number of cells reaching only 107 cells/µl post two days and 233 cells/µl post four days. The speed of cell proliferation can be derived from these facts, showing that it is greatly diminished in the highest concentration while only diminished slightly in the middle values. These factors are, of course, necessary to take into account when considering to launch a new medicine. The experiment also implies that the dosage should not exceed a certain amount in order not to affect the cell proliferation negatively. This medicine could also, depending on desire, positively lower the speed of cell proliferation.

The limited cell proliferation in the 0.1 M of NACA is probably due to the high concentration of dimethyl sulfoxide (DMSO) which has cytotoxic effects on cells. Therefore another chemical needs to be used as a dilutent in future cell culture experiments. The number of cells in the 0.001 M well was slightly divergent, this is probably due to the randomness of the placement of the cells in the Bürker chamber, hence the obtained amount.

The essential future research is required to focus on establishing the toxicity of NACA during sustained or permanent treatment of a patient. Future studies also involves establishing the exact effect of NACA on cell proliferation through large scale experiments. Toxicity and effect on cell proliferation in more fragile cells also needs to be conducted in order for NACA to, at some point, be prescribed as a medicine for oxidative stress.

NACA will probably also be used in the treatment of psychiatric and various other disorders and diseases where its effects are requested. NACA could serve as medicine against oxidative stress, antagonizing the cardiovascular diseases and prolonging the life expectancy of schizophrenic patients.

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References

[1] W. Newcomer J. Medical Risk in Patients With Bipolar Disorder and Schizophrenia. J Clin Psychiatry. 2006; 67(suppl 9):25-30.

[2] Ghanizadeh A, Berk M. Molecular Hydrogen: an overview of its neurobiological effects and therapeutic potential for bipolar disorder and schizophrenia. Medical Gas Research. 2013; 3(1): 11. Owe-Larsson B, Ekdahl K, Edbom T, Ösby U, Karlsson H, Lundberg C, Lundberg M. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011; 35: 1117-1121.

[3] Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. Journal of psychiatry and neuroscience. 2011; 36(2): 78-86.

[4] Goldstein G. N-acetylcysteine amide (NAC amide) for the treatment of diseases and conditions associated with oxidative stress.