

N-Acetylcysteine Amide (NACA) Reduces Cell Death after Oxidative Stress in a Porcine Embryonic Kidney Cell Line

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Abstract

Introduction: Oxidative stress may have detrimental effects on different structures of the cells, such as the DNA. Recently, we have published a study demonstrating that N-Acetylcysteine amide (NACA) displayed anti-inflammatory properties on the brain after exposure to oxidative stress in an established neonatal piglet model, imitating perinatal asphyxia. As different clinical studies have shown an association between the severity of hypoxic-ischemic encephalopathy and damage of the kidneys, we investigated a possible protective effect of NACA against H₂O₂-induced oxidative stress using a porcine epithelial-like embryonic kidney cell line (EFN-R). **Objective:** To investigate a potential protective effect of NACA on cells of a porcine embryonic kidney cell line exposed to H₂O₂. **Methods:** We subjected the cells to different concentrations of H₂O₂ for variable time periods, seeking the optimal dose-response for the experiments. Based on the results of these investigations, we exposed the cells to 100 μMol of H₂O₂ and/or 750 μM of NACA for 24 hours. Some of the cells would receive NACA either one hour before or one hour after exposure to H₂O₂. **Results:** The viability of the investigated EFN-R cells revealed that both, the group treated with NACA before exposure to H₂O₂ and the group treated with NACA after exposure to H₂O₂, exhibited significantly higher cell viability compared to the H₂O₂ group (p < 0.001 and p < 0.01, respectively). **Discussion:** The increased viability of the cells may indicate that NACA could play an important role in reducing oxidative stress. Taking the results from our previous study into consideration, our findings may streng-

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then the theory that NACA may have organ protective properties for neonates exposed to oxidative stress.

Keywords

N-Acetylcysteine Amide (NACA), Cell Lines, Oxidative Stress

1. Introduction

Reactive oxygen species (ROS) are important in different processes of the organism, including cell-signaling [1]. However, during oxidative stress, too much ROS is produced, which may have detrimental effects on different structures of the cells. ROS play a role as a mediator of apoptosis and may induce damage to the DNA. One important member of ROS is H_2O_2 , which may be harmful to the DNA and leads to cell injury.

Recently, we have published a study demonstrating that NACA displayed anti-inflammatory properties after exposure to oxidative stress in an established neonatal piglet model, imitating perinatal asphyxia [2]. Our piglet model has been established for many years and several authors have shown a significant increase in markers of oxidative stress after the inflicted asphyxia [3]. As different clinical studies have shown an association between the severity of hypoxic-ischemic encephalopathy and damage of the kidneys [4] [5], we decided to investigate possible protective effects of NACA using the porcine epithelial-like embryonic kidney cell line EFN-R exposed to H_2O_2 . Previous studies have shown that NACA may reduce the injury in epithelial kidney cells exposed to toxic doses of the antibiotic Gentamycin and the contrast agent Iohexol [6] [7]. Prior to the experiments with NACA, dose-response investigations of H_2O_2 were conducted to estimate the appropriate dose for the treatment of cells of a cell line.

2. Objective

To investigate a potential protective effect of NACA on cells of an embryonic kidney cell line exposed to H_2O_2 .

3. Methods

Cell culture: The porcine epithelial-like embryonic kidney cell line EFN-R (catalogue number CCLV-RIE 86) was generated and provided by courtesy of the Friedrich-Loeffler Institute, Federal Research, Institute for Animal Health, Greifswald-Insel Riems, Germany.

EFN-R cells were grown using Dulbecco's modified eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, Missouri, USA), 10% fetal bovine serum (FBS) and 1% streptomycin (Sigma-Aldrich, St. Louis, Missouri, USA). The cells were incubated in a cell chamber at $36.8^\circ C$ and 5% CO_2 . Cells were cultured when they were approximately 80% confluent. The confluence describes the percentage of

cells in growth. For subculturing, the cells were washed with PBS, trypsinated and incubated in the cell chamber for 3 minutes. Cells were then centrifuged at 3000 rpm for 3 minutes, and the pellet was dissolved in fresh cell medium.

A Bürker-chamber was used to calculate cell numbers. 100 µl of cell suspension together with 900 µl tryptan blue solutions (Life Technology, UK) was added onto a Bürker-chamber glass plate and covered with a slide and placed under a microscope. The amount of cells was counted in minimum of five squares, and the average value of number of cells per square was calculated. The cells were counted only on the top and the left edge of each square, to avoid cells be counted twice. The desired amount of cells was adjusted and transferred to plates for further experiments.

MTT: For measuring the number of viable cells the MTT-test was conducted. The MTT-test is a reliable, simple, and established method to measure cytotoxicity, proliferation, and activation in cell lines [8]. The MTT (3,4,5 dimethylthiazol-2,5 diphenyl tetrazolium, Sigma-Aldrich, St. Louis, Missouri, USA) viability assay is based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert MTT to a purple formazan precipitate. These mitochondrial succinate dehydrogenases may reflect the number of viable cells present. The resulting crystals are subsequently dissolved using dimethyl sulfoxide (DMSO) and the optical density of each well is measured using a multiscan acent plate reader (Thermo Electron Corporation, Waltham, Massachusetts, USA). Cells were exposed to H₂O₂ for 1 hour. After incubation, the medium was removed and 200 µl MTT was added and incubated for 1 hour. MTT was removed and 100 µl of DMSO was added. The cells in the control group received DMEM.

Cell line experiments: The EFN-R cells were used as model system for stress experiments. 150.000 cells were seeded in each well in a 12-wells plate. The cells were starved for 24 hours and the confluent cells were treated with H₂O₂ for various incubation time and concentrations. In the dose-response investigation of H₂O₂ we sought the preferable dose of H₂O₂ to be used in the main experiment. The cell plate was divided into four groups with triplicates, and each group was treated with different concentrations of H₂O₂ (**Figure 1**).

Statistics: The analyses were performed using SPSS software v21 (SPSS Inc. Chicago, Illinois, USA). The data were analyzed using the Kruskal-Wallis test and Mann-Whitney U test for variables with non-normal distributions. For normal distributions, Student *t-test* and ANOVA were performed.

4. Results

EFN-R cells, exposed to H₂O₂ at various concentrations and for different time periods, revealed a decline in cell viability with increasing concentrations and exposure time.

Based on the results of the experiment described above, we decided to subject the EFN-R cells to 100 µMol of H₂O₂ and/or 750 µM of NACA for 24 hours.

Our second experiment revealed that cells exposed to NACA 1 hour after treatment with H₂O₂ had a lower mortality than cells subjected to H₂O₂ alone,

$p < 0.01$. Also the group subjected to NACA 1 hour before exposure to H_2O_2 displayed significantly higher viability 24 hours later ($p < 0.001$) (Figure 2).

5. Discussion

In this study, our aim was to investigate a possible protective effect of NACA to a porcine embryonic kidney cell line exposed to oxidative stress by H_2O_2 treatment.

The dose of $750 \mu M$ of NACA was decided because of previous reports demonstrated that NACA was protective to neural cells exposed to oxidative stress induced by Glutamate [9].

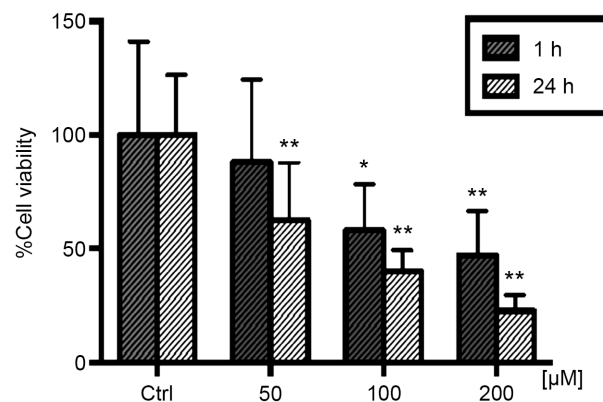


Figure 1. Effect of different concentration of H_2O_2 at different time points, measured by the MTT-assay. Cells were treated with different concentrations (50, 100, and $200 \mu Mol$) of H_2O_2 and incubated either for 1 ($n = 8$) or 24 ($n = 9$) hours. Viable cells were assessed by MTT-assay, presented in percentage compared to control for the representative time point. Values represent means \pm standard deviation (SD). Statistically different values of * $p < 0.05$, ** $p < 0.01$ were calculated with t-test and compared to cells without H_2O_2 treatment (control).

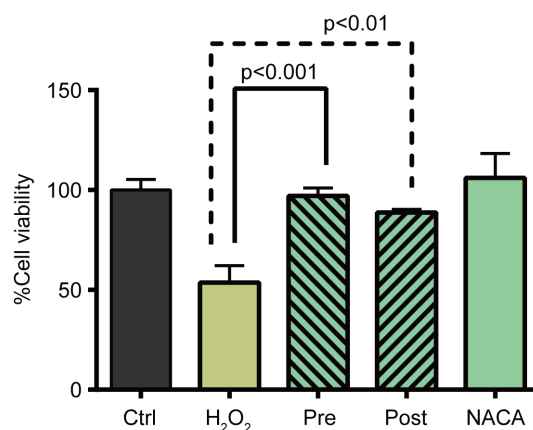


Figure 2. Cells exposed to H_2O_2 had a significantly higher mortality rate compared to the control group not exposed to H_2O_2 ($p < 0.001$). The NACA group was exposed to NACA, but not H_2O_2 . Evaluation of the viability of the EFN-R cells revealed that both the group treated with NACA before exposure to H_2O_2 (Pre) and the group treated with NACA after exposure to H_2O_2 (Post), exhibited significantly higher cell viability compared with the H_2O_2 group ($p < 0.001$ and $p < 0.01$, respectively).

Even though several studies have shown advantageous outcomes of NACA in different cell cultures subjected to oxidative stress [10] [11], we are, to our knowledge, the first who confirm the positive effects using a porcine epithelial-like embryonic kidney cell line.

6. Conclusions

The augmented viability of the EFN-R cells may indicate that NACA could play a crucial role when reducing oxidative stress.

Taking the results from our previous study into consideration, we suggest that NACA may have organ protective properties for neonates exposed to perinatal oxidative stress.

We underscore that our results are based on experiments on isolated cells and more studies should be accomplished before considering transferring them into a clinical trial.

Conflict of Interests

There are no conflicts of interests to declare.

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