Quantification of DNA Damage in Bladder Muscle and Assessing Repair with Insoine and Olaparib

Noëlle Alena Rychen, BMA 21-24

Biomedizinische Analytik HF

University of Bern, Department for BioMedical Research

1. Abstract

The study investigates the effects of Inosine and Olaparib on DNA damage in human fibroblast cells caused by H_2O_2 . Inosine, known for its potential to inhibit excessive activation of poly (ADP-ribose) polymerase (PARP), showed significant protective effects by reducing DNA damage when used as a pretreatment, with higher doses offering greater protection. Posttreatment with Inosine also decreased DNA damage, albeit less effectively than pretreatment. Olaparib, a PARP inhibitor, increased DNA damage when used posttreatment, supporting its role in obstructing DNA repair. The combination of Inosine and Olaparib resulted in a counteractive effect on DNA damage. Future research should explore the use of viral vectors to knockdown PARP1, comparing this with Olaparib treatment to gain further insights into DNA repair mechanisms.

2. Introduction

The motivation for this diploma thesis was to quantify DNA damage in fibroblastic cells for the investigation of spinal cord injuries. Previous studies have shown that after spinal cord injury the cells if the bladder suffer from DNA damage. A Spinal cord Injury occurs when the nerves and nerve fibres that transmit signals between the brain and the body are damaged. This is leads to loss of sensory and motor functions below the level of Injury. DNA damage can occur as single-strand breaks (SSBs) or double-strand breaks (DSBs). Poly (ADP-ribose) polymerases (PARP) is an enzyme which plays an important role in the repair of SSBs. Olaparib is a small molecule which is classified as a member of PARP inhibitors, that means it inhibits the cell repair mechanism. Inosine is naturally presented in RNA and plays an important role in RNA editing. [1] [2] [3] [4] [5]

3. Aims and leading questions

Previous studies have shown significant DNA damage in bladder smooth muscle cells (SMC) following spinal cord injury (SCI) in rats, mice, and patients with neurogenic bladder diseases. This study aims to evaluate the potential of Inosine and Olaparib in repairing DNA damage in human fibroblast cells (CCD1) after H_2O_2 exposure.

In order to achieve this aim following leading question was formulated:

- Does Inosine or Olaparib either enhances or inhibits the efficacy of DNA damage repair in human fibroblast cells following H_2O_2

4. Methods and Materials

The Comet Assay is a popular technique for assessing DNA damage within individual cells. The Comet Assay visually demonstrates DNA damage by causing fragmented DNA strands to migrate away from the nucleus when subjected to an electric field, forming a distinctive "comet-like" appearance under a microscope. The extent of DNA damage is then quantified by measuring the length and intensity of the "comet tail." Longer and more intense tails indicate greater DNA damage, providing valuable insights into the level of genetic injury within the tested cells. The Comet Assay encompasses two distinct variants: the alkaline Comet Assay and the neutral Comet Assay. Among these, the alkaline Comet Assay is the more prevalent and straightforward to conduct. It is capable of detecting both single and double-strand DNA breaks. In contrast, the neutral Comet Assay exclusively identifies double-strand DNA breaks. Consequently, the neutral Comet Assay exhibits lower sensitivity compared to the alkaline counterpart. [6]

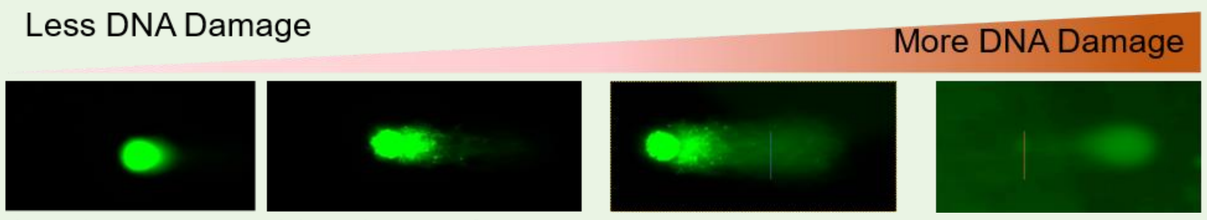


Figure 1: Comet assay Images Depicting Varying Degrees of DNA Damage

5. Results

Pre- and Posttreatment with Inosine

24 h pretreatment with 100µM Inosine slightly but noticeably reduced the tail moment.

Different concentrations of Inosine (200µM, 500µM, 1mM)

The conditions Pre- and Posttreatment with 1mM Inosine showed reductions in tail moments

Olaparib treatment

The most significant DNA damage is observed in cells posttreated with Olaparib during 24 h after H_2O_2 exposure.

Different concentrations of Olaparib

Significantly higher tail moments were observed in 100µM Olaparib posttreatment.

6. Discussion

Our results demonstrate that Inosine exerts a dual role in DNA damage protection and repair. The protective effect of Inosine can be attributed to its ability to modulate cellular antioxidant defenses, potentially through the inhibition of excessive PARP activation, as suggested by previous studies. Higher concentrations of Inosine (1mM) were more effective in mitigating DNA damage, underscoring a dose-response relationship. This dose-dependent protection aligns with the notion that sufficient concentrations of Inosine can more effectively inhibit excessive PARP activation and enhance the cellular repair machinery.

The dose-dependent studies with Olaparib revealed that higher doses provided greater DNA damage and significantly inhibited time-dependent spontaneous DNA repair, indicating its potential as a positive control to study and target PARP pathway.

References

[1] Bennett et al, 2024, Spinal cord injuries
[2] Martin, 2008, DNA damage
[3] Javle & Curtin, 2011, PARP
[4] Bochum et al., 2018, Olaparib
[5] Kim & Jo, 2022, Inosine
[6] Collins, 2004, Comet Assay

Figures

Fig. 1 Hashemi Gheinani, A. (2023) Comet Assay Images Depicting Varying Degrees of DNA Damage