In vivo and *in vitro* study of the effects of olaparib on experimental colitis and intestinal epithelial barrier integrity

PhD Thesis

Dominika Kovács

Interdisciplinary Medical Sciences Doctoral School

Molecular and Cellular Biochemistry Program

Doctoral School and Program Leader: Prof. Dr. Ferenc Gallyas

Supervisor: Dr. Balázs Radnai

University of Pécs

Medical School

Department of Biochemistry and Medical Chemistry

2023

1. Introduction

1.1. General characterisation of inflammatory bowel diseases

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic, inflammatory diseases of the gastrointestinal tract. They are complex, multifactorial conditions. The aetiology of IBD is not yet fully understood, but genetic and environmental factors, disruption of intestinal barrier function and abnormal immune response to the intestinal flora are known to play role in the developement.

The main goal of IBD treatment is to achieve endoscopic and histological remission, which is a highly complex process associated with suppression of inflammation and improvement of intestinal barrier function. The major classes of medications currently used are aminosalicylates, steroids, immunosuppressants, monoclonal antibodies (biologic therapy). Although the range of treatment options is constantly expanding, most of the current and investigational drugs act directly on an element of the immune system, targeting a specific point of an immune process or inflammatory cascade. Despite the fact that the main goal of the therapy would be to heal the mucosa, there are no approved agents that directly target the epithelial barrier.

1.2. Role of mitochondria in the development of inflammatory bowel diseases

Due to the high energy demand of intestinal epithelial cells, dysfunction of mitochondrial energy production has a strong impact on cell viability and compromising the integrity of the intestinal barrier. As early as 1980, Roediger described ulcerative colitis as a cellular energy deficiency disease. The term was coined because of the reduced oxidation of butyrate, a nutrient considered to be the main source of energy for colonocytes, measured in the intestinal epithelial cells of patients with acute ulcerative colitis. Today, Roediger's hypothesis has been confirmed by a number of other studies. Schneider and his colleagues have shown in colonic samples from IBD patients that the expression of all respiratory chain complexes are reduced. In colonic samples from patients with ulcerative colitis, the enzyme activities of Complex II, III and IV are also lower and the intestinal mucosa is characterised by significantly lower ATP levels. In IBD patients, mitochondrial lesions such as swelling, loss of outer and inner membrane integrity are observed even before the onset of inflammation, suggesting that mitochondrial dysfunction is an early event in the development of the disease.

In addition to energy production, mitochondria are also a major source of reactive oxygen species (ROS). When electron migration in the respiratory chain is disturbed, i.e. from a Complex to an oxygen molecule, partially reduced oxygen derivatives are released. Approximately 1-2% of the oxygen used in the respiratory chain is converted into superoxide anions. The elimination reaction, catalysed by superoxide dismutase, produces hydrogen peroxide (H_2O_2), a membrane-permeable oxygen derivative that damages proteins, lipids and DNA. The role of H_2O_2 in the pathogenesis of ulcerative colitis is demonstrated by the significantly higher levels of H_2O_2 in both inflamed and intact mucosa compared to samples from healthy intestine. H_2O_2 generated in colonic epithelial cells easily leaks into the extracellular space and damages the tight junction proteins that maintain the cell-cell contact. This leads to an increase in paracellular permeability, translocation of luminal bacteria into the lamina propria, and neutrophilic infiltration of intestinal tissue.

1.3. Metabolism of colonic epithelial cells during inflammation

Short-chain fatty acids produced by bacterial fermentation of dietary fibers play a prominent role in the metabolism of colonic epithelial cells. Among acetate, propionate and butyrate, butyrate is considered the primary energy source for colonocytes. Butyrate degradation proceeds through the β-oxidation cycle that produces acetyl-CoA, which is further metabolized in the citrate cycle, followed by ATP production via oxidative phosphorylation.

Thus, under normal conditions, energy production in colonocytes is predominantly via β-oxidation, so the cells are characterised by high oxygen consumption. This maintains a state of epithelial hypoxia, limiting the amount of oxygen entering the intestinal lumen, which favours the anaerobic bacteria responsible for butyrate production. The homeostatic state mentioned above is called C2 phenotype. However, during inflammation, there is a polarization of the cells towards C1 phenotype, i.e. their energy production is shifted from oxidative phosphorylation towards aerobic glycolysis. Although there is sufficient oxygen available to the cells and anaerobic conditions do not prevail, glucose is not fully oxidized and its fate is not determined by oxygenation. The pyruvate formed in glycolysis is not further oxidized to acetyl-CoA and enters the citrate cycle, and ATP production via oxidative phosphorylation occurs, but is reduced to lactate in a reaction catalyzed by the enzyme lactate dehydrogenase, while an NADH is oxidized to NAD^+.

The metabolic switch that occurs as a result of inflammation, therefore, means high glucose consumption and lactate production by colonocytes and low oxygen consumption. One consequence is a loss of epithelial hypoxia, an increase in the amount of oxygen entering the intestinal lumen, leading to bacterial dysbiosis and colonic dysfunction. The primary source of ROS involved in the pathogenesis of IBD is the mitochondrial respiratory chain itself. Since colonocytes produce ATP primarily via aerobic glycolysis during inflammation, i.e. the mitochondrial respiratory chain is inhibited, this provides a form of protection against increased ROS production. Colon biopsies from IBD patients also showed significantly higher mRNA expression of glycolysis enzymes such as aldolase A, phosphoglycerate mutase, enolase, and pyruvate kinase compared to the control group, while citrate cycle enzyme malate dehydrogenase expression was decreased, supporting a metabolic shift in colonic epithelial cells. Furthermore, the increased aerobic glycolysis was confirmed by significantly elevated fecal lactate levels in IBD patients and significantly elevated serum lactate levels in active phase of Crohn's disease.

1.4. Poly(ADP-ribose)-polymerase-1 enzyme

Poly(ADP-ribose)-polymerase enzymes (PARPs) catalyze the post-translational modification of certain target proteins (poly-ADP-ribosylation (PARylation)). PARP is a family of 17 enzymes. The most studied member is the PARP-1 isoform, which was the first to be discovered and is responsible for the majority of PARP activity in eukaryotic cells. It is a 113 kDa protein, which is activated by DNA damage and is located in the nucleus. The enzyme cleaves NAD^+ into nicotinamide and ADP-ribose. The released ADP-ribose monomers are then used to build PAR polymers,, which bind to the corresponding acceptor proteins. The negatively charged PAR polymers are able to alter the structure of their target proteins, affecting their interactions.

One of the main targets of PARylation is the PARP-1 protein itself, which is then called automodification, leading to inactivation of the enzyme. Heteromodification is the process where the PAR polymer binds to other proteins such as histones, transcription factors, proteins involved in DNA repair, cell cycle regulation such as DNA ligases, DNA topoisomerases I and II, p53 or nuclear factor-kappa B (NF-κB). In terms of target proteins, PARP-1 plays a role in DNA repair, replication, transcription, chromatin organisation and is able to influence cell proliferation, differentiation, metabolism and cell death through these cellular mechanisms.

PARP-1 is classically activated by single- and double-stranded DNA breaks, which can be caused by free radicals, other reactive oxygen and nitrogen derivatives, ionizing radiation, or DNA alkylating agents. The effect of PARP-1 activation on cells is significantly influenced by the extent of DNA damage. In the presence of minor stress, PARP-1 contributes to repair damaged DNA and thereby maintains genome integrity through its role in various DNA repair mechanisms. In contrast, much higher levels of stress and the resulting extensive DNA damage result in excessive activation of PARP-1, leading to depletion of the cell's NAD^+ and ATP stores, leading to necrosis.

NAD^+ is a key proton and electron acceptor for dehydrogenase enzymes involved in catabolic processes. Since glycolysis and the citrate cycle are the two major NAD^+ users, supplying NADH to the respiratory chain and thus ensuring ATP synthesis, NAD^+ depletion due to PARP-1 hyperactivation leads to a slowdown of these pathways, a drastic decrease in ATP levels and eventually cell death. The glycolytic block resulting from PARP-1 overactivation is not merely due to the depletion of NAD^+ stores, but is directly related to PARP-1's inhibition of the first step in glycolysis, the hexokinase I isoform that catalyzes glucose phosphorylation. This mechanism, in addition to NAD^+ deficiency, also contributes to mitochondrial dysfunction and subsequent cell death due to PARP-1 overactivation.

1.5. PARP as a therapeutic target

A better understanding of the role of the PARP-1 enzyme in DNA damage repair has raised the possibility of using PARP inhibition in anti-tumor therapy. This has spurred the development of new-generation inhibitors, which has led to dozens of clinical trials and now a growing number of PARP inhibitor registrations. The starting point for these developments was the discovery that cells carrying BRCA1 and BRCA2 mutations, i.e. homologous recombination (HR)-deficient cells, are more sensitive to PARP inhibitors than HR-normal cells. Repair of single-stranded DNA breaks in HR-defective cells would be achieved by the mechanism of base excision repair (BER), but BER does not work efficiently when the PARP enzyme is inhibited. Hence, BRCA1/2 mutant cells treated with PARP inhibitors, where neither HR nor BER function properly, have insufficient repair of DNA damage leading to cell death. In normal cells with a functional HR mechanism, PARP inhibition does not cause damage. It was this phenomenon of synthetic lethality that led to the breakthrough of the first PARP inhibitor, olaparib (Lynparza), approved for the treatment of BRCA mutation-positive ovarian cancer. Since then, it has been approved for several tumor types and new PARP

inhibitors such as veliparib, rukaparib, niraparib and talazoparib have been registered and are in several clinical trials.

Although current clinical trials are almost exclusively focused on oncological diseases, there is a need to test PARP in non-oncological indications, as preclinical studies have shown that PARP inhibition is effective in a number of disease models where oxidative stress, DNA damage, and inflammation are involved in the pathomechanism.

1.6. New generation PARP inhibitor: olaparib

Olaparib is a competitive inhibitor of PARP-1, PARP-2, and PARP-3 isoforms and can inhibit the enzymes by blocking the active site through its NAD+ analog. In recent years, the US Food and Drug Administration (FDA) has approved olaparib (Lynparza) for use in several types of cancer: ovarian, fallopian tube, peritoneal, breast, pancreatic, and prostate tumors with BRCA mutations. Used as monotherapy, olaparib is well tolerated, with side effects in the mild to moderate category, which generally do not require discontinuation of therapy. The most common adverse reactions ($\geq$ 10%) are fatigue, nausea, vomiting, diarrhea, headache, decreased appetite, anemia, neutropenia, thrombocytopenia, and lymphopenia. In preclinical safety studies, olaparib was shown to be clastogenic in mammalian cells *in vitro* and to induce micronuclei in bone marrow in rats *in vivo*. It is important to note, however, that its effects on DNA integrity and chromosome stability have not been investigated in humans, and thus no data on its genotoxicity potential are available.

1.7. Effect of PARP inhibition on experimental colitis

The protective effects of PARP inhibitors and knockout of the PARP-1 gene on experimental colitis have been demonstrated in a variety of animal models. In a rat model of 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitis, the PARP inhibitors 3-aminobenzamide (3-AB) and 1,5-dihydroxyisoquinoline (1,5-DIQ) reduced severe symptoms (weight loss, bloody diarrhea), tissue damage, myeloperoxidase activity, and the proportion of apoptotic colonic epithelial cells, which is thought to be due to inhibition of NF-κB and activation protein-1 (AP-1) DNA binding. In a TNBS-induced colitis experiment conducted by another group, the anti-inflammatory effects of 3-AB and 1,5-DIQ are attributed to the attenuation of neutrophil infiltration and reduction of cyclooxygenase-2 and prostaglandin E2 expression. The inhibitors commonly used in animal studies mentioned above belong to a group of first and second-generation PARP inhibitors. Their disadvantage is that they are only

effective at higher concentrations, are less specific and have not been clinically tested in oncological or other diseases. The third generation of small molecule PARP inhibitors (olaparib, veliparib, rukaparib, niraparib, talazoparib), which are the result of intensive development, are potent even at nanomolar concentrations, are competitive inhibitors, show high specificity for PARP-1,-2,-3 isoforms and are in the clinical application phase for various tumor types. Although olaparib has been tested *in vivo* and *in vitro* in a number of non-oncological disease models (septic shock, asthma, acute renal failure, aging), its effect on experimental colitis has not been investigated.

2. Aims

The development of third-generation PARP inhibitors and their use in oncological diseases has led to a need for their use in non-oncological diseases, including inflammatory bowel diseases. Although data are available on the effects of PARP inhibition in experimental colitis, these studies have only tested PARP inhibitors that have not progressed to clinical trials. For this reason, we investigated the effect of olaparib in an animal model of Crohn's disease. In the first half of my Ph.D. thesis, I sought to answer the following questions:

1. Does olaparib treatment reduce TNBS-induced symptoms in a mouse model of Crohn's disease?

2. What is the effect of olaparib on ulcerative lesions in the colon and on the integrity of the intestinal mucosa?

3. How does olaparib affect the production of pro- and anti-inflammatory cytokines and the levels of serum biomarkers that can monitor Crohn's disease activity?

In this work, we have paid particular attention to the epithelial barrier, the energy production of the intestinal epithelial cells, which plays a key role in the development of Crohn's disease. We complemented our *in vivo* intestinal mucosal permeability studies with additional *in vitro* experiments. In the second part of my thesis, I sought to answer the following questions:

4. Does olaparib directly affect the integrity of the Caco-2 single-cell layer which was used as a model for the intestinal barrier? Does it reduce oxidative stress-induced barrier damage?

5. Does olaparib have a protective effect on the viability of intestinal epithelial cells?

6. How does olaparib affect the energy production of intestinal epithelial cells during oxidative stress?

3. Results

3.1. Examination of the effect of olaparib in a mouse model of TNBS-induced colitis

3.1.1. Body weight change

One of the main symptoms of TNBS-induced colitis is diarrhea and dramatic weight loss. TNBS treatment resulted in a 12% reduction in body weight from the baseline, compared to a 10% reduction with 20 mg olaparib and a 5% reduction with 50 mg olaparib, suggesting that olaparib dose-dependently reduced weight loss, but the difference was not significant.

3.1.2. Macroscopic inflammatory parameters

The scoring of macroscopically evaluable histological lesions caused by TNBS treatment took into account the extent of ulceration, the number of adhesions, the shortening of the colon and the thickening of the intestinal wall, as well as the consistency and blood content of the stool. TNBS treatment caused a significant degree of damage compared to the control group. It resulted mainly in more extensive ulcerative lesions in the mid-colon, with oedematous thickening of the intestinal wall and hyperemia. Olaparib dose-dependently reduced the severity of symptoms. The difference was significant for the 50 mg treatment. In this case, there was such a significant reduction in lesion severity that no statistically detectable difference in macroscopic lesions was observed compared to the control group.

Among the parameters taken into account for the scoring, the change in the number of ulcers and their extent should be highlighted. The higher dose of olaparib significantly reduced both the number and size of ulcers.

3.1.3. Intestinal mucosal permeability

Impairment of intestinal mucosal barrier function was assessed by measuring the concentration of fluorescein isothiocyanate-labelled dextran (FITC-dextran) administered intracolonally and subsequently released into the circulation. Mice treated with TNBS showed greater permeability of the intestinal mucosa compared to the control group. Treatment with 50 mg olaparib significantly reduced the intestinal wall permeability-enhancing effect of TNBS, thus improving intestinal mucosal barrier function. No difference in FITC-dextran concentration was observed between the control and olaparib-treated groups.

3.1.4. Histopathological examination

The main histological features of the colon of TNBS-treated animals include ulceration, intense infiltration of immune cells, extensive fibrosis and oedematous thickening of the submucosa. Our histopathological study showed that the TNBS treatment indeed caused a severe thickening of the submucosal layer of the colon, which was greatly reduced by treatment with 50 mg olaparib.

3.1.5. Inflammatory cytokines

To investigate the potential anti-inflammatory effect of olaparib, the levels of pro- and anti-inflammatory cytokines were measured in colonic samples.

Interleukin-1β (IL-1β) levels increased by 62% after TNBS treatment compared to the control group. The increase was only 11% with olaparib treatment and no significant change was observed compared to the control group. TNBS treatment resulted in a 136% increase in IL-6 levels compared to the control group. This significant increase in cytokine production was effectively reduced by olaparib treatment by 111%. Increased tumour necrosis factor alpha (TNF-α) levels in the intestinal tissue are known to be associated with the activity and severity of inflammatory bowel disease. Nevertheless, no significant changes in TNF-α levels were observed following TNBS treatment. Our anti-inflammatory IL-10 levels decreased by 12% in the TNBS-treated group compared to the control group, whereas olaparib resulted in a significant increase.

3.1.6. Hematological parameters

Of the twenty values examined in the hematological analysis, our TNBS treatment caused a significant difference in two parameters compared to the control group. The lymphocyte count was decreased, whereas this decrease was significantly smaller when olaparib was given. The monocyte count was increased by TNBS, which was effectively reduced by olaparib treatment.

Based on absolute cell counts obtained during blood testing, four serological markers that can be used in the diagnosis of inflammatory bowel disease and monitoring of disease activity were investigated: lymphocyte-to-monocyte ratio (LMR), neutrophil-to-monocyte ratio (NMR), platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR).

The effect of TNBS treatment on each marker showed differences typical of Crohn's disease patients. The TNBS treatment resulted in a 6-fold increase in NLR and a 2-fold increase in PLR compared to the control group, which was effectively reduced by olaparib treatment in both cases. No difference in PLR was observed between the olaparib and control groups. The LMR was reduced by TNBS treatment to one-sixth of the control level, while olaparib treatment was able to attenuate this reduction. The decrease in NMR due to TNBS treatment was not significant, but olaparib resulted in an improvement in NMR.

3.2. Study of the effects of olaparib on Caco-2 cell culture

3.2.1. Detection of PARP isoforms in Caco-2 cells

As olaparib is a potent inhibitor of PARP-1 (IC_{50} = 5 nM), PARP-2 (IC_{50} = 1 nM) and PARP-3 (IC_{50} = 4 nM) isoforms, we first examined the basal mRNA expression of all three isoforms in untreated Caco-2 cells by real-time PCR. To eliminate possible quantitative differences between samples, β-actin gene expression was also measured and the results were normalized to this. All three PARP isoforms were detected in Caco-2 cells, but their expression levels were significantly different. PARP-1 mRNA was the highest in the cells, followed by PARP-2 and then PARP-3 isoforms.

3.2.2. Effect of olaparib on epithelial barrier integrity

Since in our animal studies olaparib had a spectacularly improved macroscopic appearance of the colon and preserved the barrier function of the intestinal mucosa, we first investigated the effect of olaparib on the intestinal epithelial barrier in our *in vitro* experiments. For this purpose, we used the widely accepted and used confluent Caco-2 single-cell layer. An important role in the pathomechanism of IBD has been attributed to increased intestinal permeability, which is largely due to oxidative damage to epithelial cells. Reactive oxygen derivatives, including H_2O_2, lead to DNA breakage and hence PARP activation.

In this context, we first investigated the barrier-damaging effects of different H_2O_2 concentrations (100, 200, 500, and 1000 μM) using the impedance-based xCelligence device. The Cell Index value measured by the instrument correlates well with the number of viable, plated cells. Lower concentrations such as 100, 200, and 500 μM did not result in a spectacular decrease in the Cell Index value compared to the untreated group, thus not affecting the integrity of the cell layer. However, 1 mM H_2O_2 caused a large decrease in the

Cell Index, i.e. it was found to be capable of damaging the barrier integrity, so further experiments were performed with this concentration.

Next, we investigated whether olaparib could attenuate the damaging effects of H_2O_2. With 30 min olaparib pretreatment, the Cell Index decrease induced by 1 mM H_2O_2 was found to be much smaller, i.e. it attenuated the loss of barrier integrity.

Another method was also used to investigate the barrier function of the epithelial barrier, where the permeability of the cell layer was measured using a 4 kDa FITC-dextran marker. For the control group, we measured an extremely low permeability to the marker molecule, which was increased by approximately 27-fold by 1 mM H_2O_2 treatment. The olaparib treatment remarkably reduced the permeability of the cell layer to control levels, confirming the result observed in our impedance-based measurements. No differences were observed between the control group and the olaparib-pretreated group in the permeability assay.

To further confirm our results, light microscopy images of the cell layers were taken after 24 hours of treatment. The cell layer morphology was strongly altered by 1 mM H_2O_2. Tight junctions between the cells were lost, the cells became rounded and most of them floated. In contrast, the cell layer pretreated with olaparib showed the same pattern as the control culture, i.e. olaparib preserved the barrier function of the cell layer.

3.2.3. Effect of olaparib on cell viability

To determine whether the treatment used in barrier integrity and permeability assays have an effect on the viability of Caco-2 cells forming the monolayer, a flow cytometric assay was performed using annexin V/7-aminoactinomycin D (7-AAD) labeling. This dual labeling allows the separation of live, early and late apoptotic/necrotic cells.

H_2O_2 treatment for 24 h reduced the proportion of live cells by 37%, while total apoptotic cells increased by 32%. In terms of the proportion of apoptotic cells, hardly any early apoptotic cell population was detected, the treatment mainly increased the proportion of late apoptotic cells, and this population also includes cells undergoing necrosis, as annexin V/7-AAD staining does not allow the separation of late apoptotic and necrotic cells. H_2O_2 treatment combined with olaparib pretreatment resulted in only an 11% reduction in live cells and only a 6% increase in late apoptotic/necrotic cells. Olaparib alone did not cause any significant difference in any cell population compared to the control group.

Our results suggest that our 1 mM H_2O_2 treatment resulted in high cell death and olaparib had a protective effect.

3.2.4. Metabolic effects of olaparib

3.2.4.1. Effect of olaparib on glycolysis

During inflammation, proinflammatory signals alter the metabolism of colonic epithelial cells. The energy production of cells, which until now have mainly oxidized butyrate, is shifted towards glycolysis with lactate production. Indeed, in colonocytes from patients with ulcerative colitis, a decrease in butyrate oxidation is observed, while cellular glucose utilization and lactate production are increased. Hence, we investigated the effect of olaparib on glycolysis in Caco-2 cells treated with H_2O_2 by measuring extracellular acidification.

H_2O_2 drastically decreased the basal acidification rate, whereas olaparib resulted in a significant increase. Glycolytic activity can be investigated by oligomycin injection. By inhibiting mitochondrial ATP synthesis, cellular metabolism is shifted towards anaerobic glycolysis with lactate production, which is reflected in a decrease in extracellular pH. Oligomycin caused a strong increase in the rate of acidification in both control and H_2O_2 + olaparib treated groups, i.e. cells were able to undergo metabolic transition. The increase in extracellular acidification rate in response to oligomycin was significant in both groups. In contrast, in cells treated with H_2O_2 alone, no acidification occurred in response to oligomycin, i.e. the cell was unable to compensate for the loss of mitochondrial energy production by glycolysis.

3.2.4.2. Effect of olaparib on mitochondrial respiration

The primary energy source for colonocytes is butyrate, produced during bacterial fermentation, which is essential for maintaining barrier function. ATP production from butyrate requires intensive mitochondrial respiration and oxidative phosphorylation. PARP-1 activation by excessive oxidative stress is known to decrease mitochondrial membrane potential, Complex I function, mitochondrial oxidation and ATP production, and elevated levels of mitochondrial ROS. Impaired regulation of the electron transport chain has been observed in IBD patients. Hence, we investigated the effect of PARP inhibition by olaparib on mitochondrial function in H_2O_2-treated Caco-2 cells by measuring oxygen consumption.

H_2O_2 treatment reduced basal cell respiration to 44%, which was not affected by olaparib treatment. After inhibition of F_O-F_1-ATP synthase by oligomycin, the oxygen consumption of cells for ATP synthesis was calculated to be 61% lower in the H_2O_2 treatment compared to the control group. For olaparib treatment, this reduction was 54%, but the difference was not significant. H_2O_2 caused a 13.5% decrease in mitochondrial coupling efficiency, whereas olaparib treatment caused a lower decrease of only 2.5%. No significant difference was observed between the control and H_2O_2 + olaparib-treated groups.

In the next step, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) abolished the proton motor force and increased mitochondrial oxygen consumption, thus allowing the maximum oxygen consumption of the cells to be measured. H_2O_2 treatment reduced this value by 73.5% compared to the control group, while in the presence of olaparib this reduction was significantly smaller (52%). As a final step in the measurement, the respiratory chain was inhibited with a mixture of rotenone and antimycin A, which minimized the oxygen consumption of the cells. This step provides an opportunity to test the reserve respiratory capacity of the cells. The H_2O_2 treatment led to an extremely low reserve respiratory capacity of the cells of 7%, whereas this value was much higher with olaparib treatment, at 49.5%. If the oxygen consumption measured after inhibition of F_O-F_1-ATP synthase by oligomycin is subtracted from the non-mitochondrial oxygen consumption measured after the addition of rotenone and antimycin A, the value of proton leakage is obtained. In our experiments, H_2O_2 treatment resulted in a lower proton leakage compared to the control, which was further reduced by olaparib treatment.

4. Summary

In this study, I investigated the effects of new-generation PARP inhibitor olaparib on epithelial barrier integrity, a key player in the development of inflammatory bowel disease, and on colonic epithelial cell metabolism in vitro.

In the first part of the study, animal experiments were performed to demonstrate the protective effect of PARP inhibition by olaparib in TNBS-induced experimental colitis. Olaparib was effective in reducing macroscopic lesions of the colon, the number and extent of ulcers, submucosal thickening, and colon permeability. It reduced the levels of intestinal proinflammatory cytokines IL-6 and IL-1β and increased the anti-inflammatory IL-10. Furthermore, it positively influenced the levels of different serum biomarkers NLR, PLR, and LMR, which are also characteristic of Crohn's disease.

Following the protective effect observed in intestinal permeability studies, we performed *in vitro* studies on Caco-2 single-cell layer focusing on the intestinal barrier as a potential target of olaparib. We have found that olaparib maintains glycolytic energy production of intestinal epithelial cells during oxidative stress, which is the main source of ATP during the metabolic polarization of cells under inflammation. Olaparib protects against H_2O_2-induced cell death and preserves barrier integrity. All this suggests that olaparib was able to reduce the severe symptoms of experimental colitis by protecting the intestinal epithelial cells and maintaining the mucosal barrier function.

With these results, we aim to highlight the potential of PARP inhibition by olaparib in the treatment of Crohn's disease and to contribute to the review of the use of olaparib in human therapy.

5. List of publications

Publication related to the thesis

Kovács, D., Vántus, V. B., Vámos, E., Kálmán, N., Schicho, R., Gallyas, F., & Radnai, B. (2021). Olaparib: A clinically applied PARP inhibitor protects from experimental Crohn's disease and maintains barrier integrity by improving bioenergetics through rescuing glycolysis in colonic epithelial cells. *Oxidative Medicine and Cellular Longevity, 2021*.

IF: 7,310

Further publications

Garai, J., Radnai, B., Vámos, E., Kovács, D., Vántus, V. B., Rumbus, Z., Pákai, E., Garami, A., Gulyás-Fekete, G., & Agócs, A. (2023). Synthesis and evaluation of a new class of MIF-inhibitors in activated macrophage cells and in experimental septic shock in mice. *European Journal of Medicinal Chemistry*, *247*, 115050. **IF: 7,088**

Andreidesz, K., Koszegi, B., Kovacs, D., Bagone Vantus, V., Gallyas, F., & Kovacs, K. (2021). Effect of Oxaliplatin, Olaparib and LY294002 in Combination on Triple-Negative Breast Cancer Cells. *International Journal of Molecular Sciences*, *22*(4), 2056. **IF: 6,208**

Andreidesz, K., Szabo, A., Kovacs, D., Koszegi, B., Bagone Vantus, V., Vamos, E., Isbera, M., Kalai, T., Bognar, Z., & Kovacs, K. (2021). Cytostatic Effect of a Novel Mitochondria-Targeted Pyrroline Nitroxide in Human Breast Cancer Lines. *International Journal of Molecular Sciences*, *22*(16), 9016. **IF: 6,208**

Horvath, O., Ordog, K., Bruszt, K., Kalman, N., Kovacs, D., Radnai, B., Gallyas, F., Toth, K., Halmosi, R., & Deres, L. (2021). Modulation of Mitochondrial Quality Control Processes by BGP-15 in Oxidative Stress Scenarios: From Cell Culture to Heart Failure. *Oxidative Medicine and Cellular Longevity*, *2021*. **IF: 7,310**

Garai, J., Krekó, M., Őrfi, L., Jakus, P. B., Rumbus, Z., Kéringer, P., Garami, A., Vámos, E., Kovács, D., & Bagóné Vántus, V. (2021). Tetralone derivatives are MIF tautomerase inhibitors and attenuate macrophage activation and amplify the hypothermic response in endotoxemic mice. *Journal of enzyme inhibition and medicinal chemistry*, *36*(1), 1356–1368. **IF: 5,756**

Ordog, K., Horvath, O., Eros, K., Bruszt, K., Toth, S., Kovacs, D., Kalman, N., Radnai, B., Deres, L., & Gallyas Jr, F. (2021). Mitochondrial protective effects of PARP-inhibition in hypertension-induced myocardial remodeling and in stressed cardiomyocytes. *Life Sciences*, *268*, 118936. **IF: 6,780**

Ramadan, F. H., Szabo, A., Kovacs, D., Takatsy, A., Bognar, R., Gallyas Jr, F., & Bognar, Z. (2020). Involvement of Mitochondrial Mechanisms in the Cytostatic Effect of Desethylamiodarone in B16F10 Melanoma Cells. *International Journal of Molecular Sciences*, *21*(19), 7346. **IF: 5,924**

Szabo, A., Sumegi, K., Fekete, K., Hocsak, E., Debreceni, B., Setalo Jr, G., Kovacs, K., Deres, L., Kengyel, A., Kovacs, D., Mandl, J., Nyitrai, M., Febbraio, M.A., Gallyas, F., Sumegi, B. (2018). Activation of mitochondrial fusion provides a new treatment for mitochondria-related diseases. *Biochemical Pharmacology*, *150*, 86–96. **IF: 4,825**

Total Impact Factor: 57,409

First-author conference posters

Kovács, D., Bagóné Vántus, V., Sümegi, B., Gallyas, F., Radnai, B. Effect of clinically used PARP inhibitor olaparib on oxidative stress-induced epithelial barrier dysfunction *in vitro.* International Student Congress (ISC) of the Medical University of Graz, Graz, 2019.

Kovács, D., Bagóné Vántus, V., Sümegi, B., Gallyas, F., Radnai, B. Clinically used PARP inhibitor olaparib protects against oxidative stress-induced epithelial barrier dysfunction *in vitro.* Hungarian Molecular Life Sciences, Eger, 2019.

Kovács, D., Bagóné Vántus, V., Sümegi, B., Gallyas, F., Radnai, B. A PARP inhibitor olaparib védő hatása kísérletes Crohn modellben és mesterséges epitheliális határrétegen: célpont a mitokondrium. 49. Membrán-Transzport Konferencia, Sümeg, 2019.

Kovács, D., Vámos, E., Sümegi, B., Gallyas, F., Radnai, B. The Effect of PARP Inhibitor Olaparib on Oxidative Stress-induced Epithelial Barrier Disruption and Mitochondrial Dysfunction.Medical Conference for PhD Students and Experts of Clinical Science, Pécs, 2018.

Kovács, D., Vámos, E., Sümegi, B., Gallyas, F., Radnai, B. PARP Inhibitor Olaparib Protects Against Oxidative Stress-Induced Epithelial Barrier Dysfunction and Inhibits

Proinflammatory Macrophage Activation. 7th Interdisciplinary Doctoral Conference, Pécs, 2018.

Kovács, D., Vámos, E., Sümegi, B., Gallyas, F., Radnai, B. Az olaparib (PARP inhibítor) véd az oxidatív stressz indukálta epithel barrier diszfunkció ellen és gátolja a gyulladásos makrofág aktivációt. 48. Membrán-Transzport Konferencia, Sümeg, 2018.

Kovács, D., Vámos, E., Götzer, M., Balogh, P., Radnai, B. A mitokondriális ciklofilin D szerepe kísérletes ulceratív kólitiszben. Doktoranduszok a Klinikai Kutatásban Konferencia, Pécs, 2018.

Co-authored conference posters

Bagóné Vántus, V., Kovács, D., Vámos, E., Deák, P., Vass, I., Gallyas, F., Radnai, B. A PARP inhibitor talazoparib hatásának vizsgálata TNBS indukálta kísérletes Crohn betegség modellben és mesterséges epitheliális barrieren. 51. Membrán-Transzport Konferencia, Sümeg, 2023.

Vámos, E., Bagóné Vántus, V., Kovács, D., Deák, P., Kőszegi, B., Kálmán, N., Vass, I., Gallyas, F., Radnai, B. A MIF tautomeráz inhibitor KRP 6 gátolja a gyulladásos makrofág aktivációt és védi a mitokondriális energiatermelést. 51. Membrán-Transzport Konferencia, Sümeg, 2023.

Bagóné Vántus, V., Kovács, D., Vámos, E., Deák, P., Vass, I., Gallyas, F., Radnai, B. Investigation the effects of the PARP inhibitor Talazoparib in a TNBS induced Inflammatory Bowel Disease Mice Model. Annual Meeting of the Hungarian Biochemical Society, Pécs, 2022.

Vámos, E., Bagóné Vántus, V., Kovács, D., Deák, P., Kőszegi, B., Kálmán, N., Vass, I., Gallyas, F., Radnai, B. Effect of KRP 6, a novel MIF tautomerase inhibitor on macrophage activation and mitochondrial function. Annual Meeting of the Hungarian Biochemical Society, Pécs, 2022.

6. Acknowledgements

I would like to take this opportunity to thank Professor Ferenc Gallyas, for making it possible for me to participate in the doctoral program and for providing the necessary conditions for my research. I would also like to thank my supervisor Dr. Balázs Radnai for allowing me to learn animal and cell culture methods as a member of the IBD team, for his professional guidance, and for his help in the preparation of my publications and dissertation. I am grateful to Dr. Vántus Viola Bagóné for her help and active participation in animal experiments, her professional insights, and her friendship. I would like to thank the staff of the Institute of Laboratory Medicine for performing the blood count test. I would like to thank Dr. Nikoletta Kálmán for her work in gene expression studies and all my colleagues who helped me in any way.

I would also like to thank my parents for their support and help and for being there for me in any way. Finally, I would like to thank my husband and my son for everything, especially for their motivation.