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EFFECT OF RESVERATROL ON REGULATED CELL DEATH OF ENTEROCYTES UNDER CONDITIONS OF OXIDATIVE STRESS *in vitro*

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Aim. **The study purposed** to evaluate a concentration-dependent effect of Sirtuin 1 (SIRT1) modulators on cell death of enterocytes in the ileum of the small intestine under conditions of oxidative stress.

Materials and Methods. The experiments were conducted using female Alba mice. *Isolation of enterocytes* was carried out according to Kimura et al. 2015. *Modeling of oxidative stress* (OS) by adding hydrogen peroxide to phosphate-buffered saline: "weak" (50 μM); "medium" (250 μM); "strong" (500 μM). Apoptotic and necrotic death of enterocytes was assessed by morphological characteristics using the in vivo method of the two-color fluorescent nucleic acid dye Hoechst 33342 and propidium iodide. *Substances used*: Resveratrol — SIRT1 activator and Ex527 — specific SIRT1 inhibitor (2 μM, 20 μM, 100 μM).

Results. An increase in the number of live cells ($P < 0.01$) and a decrease in apoptotic ($P < 0.01$) and necrotic ($P < 0.01$) cells was observed with increasing Resveratrol concentration (from 2 to 200 μM) under OS conditions.

Conclusion. The obtained results suggest that Resveratrol reduces cell death of enterocytes, involving activation of SIRT1 under oxidative stress conditions *in vitro*. Further research is needed to confirm the effectiveness of this strategy and to establish protocols for the inclusion of antioxidants in treatment regimens for inflammatory bowel disease.

Keywords: oxidative stress, Sirtuin1, enterocytes, apoptosis, necrosis.

Inflammatory bowel disease (IBD) is a chronic, immune-mediated disorder that affects the gastrointestinal tract [1, 2]. Although the exact etiology of IBD remains unclear, dysfunctional intestinal immunoregulation is believed to be the underlying cause. Among the immunoregulatory factors, reactive oxygen species are produced at abnormally high levels in IBD. The role of oxidative stress (OS) in the pathophysiology of IBD, its diagnostic targets, and the potential use of antioxidant therapy for the prevention and treatment of IBD are under active investigation [3].

Sirtuins (SIRT1 — SIRT7) are nicotinic adenine dinucleotide (+)-dependent histone deacetylases that regulate essential signaling pathways and are involved in numerous biological processes. The role of SIRT1 in OS is under active investigation [4]. SIRT1 can be activated by Resveratrol (Res, 3,4′,5-trihydroxy-trans-stilbene), naturally occurring polyphenolic compound with anti-oxidant, anti-inflammatory and anti-apoptotic effects [5].

Regulated cell death (RCD) plays a vital role in development, tissue homeostasis, inflammation, immunity, and in the development of various pathophysiological conditions. Currently, the study of

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RCD is promising as one of the possible new therapeutic strategies for prevention of both functional intestinal disorders themselves and the negative consequences of OS.

The study aimed to evaluate a concentration-dependent effect of SIRT1 modulators on cell death of enterocytes in the ileum of the small intestine under conditions of oxidative stress.

Methods. The experiments were conducted using female Alba mice (weighing 18–22 g) and approved by the ethics committee of Bogomoletz Institute of Physiology.

Isolation of enterocytes was performed according to Kimura Y et al. 2015 [1].

O*xidative stress* was simulated using hydrogen peroxide (H_2O_2). By adding calculated amounts of H_2O_2 (88597, Sigma-Aldrich, USA) to phosphate-buffered saline (PBS), pH 7.4, OS was obtained: "weak" (50 µM); "medium" (250 µM); "strong" (500 µM).

Apoptotic and necrotic death of enterocytes was assessed by morphological characteristics using the in vivo method of the two-color fluorescent nucleic acid dye Hoechst 33342 and propidium iodide.

Substances used: Res — SIRT1 activator (2 µM, 20 µM, 100 µM, Carl Roth GmbH Co. KG, Germany) and Ex527 — specific SIRT1 inhibitor (2 µM, 20 µM, 100 µM, Sigma). The results were statistically processed in the Graph Pad Prism version 10.3.0 (507) (GraphPad Software, California USA). Differences were considered statistically significant at $P < 0.05$.

Results and Discussion. A decrease in the number of live cells and an increase in necrotic and apoptotic cells of the ileum were found under the influence of Ex527. No significant changes were found under the influence of Res.

Under conditions of "weak" OS, there were lower live cells to 78.13±1.96% and raised cells with signs of apoptosis to 14.57±2.07 ($n = 6$, $P < 0.05$).

Under conditions of "medium" OS, there is a decrease in live cells to 66.00±1.69% ($n = 6$, $P < 0.05$), an increase in cells with signs of necrosis to 11.88±1.46% ($n = 6$, $P < 0.05$) and apoptosis to 22.13±2.23% ($n = 6$, $P < 0.01$) compared to the control.

Under conditions of "strong" OS, there is a decrease in live cells to 50.75±1.67%, an increase in cells with signs of necrosis to 19.13±1.96%, and apoptosis to 30.13±1.25% ($n = 6$, $P < 0.01$) compared to the control.

It was found that post-incubation with the SIRT1 activator — Res (20 µM) after OS (250 µM) causes an increase in the percentage (of live cells to 75.13±3.31% ($P < 0.05$) and reduces the proportion of cells with signs of apoptosis to 16.13±2.30% ($P < 0.05$) and necrosis to 8.7 ±1.39% ($P < 0.05$, Figure).

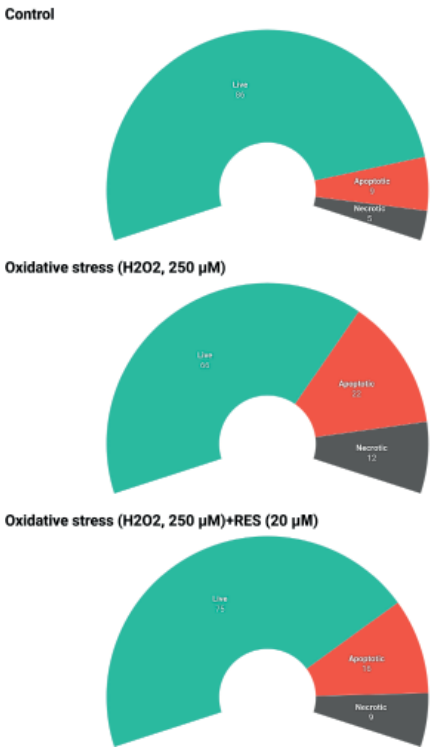

Figure. **Viability of enterocytes of the ileum of the small intestine under experimental conditions:** *1* — control; *2* — oxidative stress; *3* — Resveratrol under oxidative stress

Thus, under conditions of OS, the use of the activator Res causes an increase in the percentage of living cells and a decrease in cells with signs of apoptosis and necrosis.

Conclusions. Resveratrol reduces cell death of enterocytes, involving activation of SIRT1 under oxidative stress conditions *in vitro*. Further research is needed to confirm the effectiveness of this strategy and to establish protocols for the inclusion of antioxidants in treatment regimens for inflammatory bowel disease.

Authors' Contribution

Lytvynenko A.P. — data acquisition, analysis, interpretation; Blashkiv O.T. — data acquisition; Voznesenskaya T.Yu — research design.

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