

SHORT THESIS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (PhD)

**The role of the natural bioactive molecules pterostilbene and
cholecystokinin in regulating complex cellular damage
mechanisms in various animal models**

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1. INTRODUCTION

In recent decades, the incidence of chronic, noncommunicable diseases has shown an upward trend worldwide, which not only increases the burden on healthcare systems but also fundamentally shapes patterns of morbidity and mortality. A significant proportion of these conditions are not the result of a single, clearly identifiable causative factor, but rather the outcome of complex, interrelated pathobiological processes. The clinical presentation of these diseases is often organ-specific, yet the underlying molecular and cellular mechanisms overlap in many respects.

One of the fundamental challenges of modern biomedical research is therefore to uncover the common biological determinants underlying diseases with different clinical presentations, and to determine how these can be influenced. This approach increasingly focuses on the processes that shape the responses of cells to damaging effects. Persistent oxidative stress and inflammatory signaling lead to structural changes and functional disturbances in numerous organ systems, regardless of whether the triggering stimulus is vascular, metabolic, or toxic in nature. If these processes exceed the adaptive capacity of the given tissue, the damage becomes progressive and leads to long-term loss of function. However, the outcome of biological responses depends not only on the nature of the damaging effect, but also on its temporal course, intensity, and the current state of the tissue environment.

Cell types and tissues with high metabolic demands or that are constantly undergoing renewal are particularly sensitive to these processes. The retina, which is considered part of the peripheral nervous system, and the cell formation occurring in the bone marrow are anatomically and functionally distinct systems; yet they share the common trait of heightened vulnerability to harmful environmental influences. In the retina, oxidative and inflammatory stress can lead to functional impairment and cell death, while in the bone marrow, the involvement of progenitor cells may manifest as impaired hematopoiesis and increased sensitivity to external interventions—such as cytotoxic treatments.

Experimental findings from recent years suggest that these responses cannot be considered uniform or linear. Ischemic, metabolic, and cytotoxic effects are often present simultaneously, and their interaction results in complex biological responses that can only be interpreted by considering multiple factors together. The nature and extent of biological responses to injury thus depend not only on the nature of the

damaging stimulus, but also on the condition of the tissue in question and the timing of the intervention.

All of this justifies the use of experimental approaches that allow the nature and consequences of biological responses to injury to be examined in different organ systems. This dissertation examines the cellular-level characteristics and modifiability of these responses in various experimental models through two distinct series of experiments.

2. AIMS

We have defined the following objectives:

1. To investigate the retinal protective effects of pterostilbene under healthy conditions and following ischemia-reperfusion injury, with particular focus on retinal ganglion cell survival, the development of glial reactions, inflammatory and stress-related signaling processes, as well as functional changes measurable by electroretinography.

2. The aim of the study is to elucidate the role of cholecystokinin (CCK) signaling in the development of cytotoxic vulnerability in bone marrow granulocyte-macrophage progenitor cells (CFU-GM). In this study, we analyzed the effects of CCK-1 receptor deficiency and pharmacological CCK antagonism, and examined their interaction with carboplatin-induced cytotoxic stress.

3. MATERIALS AND METHODS

Ethics

The experimental protocols were registered by the Workplace Animal Experimentation Committee of the University of Debrecen (registration numbers: 13/2022/DEMÁB and 14/2022/DEMÁB) and approved by the Department of Food Chain Safety and Animal Health, Agricultural Division, Hajdú-Bihar County Government Office.

From Nature to Treatment: The Impact of Pterostilbene on Mitigating Retinal Ischemia–Reperfusion Damage by Reducing Oxidative Stress, Inflammation, and Apoptosis

Animals and Groups

We employed male adult Sprague Dawley (SD) rats, aged 18 weeks and weighing between 500 and 600 g, obtained from Charles River Laboratories International, Inc. (Wilmington, MA, USA). These rats were kept under standard conditions, with temperatures maintained between 22 and 24 °C, at the Department of Pharmacology and Pharmacotherapy, University of Debrecen, Hungary. A 12 h light–dark cycle was applied, and the rats could access water and standard rat chow freely. Prior to the commencement of the study, a two-week preliminary adaptation phase was provided. After acclimatization, the rats were randomly allocated into two groups (n = 10/group): one receiving vehicle (mucilage) treatment and the other receiving pterostilbene treatment. Mucilage was made from a viscosity enhancer, hydroxyethylcellulose, and water according to a Hungarian standard recipe published as an official preparation formula in *Formulae Normales VIII*—a Collection of Standard Recipe Samples. It is a colorless or slightly yellowish, transparent, almost odorless, tasteless viscous fluid. It mixes with water in all proportions. It was purchased from the University Pharmacy. Pterostilbene (source of pterostilbene: Apollo Scientific, product code: BIP1700, purity: ≥97% (HPLC), form: white solid, synonyms: *Pterocarpus marsupium*, 3',5'-dimethoxy-resveratrol, 3,5-dimethoxy-4'-hydroxystilbene, 4-[(1E)-2-(3,5-dimethoxyphenyl) ethenyl] phenol, empirical formula: C₁₆H₁₆O₃) was administered at a dosage of 5 mg/kg/day, once daily, for a duration of five weeks. The substances were administered through a gastric tube to ensure accurate dosing. We followed institutional and ethical guidelines to use as few animals as possible, taking into account potential losses. In accordance with animal ethics principle 3R, ischemia–reperfusion injury was specifically induced in the left eye of rats. The unblinded right eyes served as healthy

controls. This approach not only reduced the overall number of animals but also helped to normalize any unintended systemic effects of pterostilbene between the treated and untreated groups.

Ocular Ischemia–Reperfusion

General anesthesia was achieved with ketamine–xylazine (100/10 mg/kg), which was followed by the administration of a local anesthetic agent (oxibuprocaine, Humacain 4 mg/mL eye drops, Teva Ltd., Debrecen, Hungary). Subsequently, ischemia was induced in the left eyes of the SD rats using a methodology previously reported.

Briefly, bent forceps were used to gently protrude the left eye of each SD rat, and a polyester fiber surgical suture (Mersilene, 2 mm, Ethicon Inc., Cincinnati, OH, USA) was used to create a slip knot behind the eye bulb. To effectively restrict blood supply to the retina for a desired time period, this slip knot was tightened around the blood vessels of the eye. Ischemia induction was verified macroscopically by fundoscopic examination with an ophthalmoscope. During anesthesia, the rats' eyes were protected from dehydration using a specialized eye gel (Vidisic, Bausch & Lomb, Berlin, Germany). The ischemic state lasted 60 min after which the slip knot was loosened to restore the flow of blood in retinal arteries. The visual confirmation of sufficient reperfusion was performed using ophthalmoscopy.

Electroretinography

Hand-held Multi-species ElectroRetinoGraph, a Ganzfeld-type flash electroretinograph (HMsERG, OcuScience, Henderson, NV, USA), was utilized for both stimulus generation and data acquisition. The ERG measurements adhered to previously described methodologies. The rats, divided into vehicle-treated ($n = 10$) and pterostilbene-treated ($n = 10$) groups, were anesthetized with a mixture of ketamine–xylazine (100/10 mg/kg). Upon achieving deep anesthesia, mydriasis was induced using topical cyclopentolate (Humapent, Teva Ltd., Debrecen, Hungary), following which the animals were darkadapted for 20 min. During the procedure, the animals were positioned in a prone position on a heated pad (ATC 2000, WPI, Sarasota, FL, USA) to maintain a constant body temperature of 37 °C. Gold-coated corneal contact lens electrodes (ERG-jet Contact Lens Electrode, Fabrinal SA, La Chaux-De-Fonds, Switzerland) were placed on each eye, with reference and ground stainless steel needle electrodes inserted subcutaneously above the jaw and tail base,

respectively. Conductive gel was applied to the cornea to ensure optimal electrical contact and hydration throughout the procedure.

ERGs were recorded from both eyes simultaneously using a Ganzfeld dish. Single-flash images were acquired under both dark-adapted (scotopic) and light-adapted (photopic) conditions. The bandpass filter width was set to 1–300 Hz. The single white flash stimulus intensity ranged from -2.5 to $1 \log \text{cd}\cdot\text{s}/\text{m}^2$, and light adaptation was performed with a $30 \text{ cd}\cdot\text{s}/\text{m}^2$ backlight for 10 min before recording photopic responses. At each flash intensity, 10 responses were averaged, with the interval between stimuli varying between 2 and 20 s depending on the flash intensity. Data analysis was performed using software provided by the ERG system manufacturer. The dark-adapted oscillatory potential (OP) measurements were derived from ERG waveforms recorded for $3000 \text{ mcd}\cdot\text{s}/\text{m}^2$ flash stimuli, with bandwidth filtering of 100–300 Hz after recording. For the measurement of OP amplitudes, the highest positive and the lowest negative peaks were measured from a baseline set to $0 \mu\text{V}$. The absolute values of the two numbers were then summed. The implicit time is the time required for the highest positive peak to be formed after the flash. Four individual OP averages were given for each eye. Data analysis was performed using software provided by the ERG system manufacturer (ERGView 4.380, Ocuscience, Henderson, NV, USA).

Western Blot

Immediately following euthanasia, both eyes of the animals were carefully removed from the orbit and promptly immersed in liquid nitrogen for preservation until subsequent molecular biological examinations ($n = 4$ per group). Whole-eye samples were then pulverized and homogenized with a homogenization buffer comprising 25 mM Tris, 25 mM NaCl, 1 mM Na-orthovanadate, 10 mM NaF, 10 mM Na-pyrophosphate, 10 mM okadaic acid, 0.5 mM EDTA, 1 mM PMSF protease inhibitor cocktail, and distilled water, all sourced Life 2024, 14, 1148 5 of 13 from Sigma-Aldrich Merck KGaA, Darmstadt, Germany. The resulting solution was further homogenized using a disperser (IKA-WERKE, Staufen, Germany). Following centrifugation at 2000 rpm for 10 min at 4°C , the cytosolic and mitochondrial proteins were collected with the supernatant, while the pellet containing the nuclear fraction was dissolved and incubated for 1 h in homogenization buffer supplemented with Triton X 100 surfactant (Sigma-Aldrich Merck KGaA, Darmstadt, Germany). Following the initial centrifugation step (14,000 rpm, 10 min, 4°C), the supernatant containing nuclear proteins was carefully aspirated. Subsequently, the supernatant containing cytosol and mitochondria

underwent further centrifugation (10,000 rpm for 20 min at 4 °C), and the resulting supernatant, containing the cytosolic fraction, was collected. The total protein concentration in 10 µL of the supernatants (nuclear and cytoplasmic) was measured using a spectrophotometer (FLUOstar Optima, BMG Labtech, Ortenberg, Germany) and a BCA assay kit (QuantiPro BCA Assay Kit, Sigma-Aldrich Merck KGaA, Darmstadt, Germany). The remaining volume was either mixed with Laemmli sample buffer (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) or stored at –80 °C for further analysis.

Proteins were separated by SDS–polyacrylamide gel electrophoresis (12% gel, 25 mA for approximately 220 min). Subsequently, the blotting of proteins to a nitrocellulose membrane (GE Healthcare, Darmstadt, Germany) was performed at 25 V for 90 min. The membrane was then blocked in a 5% BSA solution (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) and incubated overnight at 4 °C with primary antibodies in TBST solution. The primary antibodies used included anti-histone H3 (detecting histone 3, ~17 kDa), anti-betaactin (detecting beta-actin, ~42 kDa), anti-HSP70 (~70 kDa), anti-PARP1 (~113 kDa), GFAP (55, 48 kDa), and anti-NFκB (~50 kDa) antibodies. The membranes were then washed with TBST for 3 × 10 min and incubated with secondary antibodies (anti-mouse or antirabbit) conjugated with horseradish peroxidase enzyme. The blots were visualized using WesternBright™ enhanced chemiluminescent substrate (Advansta Inc., Menlo Park, CA, USA) and a LiCor C-Digit blot scanner (LI-COR Inc., Lincoln, NE, USA). Scanned images were analyzed using Image Studio Digits software (version 5.2, LI COR Inc., Lincoln, NE, USA), including normalization to the background and standardization to a housekeeping protein (histone H3 or beta-actin). Three Western blots from all treatment groups were analyzed.

Histology

Following euthanasia, the eyes of four animals per group were promptly excised from the orbit, with the upper portion of each eyeball marked for subsequent orientation. Subsequently, the eye bulbs were infused with and immersed in 4 °C paraformaldehyde solution (PFA, pH 7.4, 4% in phosphate buffer containing 10 g paraformaldehyde, 50 µL 10 N NaOH, 25 mL 10 × PBS, and 200 mL ddH₂O) for 24 h to ensure the proper fixation of the retina. The following day, corneas were removed to ensure the thorough removal of PFA, and tissue samples were washed in water for 1 h. The tissue specimens were then preserved in 70% alcohol until further histological processing. Histological processing involved sequential dehydration in 70%, 90%, and 100% ethanol, followed by clearing in xylene and

infiltration/embedding in Histowax (Histolab Products AB, Gothenburg, Sweden). Subsequently, the paraffin-embedded eye tissue blocks were sectioned frontally with a microtome into 5 μ m thick sections. Sections near the optic disc were selected for further processing. After deparaffinization and the rehydration of the sections, hematoxylin–eosin (H & E) staining was performed as follows: The sections were stained for 1.5 min with hematoxylin (Gill-type, GHS2128, Sigma-Aldrich Merck KGaA, Darmstadt, Germany). Following staining, sections were rinsed in running tap water until they turned blue, followed by a 3 s staining with eosin (Eosin Y, Alcoholic solution, 3801600E, Leica Biosystems Richmond, Inc., Mumbai, Maharashtra, India). Images were captured from the inferior part of the retina, near the optic disc, using a Nikon Eclipse 80i microscope equipped with a DS-Fi3 Microscope Camera, utilizing a 40 $\times$ objective lens (Nikon Plan Fluor 40 $\times$ /0.75 DIC M/N2 ∞ /0.17 WD 0.66, Nikon Europe B.V., Amstelveen, The Netherlands). Measurements were conducted using Nikon NIS-Elements BR microscope software (Ver5.41.00).

Statistical Analysis

The results are presented as means $\pm$ standard error of the mean (SEM). The normality of the data within the groups was assessed using the D'Agostino–Pearson test. Differences in means among the groups were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. We applied Mann–Whitney tests for statistical analysis in instances where this method was applicable. A *p*-value less than 0.05 was considered statistically significant. GraphPad Prism software for Windows (version 7.00, GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis.

CCK-1R-selective and non-selective cholecystokinin antagonists, lorglumide, and proglumide increased toxicity of carboplatin to granulocyte–macrophage progenitor cells (CFU-GM) of bone marrow of rats

Animals

Long Evans Tokushima Otsuka (LETO) and Otsuka Long Evans Tokushima Fatty (OLETF) rats were kindly provided by Kazuya Kawano from Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). All rats were males and 8 weeks old. The body weights of LETO rats were 256 ± 28 g, and the body weights of OLETF rats were significantly higher, 331 ± 34 g ($P < 0.01$). Blood sugar levels were in the normal range in both groups. The rats were fasted overnight with

allowed access to tap water “ad libitum” before blood glucose concentration was determined by glucometer (Accu-Chek, Roche Diagnostics, Budaörs, Hungary).

The animals were housed in an animal room with 12-h light and dark periods a day, a temperature of 23 ± 2 °C, a relative humidity of $60 \pm 10\%$ with 3–5 animals per pen. They were fed commercial laboratory chow and tap water ad libitum.

Study design

Hemopoiesis was studied in the femoral bone marrow of 8-week-old obese OLETF and wild nonobese LETO rats. Altogether 10 OLETF and 10 LETO rats were used. The animals were euthanized by an intravenous overdose of thiopental sodium (100 mg/kg; Thiopental Sandoz, Sandoz Pharmaceutical PLC, Switzerland) administered via the lateral tail vein. Subsequently, the femoral bones of the rats were prepared under sterile conditions, and the bone marrow was completely flushed out with McCoy’s 5A medium (Sigma-Aldrich Merck KGaA, Darmstadt, Germany). Single cell suspensions, checked under microscope, were obtained by suspending bone marrow samples in McCoy’s 5 A medium through thin needle syringes. After Ficoll-Iodamide gradient centrifugation, the mononuclear cell fraction of bone marrow was used (see under CFU-GM assay).

Bone marrow function was characterized by measuring total femoral cellularity, colony numbers of CFU-GM, and total femoral CFU-GM content. Cellularity of the whole femoral bone marrow shows the number of the total mononuclear bone marrow cells, which is a good indicator of the intensity of hemopoiesis, including all cell lineages. Cellularity was calculated using bone marrow cell counts and volumes of samples. Colony numbers of CFU-GM progenitors were counted in special soft-gel cultures prepared according to the CFU-GM colony assay, and the total CFU-GM content of the femur was calculated by multiplying cellularity and frequency of CFU-GM indicated by colony numbers. The latter values mirror the intensity of granulopoiesis.

The bone marrow mononuclear cells of each rat were grown in normal conditions or in the presence of carboplatin at final concentrations in 0.2–3 mg/L range. Carboplatin (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) was dissolved in sterile distilled water at 50 °C in an ultrasonic bath (XUB5, Bio-Science Ltd., Budapest, Hungary) freshly in each experiment. When it completely dissolved, this solution of 1 mg/ml concentration was immediately diluted 100 times by culture medium and used for the CFU-GM assay.

In the further experiments Long Evans Tokushima Otsuka (LETO) rats were treated with CCK antagonists using equimolar doses at two levels of both proglumide and lorglumide. After 1-week acclimatization period, the rats were randomly allocated into five groups ($n = 12/\text{group}$): first group receiving vehicle (0.9% NaCl) treatment, the next two groups receiving 3 mg/kg or 10 mg/kg of proglumide (proglumide sodium, Mw: 356.39 g/mol, Sigma-Aldrich Merck KGaA, Darmstadt, Germany), and the other two groups receiving 4 mg/kg or 13 mg/kg of lorglumide (lorglumide sodium, Mw: 481.39 g/mol, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) once daily intravenously for 5 consecutive days. The used doses were $8.4 \cdot 10^{-6}$ mol/kg and $2.7 \cdot 10^{-5}$ mol/kg equimolar doses of proglumide and lorglumide with the same number of molecules at the dose levels, in the interest of the accurate comparison of their effects. Pharmacological effects can be compared at molecular levels on receptors if the same number of molecules of the studied drugs are used. Cholecystokinin antagonists were administered intravenously as a slow bolus injection in 3–4 s using special one-rat cage after submersing the tail in warm water for 1–2 min. We administered the proper doses each day at the same time with the exact same order for the rats starting at 9.00.

On the 6th day, the animals were euthanized by an intravenous overdose of thiopental sodium (100 mg/kg; Thiopental Sandoz, Sandoz Pharmaceutical PLC, Switzerland) administered via the lateral tail vein. Bone marrow was obtained and handled similarly than in the previous study to see effects of CCK antagonists on hemo- and granulopoiesis. To study whether the pre-treatment with the cholecystokinin antagonists influences sensitivity of CFU-GM progenitors against carboplatin toxicity, the bone marrow mononuclear cells of each rat were grown in normal conditions or in the presence of carboplatin of 1 or 2 mg/L final concentrations. Carboplatin was dissolved freshly in each experiment and handled similarly as previously described.

CFU GM assay

The soft-gel cultures were prepared to maintain descendants of CFU-GM progenitor cells together, which formed colonies and showed the frequency of CFU-GM among the inoculated bone marrow cells as described previously. Briefly, bone marrow cell suspensions diluted 0.9% NaCl in a 1:1 ratio, and mononuclear cell fractions were obtained by Ficoll-Iodamide (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) gradient centrifugation (1077 g/mL). Viable cells of the buffy coat were counted with the help of a hemocytometer, using 0.04% trypan blue; viability was consistently 99–100%. Inocula of $1 \times 10^5/\text{mL}$ rat bone marrow cells

were plated in petri dishes with a diameter of 35 mm (Greiner, Nürtingen, Germany) and were grown in McCoy's 5 A modified medium (Sigma-Aldrich Merck KGaA, Darmstadt, Germany) supplemented with amino acids, Na pyruvate, NaHCO₃, antibiotics (streptomycin, penicillin), 20% fetal bovine serum (Sigma-Aldrich Merck KGaA, Darmstadt, Germany), and colony-stimulating factors, namely rrGM-CSF and rhG-CSF (Sigma-Aldrich Merck KGaA, Darmstadt, Germany). Methylcellulose (Methocel, 3000–5000 centipoises; FLUKA, Buchs, Switzerland) at 1.2% was used as a support matrix for semisolid cultures. The cultures were grown in duplicates for 14 days at 37 °C at 100% relative humidity in an atmosphere containing 5% CO₂. The colonies were counted under a dissecting microscope (Olympus, Hamburg, Germany) at the end of the incubation period. Colonies were defined as groups of at least 50 cells, consisting of granulocytes and/or monocytes, verified by smears or cytospin preparations.

In vitro treatment of CFU GM progenitors of LETO rats

To see whether the effects of CCK antagonists were direct or indirect on the progenitor cells, samples of a further 10 LETO rats were used. Primary cell cultures were made from sterile bone marrow mononuclear cells of non-obese LETO rats according to the previously described method, and in these soft gel cultures, CFU-GM progenitors were grown without or with proglumide or lorglumide using 12 concentration levels in a 0.1 to 15,000 µM range.

Testing CCK 1 and CCK 2 receptors in bone marrow cells

Receptors of CCK-1 and CCK-2 were tested in femoral mononuclear bone marrow cells of LETO and OLETF rats (n = 10/group) treated with vehicle (0.9% NaCl) or 10 mg/kg of proglumide once daily orally for 4 weeks. We mixed samples of 2 rats to get enough amounts of cells to study; thus, data are originated from 5–5 samples. Then, the mononuclear bone marrow cells of the two groups of rats were cultured in modified McCoy's 5 A medium according to the previously described CFU-GM assay with colony-stimulating factors but without methylcellulose in suspension culture flasks (Greiner Bio-One Ltd. Hungary, Mosonmagyaróvár, Hungary). From the single-cell bone marrow, suspensions mononucleated cells were obtained from buffy coat after FicollIodamide gradient centrifugation (1077 g/mL) and counted by hemocytometer using trypan blue. Viable nucleated cells of 5×10^6 /mL were seeded into culture flasks (25 mL) containing 10 mL of abovementioned completed medium and were grown at 37 °C in an atmosphere containing 5% CO₂ with 100% relative humidity.

The progenitor cells have the highest proliferation rate among mononuclear bone marrow cells, and after a few days, their numbers exponentially increase. The special colony-stimulating factors of the CFU-GM assay are required for CFU-GM progenitors to survive and differentiate, and at the same time, the other cells have been much less supported. Missing methylcellulose resulted in less stimulation for CFU-GM to differentiate. Cytospin preparations of the cells in liquid cultures were checked day by day, and on the fifth day, we found increased cell numbers, which showed mononucleated cells, including some myeloblasts, without any more differentiated cells or cells with segmented nuclei. We used the non-adherent cells from these liquid cultures on the 5th day, carefully paying attention to exclude adherent stromal or more differentiated other cells. As a result of this method, we got CFU-GM enriched bone marrow cell suspension.

RNA extraction and reverse transcription

Bone marrow cells derived from abovementioned cultures were held in RNA later solution (Qiagen, Hilden, Germany) which prevents degradation of mRNA until RNA extraction. Total RNA was purified using RNA Protect Mini kit (Qiagen) according to the manufacturer's recommendations. Concentration of RNA was determined by SmartSpec™ Plus Spectrophotometer (Bio-Rad, Hercules, USA). First-strand cDNA synthesis was performed on 2 µg of total RNA using random primers (Invitrogen Life Technologies, Karlsruhe, Germany) and Superscript™ II RNase H- Reverse Transcriptase (Invitrogen) as described previously.

Real time quantitative PCR

Real-time PCR was performed using ABI PRISM® 7900 HT Sequence Detection System (Applied Biosystems Inc, Foster City, CA, USA) in 10-µl aliquots containing 2× FastStart® TaqMan Master Mix (Rox) (Roche Applied Science, Mannheim, Germany), 4.5 µl cDNA template and either 0.5 µl TaqMan® Gene Expression inventoried assay (for CCK-1 and CCK-2 receptor) or oligos for 18S rRNA (500 nM forward and reverse primer each plus 100 nM probe). The assay probes contain a 6-carboxy-fluorescein phosphoramidite (FAM dye) labeling at the 5' end of the probe and a minor groove binder plus a non-fluorescent quencher at the 3' end, while the probe for 18S rRNA was labeled with TAMRA as a quencher instead of MGB. TaqMan® Gene Expression Assay IDs were Rn00562164_m1 for CCK-1R (Acc. No.: NM_012688) and Rn00565867_m1 for CCK-2R (Acc. No.: NM_013165), respectively. The expression of the housekeeping gene 18S rRNA (Acc. No.: EF060919) served as internal control. The oligonucleotide sequences for 18S rRNA were designed using qPCR primer version 1.2 and ordered from Bio-

Science Ltd (Budapest, Hungary). Amplification conditions consisted of 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing and amplification) in an ABI PRISM® 7900 HT Sequence Detection System (Applied Biosystems).

TaqMan assay assessment

TaqMan PCR for target (CCK-1R and CCK-2R) and reference (18S rRNA) genes was run from the same RT reaction. Three replicates were run for each gene for each sample in a 384-well format plate. “No template controls” (NTC) were used to confirm that reagents were not contaminated. Ct values were calculated with the Applied Biosystems SDS 2.1 software and were analyzed using the threshold cycle (Ct) relative quantification method.

Statistical analysis

The data obtained from individual rats or samples were used for statistical analysis. The results are presented as means $\pm$ standard error of the mean (S.E.M). Differences among the groups were analyzed using Kruskal–Wallis analysis of variance followed by the Mann–Whitney U test for comparison of two groups or samples. Differences were regarded as statistically significant at $P < 0.05$ and signed by stars (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). We used the statistical package of GraphPad Prism software for Windows (version 8.0.1 GraphPad Software Inc., La Jolla, CA, USA).

For mRNA expression, data were analyzed by comparing the means of the $2^{(-\Delta\Delta CT)}$ values. The levels of CCK-1 and CCK-2 receptor transcripts were normalized to the levels of 18S rRNA (ΔCT). Then, ΔCT was normalized to the values of untreated LETO and OLETF rats ($\Delta\Delta CT$). The normalized ΔCT values for untreated LETO or OLETF rats were considered as 1 (100%). All $2^{(-\Delta\Delta CT)}$ values were expressed as the mean $\pm$ S.E.M. and were evaluated statistically by Kruskal–Wallis analysis of variance followed by the Mann–Whitney U test. Differences were regarded as statistically significant at $P < 0.05$ from its corresponding value of untreated control rats at both genes examined.

4. RESULTS

From Nature to Treatment: The Impact of Pterostilbene on Mitigating Retinal Ischemia–Reperfusion Damage by Reducing Oxidative Stress, Inflammation, and Apoptosis

Electroretinography

The electroretinography (ERG) study revealed notable findings. In both a-wave and b-wave responses, the vehicle-treated ischemic–reperfusion group (MUCI IR) exhibited the lowest amplitudes compared to other experimental groups. Specifically, the mean amplitude of b-waves was significantly diminished in the vehicle-treated ischemic–reperfusion group (MUCI IR) compared to both the vehicle-treated non-ischemic control (MUCI NO IR) and pterostilbene-treated ischemic–reperfusion (PTER IR) groups. Additionally, the mean b-wave amplitudes of the pterostilbene-treated ischemic–reperfusion (PTER IR) group demonstrated higher values than those observed in the Mucilago-treated ischemic–reperfusion (MUCI IR) group. The implicit time analysis of a-wave and b-wave responses did not reveal any statistically significant differences among the various treatment groups.

Western Blot

The results of Western blot analysis revealed a notable increase in the expression levels of glial fibrillary acidic protein (GFAP) and heat shock protein 70 (HSP70) in the MUCI IR group compared to both the MUCI NO IR and PTER IR groups. Statistical analysis demonstrated a significant difference when comparing the MUCI NO IR group with these two experimental groups, indicating a distinctive response to I/R injury. Similarly, the expression levels of poly(ADP-ribose) polymerase 1 (PARP1) and nuclear factor kappa B (NFkB) were significantly elevated in the MUCI IR group relative to the MUCI NO IR and PTER IR groups. The observed differences between the groups were statistically significant. Notably, pterostilbene treatment led to a substantial decrease in the expression levels of all four proteins when compared to animals treated with the vehicle (MUCI), irrespective of whether they were subjected to ischemic reperfusion (MUCI IR) or were in the non-ischemic control group (MUCI NO IR).

Histology

Based on histological analyses, the following can be reported: Significant differences were observed in the total retinal layer thickness among the four animal

groups. This value was found to be the smallest in the MUCI IR group, indicating that the retina thickness in this group was the thinnest compared to the other three animal groups. There was a significant difference between the ischemic (MUCI IR) and the corresponding non-ischemic (MUCI NO IR) animals' values. The average retinal layer thickness in the latter group was significantly larger, albeit not as much as in the case of non-ischemic animals receiving pterostilbene treatment (PTER NO IR). We compared the retinal thickness of non-ischemic animals in the two treatment groups (MUCI NO IR vs. PTER NO IR). The difference was significant. The same was observed in the case of ischemic eyes (MUCI IR vs. PTER IR). In animals treated with pterostilbene, significant differences in the retinal layer thickness were observed. The values of the PTER IR group were significantly smaller than those of the PTER NO IR group. It can be said that the total retinal thickness was the highest in the PTER NO IR group, considering all four groups.

CCK-1R-selective and non-selective cholecystokinin antagonists, lorglumide, and proglumide increased toxicity of carboplatin to granulocyte–macrophage progenitor cells (CFU-GM) of bone marrow of rats

Hemopoiesis in obese OLETF rats with CCK-1R deficiency and non-obese LETO rats

We studied OLETF rats to see whether the CCK-1R deficiency influences hemopoiesis. Bone marrow function was evaluated by total cellularity, CFU-GM colony formation, and CFU-GM content of femoral bone marrow. Neither cellularity nor CFU-GM colony numbers or the total femoral CFU-GM content of OLETF rats did not significantly differ from those of wild LETO rats from which OLETF rats originated. However, when we tested toxicity of carboplatin *in vitro*, decreased threshold was evaluated in CFU-GM. Dose–response curve of carboplatin was shifted to the left, and IC_{50} of carboplatin was 0.62 mg/L in CFU-GM of obese OLETF vs. 1.16 mg/L in CFU-GM of non-obese LETO rats.

Effect of proglumide and lorglumide on CFU-GM progenitors of bone marrow *in vivo*

Cellularity of femoral bone marrow shows total numbers of mononuclear cells in femoral bone marrow of rats including all type of hemopoietic and stromal stem cells with their descendants in hemopoietic lineages. Both CCK antagonists did not changed cellularities of LETO rats. Nevertheless both CCK antagonists damaged granulopoiesis *in vivo*. Differentiating granulocytes are not included in mononuclear cells of bone marrow; however, their progenitors, the granulocyte–macrophage

colony forming unit (CFU-GM), is included, as one of the populations of stem cells. Frequencies of CFU-GM as well as their total numbers in femur were less, than in control animals at $2.7 \cdot 10^{-5}$ mol/kg, the higher equimolar doses of proglumide (10 mg/kg, $P < 0.0001$) and lorglumide (13 mg/kg, $P < 0.0001$). In addition, CCK-1R-selective lorglumide decreased CFU-GM population also in the lower dose in 4 mg/kg, while proglumide had no effect in the equimolar 3 mg/kg dose.

Effect of proglumide and lorglumide pre-treatment *in vivo* on *in vitro* toxicity of carboplatin to CFU-GM progenitors

Carboplatin decreased CFU-GM colony numbers dose-dependently in bone marrow cultures of LETO rats. We found increased sensitivity of CFU-GM progenitors against carboplatin after 5-day-long administration both with proglumide and lorglumide used in equimolar doses (proglumide, 10 mg/kg; and lorglumide, 13 mg/kg, respectively). After both proglumide and lorglumide treatment of LETO rats, we observed less CFU-GM colony numbers dose-dependently, comparing with the vehicle-treated control LETO rats using *in vitro* colony assay, in which CFU-GM cells were grown in the presence of carboplatin with 1 mg/L ($P < 0.0001$ for each corresponding pair) or 2 mg/L ($P < 0.0001$) concentrations. Carboplatin in 1 mg/L concentration decreased colony numbers of CFU-GM to 28% in cultures of rats pre-treated with proglumide 3 mg/kg comparing with 11% in cultures of rats pre-treated with lorglumide equivalent 4 mg/kg dose ($P < 0.0001$). At the same time, carboplatin in 1 mg/L concentration inhibited proliferation of CFU-GM progenitors of the animals pre-treated with lorglumide completely, while 13% of CFU-GM survived after proglumide pre-treatment using $2.7 \cdot 10^{-5}$ mol/kg, the higher equimolar doses of proglumide (10 mg/kg, $P < 0.0001$) and lorglumide (13 mg/kg, $P < 0.0001$). The CCK-1R selective lorglumide had more powerful sensitizing effect to carboplatin toxicity than the non-selective proglumide.

Direct effect of proglumide and lorglumide on CFU-GM progenitors of LETO rats *in vitro*

If we cultured bone marrow mononuclear cells of LETO rats in the presence of proglumide and lorglumide in the proper dose ranges, inhibitory effects were evaluated dose-dependently on the proliferation of CFU-GM cells of the LETO rats. It is a result of direct effects on the progenitor cell population. The CCK-1R-selective lorglumide caused more powerful inhibition, than the non-selective proglumide. Dose-response curve of lorglumide was steeper and half maximal inhibitory concentrations, the IC_{50} values were 76.82 and 47.37 μM for proglumide and lorglumide, respectively.

Testing cholecystokinin receptors in bone marrow cells

For quantification of messenger RNA (mRNA) transcripts, RT-PCR method was used, and we found that both CCK-1 and CCK-2 receptors were expressed in the progenitor cells of bone marrow of LETO rats. We also used OLETF rats with CCK-1R deficiency and CCK-2 receptors were found without CCK-1 receptors on their progenitor cells.

Treatment with proglumide, a non-specific CCK antagonist in 10 mg/kg daily doses administered for 4 weeks, significantly downregulated relative mRNA levels of both CCK-1R and CCK-2R in bone marrow progenitors of the proglumide-treated LETO rats in comparison with their untreated controls. We were able to detect only CCK-2R in bone marrow progenitor cells of OLETF rats treated with proglumide by the same manner, and decrease in CCK-2 receptors was also significant after proglumide administration in OLETF rats.

5. DISCUSSION

From Nature to Treatment: The Impact of Pterostilbene on Mitigating Retinal Ischemia–Reperfusion Damage by Reducing Oxidative Stress, Inflammation, and Apoptosis

In our present study, we investigated the effects of pterostilbene on retinal I/R injury in a rat model. Our results demonstrate that pterostilbene has a significant retinoprotective effect, as evidenced by improved retinal function; preserved retinal structure; and reduced markers of glial activation, cellular stress, inflammation, and apoptosis. Pterostilbene treatment significantly enhanced retinal function, as evidenced by the increased b-wave amplitude in ERG recordings. This suggests that pterostilbene preserves the functionality of retinal cells, particularly bipolar and Müller cells, which are crucial for visual signal transmission. This indicates that pterostilbene not only protects retinal cells from immediate damage but also helps maintain their functional integrity, which is essential for visual processing. Histological analysis revealed that pterostilbene-treated rats had reduced retinal thinning and better-preserved retinal architecture compared to vehicle-treated rats. This structural preservation is critical because retinal thinning, due to the loss of retinal ganglion cells and inner retinal neurons, commonly results from I/R injury, leading to vision impairment or loss. The decreased expression of glial fibrillary acidic protein (GFAP) in pterostilbene-treated rats suggests the mitigation of glial activation, reducing the detrimental effects of gliosis on retinal neurons. Gliosis, characterized by the proliferation and hypertrophy of glial cells, can exacerbate neuronal damage and disrupt the retinal environment. By limiting this response, pterostilbene helps maintain a healthier retinal milieu, conducive to neuron survival and function. Furthermore, we found reduced levels of heat shock protein 70 (HSP70), a protein associated with cellular stress responses, indicating that pterostilbene may enhance cellular resilience to I/R-induced stress. The lower expression of HSP70 in pterostilbene-treated rats suggests that these cells experience less stress, possibly due to the antioxidant properties of pterostilbene, which help neutralize the reactive oxygen species (ROS) generated during I/R injury. The neuroprotective effects of pterostilbene observed in this study are consistent with prior research, which emphasizes its anti-inflammatory, antioxidant, and anti-apoptotic properties. Furthermore, pterostilbene has been shown to modulate inflammatory pathways by inhibiting nuclear factor kappa B (NFκB) activation, a key regulator of inflammation. Our findings of decreased NFκB expression in pterostilbene-treated rats corroborate these studies, suggesting that pterostilbene

may exert its protective effects partly by reducing inflammatory responses. NF κ B upregulates various inflammatory cytokines and adhesion molecules, contributing to secondary damage following I/R injury. By inhibiting this pathway, pterostilbene reduces the inflammatory cascade that exacerbates retinal damage. Additionally, the decrease in poly(ADP-ribose) polymerase 1 (PARP1) expression supports the idea that pterostilbene can inhibit apoptosis, as PARP1 is integral to DNA damage-induced cell death pathways. The overactivation of PARP1, in response to significant DNA damage, results in cellular energy depletion and necrotic cell death. By lowering PARP1 expression, pterostilbene likely disrupts this energy-draining cycle, thereby preserving cell viability and function.

Our study enhances the understanding of pterostilbene's protective effects by offering detailed insights into its impact on retinal I/R injury. While previous research has mainly concentrated on the systemic or neural protective effects of pterostilbene, our work emphasizes its potential as a therapeutic agent for retinal neurodegenerative conditions. The specific reduction in markers of inflammation, oxidative stress, and apoptosis underscores the multi-faceted protective mechanisms of pterostilbene. Furthermore, our findings suggest that pterostilbene's protective effects extend to preserving long-term retinal structure and function. This indicates the potential for pterostilbene to be used not only as a treatment to prevent acute damage but also as a long-term therapeutic strategy to maintain retinal health and function. The findings of this study have significant implications for treating retinal I/R injuries and related disorders. By demonstrating that pterostilbene can effectively reduce oxidative stress, inflammation, and apoptosis, this study highlights its potential as a neuroprotective therapeutic agent. These properties are particularly relevant for conditions such as diabetic retinopathy, age-related macular degeneration, glaucoma, and central retinal artery occlusion, where I/R injury is a critical pathogenic mechanism. In diabetic retinopathy, retinal I/R injury contributes to disease progression by promoting inflammation and neuronal damage. Pterostilbene's ability to mitigate these harmful processes indicates that it could serve as a valuable adjunct therapy for managing diabetic retinopathy.

This study has several limitations. It was performed on a rat model, so further research is needed to assess its applicability to humans. The dosage and administration method of pterostilbene might require optimization for clinical use, including evaluating its route of administration, bioavailability, and potential side effects. Additionally, this study primarily addressed the acute phase of retinal I/R injury; long-term studies are needed to confirm if pterostilbene's protective effects

are sustained and translate into functional vision improvements. The potential interactions of pterostilbene with other treatments for retinal diseases also need further investigation.

Obviously, future research should focus on exploring the long-term effects of pterostilbene treatment and its efficacy in different models of retinal injury. Additionally, understanding the precise molecular mechanisms by which pterostilbene exerts its protective effects will be crucial for developing targeted therapies. Clinical trials will also be necessary to confirm the safety and effectiveness of pterostilbene in human subjects. Another avenue for future research is to investigate the potential synergistic effects of pterostilbene with other neuroprotective agents. Combining pterostilbene with established treatments for retinal diseases could enhance therapeutic outcomes and provide more comprehensive protection against I/R injury.

In conclusion, our research suggests that pterostilbene, as a potential future adjunct treatment, may reduce retinal injury caused by I/R and enhance functional retinal status. By reducing oxidative stress, inflammation, and apoptosis, pterostilbene could improve visual acuity. Additionally, since numerous clinical studies have linked visual loss with cognitive decline, pterostilbene may also contribute to an improved quality of life.

CCK-1R-selective and non-selective cholecystokinin antagonists, lorglumide, and proglumide increased toxicity of carboplatin to granulocyte–macrophage progenitor cells (CFU-GM) of bone marrow of rats

Myelotoxicity is a dose-limiting side effect of chemotherapy that contributes to the narrow therapeutic windows of anticancer drugs. Each hemopoietic cell lineage is affected; however, the granulocyte–macrophage progenitor population (CFU-GM) is one of the key targets, which limits the use of the proper doses of treatment protocols because of the risk of serious neutropenia. Cells of this population are the common progenitors of granulocytes and monocytes, including phagocytes and macrophages for non-specific immune responses. The progenitor cell population has a higher proliferation rate than that of stem cell populations, which results in their increased damage during antiproliferative therapy by anticancer drugs. Myelosuppression with prolonged and more serious neutropenia associated with infections is more frequent during the chemotherapy of obese patients than in those who have normal weights. Cholecystokinin antagonists are investigated for use against the pancreas and hepatocarcinomas, the risks of which are higher with other malignancies in obesity, with poorer prognosis than in nonobese patients.

Insulin, leptin, and cholecystokinin are implicated in the control of satiety, and their dysfunctions are associated with the development of obesity and its complications. Many cytokines drive and support the proliferation, differentiation, and survival of CFU-GM progenitors and their precursor stem cells. The names of granulocyte-macrophage and granulocyte colony-stimulating factors (GM-CSF; G-CSF) are originated from their importance in granulopoiesis. It is less known that insulin and leptin also stimulate and protect hemopoietic stem cells, as well as the wide range of progenitors including CFU-GM. Leptin stimulates cholecystokinin release from the gut; cholecystokinin influences the distribution of leptin and regulates insulin release from pancreas, and among them, synergistic effects are common in many tissues. We studied whether CCK also influences hemopoiesis.

Role of cholecystokinin has not known in hemopoiesis; however, Miyamoto et al. found that transplantation with bone marrow, derived from mouse with CCK-1 receptor deficiency, produced proinflammatory macrophages and aggravated damage in diabetic kidney, which suggested that cholecystokinin influences granulopoiesis via CCK-1R signaling. We studied hemopoiesis of Otsuka Long-Evans Tokushima Fatty (OLETF) rats with CCK-1 deficiency and LETO rats from which OLETF rats originated. OLETF rats have been established as an animal model of obesity and non-insulin dependent diabetes mellitus. In our previous experiments, myelotoxicity of carboplatin was increased *in vivo* in db/db mice, the animal model of obesity-associated diabetes mellitus. Diabetes mellitus develops in OLETF rats about 18 weeks of age; therefore, we used 8-week-old rats with normal fasting blood glucose values; however, insulin resistance cannot be completely excluded, as they already had overweight. Although hemopoiesis seemed to be intact in the obese OLETF rats on the first look, sensitivity of their CFU-GM progenitors was significantly greater to toxicity of carboplatin, than that of LETO rats. Insulin resistance itself also decreases threshold of sensitivity against toxicity of carboplatin, that is why in the further experiments we used wild non-obese LETO rats to see whether CCK antagonists have effect on granulopoiesis via CCK receptors.

In our previous experiments, proglumide inhibited postprandial whole body insulin sensitization above 10 mg/kg dose, and lorglumide inhibited cerulein-induced amylase secretion of the pancreas in 10 mg/kg dose in rats. That is why we used 10 mg/kg proglumide and 13 mg/kg lorglumide equimolar doses with the same number of molecules, which seemed to be a dose affecting physiological processes in rats. To differentiate indirect effects of insulin sensitization/resistance and direct effects of CCK antagonists on bone marrow cells, we used also a lower

$8.4 \cdot 10^{-6}$ mol/kg dose, which is equal to 3 mg/kg of proglumide and 4 mg/kg of lorglumide, and furthermore, we studied direct effects of proglumide and lorglumide *in vitro* in bone marrow cell cultures, too. We found inhibitory effects of CCK antagonists on CFUGM both *in vivo* and *in vitro*. Not only the non-selective proglumide, but also the CCK-1R-selective lorglumide damaged CFU-GM cells; in addition, lorglumide had more powerful effect than proglumide. Lorglumide damaged granulopoiesis in 4 mg/kg dose, while proglumide had no effect in the equimolar dose. In higher doses, both CCK antagonists inhibited CFU-GM cells. Cholecystokinin antagonists inhibited CFU-GM progenitor cells dose-dependent manner *in vitro*, too, which confirmed direct effects on CFU-GM progenitors; IC₅₀s were 47.37 vs. 76.82 μ M, respectively.

However, both CCK receptors have a role in carcinogenesis in pancreas malignancies; the CCK-1 receptor might have a more specific role, as the highly selective CCK-2R antagonist, Z-360, had no effects in patients with metastatic pancreatic cancer in a phase II study. Both proglumide and lorglumide were successful against pancreas carcinoma in animal experiments *in vivo*. Devazepide, a CCK-1R selective antagonist with a different chemical structure than lorglumide and proglumide, was also successful in decreasing the invasiveness of pancreatic cancer cells in mice, which suggested a specific effect via CCK- 1R. All in all, the effective antitumoral doses from animal experiments corresponded to those that inhibited the proliferation of CFU-GM in normal bone marrow in our experiments. Lorglumide inhibited carcinogenesis at a 10 mg/kg parenteral dose in the pancreas of rats, and proglumide was effective against pancreas and hepatocarcinoma at 0.1 mg/ml in drinking water, which meant approximately 30 mg/kg oral dose in mice. In addition, pre-treatment with CCK antagonists sensitized CFU-GM against carboplatin.

Platinum-based drugs have become a mainstay of cancer therapy, approximately half of all patients undergoing chemotherapeutic treatment receive a platinum drug. Carboplatin has some advantages among platina derivatives as it has relatively mild non-hematological toxicities including nephro- and neurotoxicity. In combination with paclitaxel, carboplatin is used for many solid tumors, e.g., ovarian, endometrial, non-small cell lung, and cervical cancers and seemed to be successful in treatment of advanced pancreas adenocarcinomas and hepatocellular carcinomas. The dose-limiting side effect of carboplatin is myelotoxicity with thrombocytopenia and severe neutropenia. In combination, paclitaxel may ameliorate thrombocytopenia but not neutropenia caused by carboplatin. The CCK-1R-selective lorglumide pre-

treatment resulted in greater increase in sensitivity of CFU-GM and aggravated toxicity of carboplatin more, than the non-selective proglumide.

Proglumide, a non-selective CCK receptor antagonist, decreased migration of neutrophils and macrophages into infectious focus in sepsis. Cholecystokinin-2 receptor was determined in human peripheral blood mononuclear cells and on polymorphonuclear leukocytes obtained from peripheral blood. Both CCK-1R and CCK-2R are found in rat pulmonary interstitial macrophages, and lipopolysaccharide stimulates their gene expressions. In peritoneal macrophages, CCK-1R and not CCK-2R were detected and gene expression of CCK-1R increased by lipopolysaccharide-stimulation. Anti-inflammatory and immunomodulatory effects of cholecystokinin are realized mostly via CCK-1R, including attenuation of iNOS expression as well as NF kappa B signaling or inhibition of phagocytosis and tissue infiltration with macrophages, which may be useful in sepsis and devazepide, a CCK-1R selective antagonist, inhibiting some of these effects.

However, in the HL-60 promyelocytic leukemic cell line, CCK-2R is found; there are no data whether they are also found on the normal hemopoietic cells. In our results, we presume that not only CCK-2R, but also CCK-1R are present in normal CFU-GM progenitors and protect them. We were able to detect both CCK-1 and CCK-2 receptors in the CFU-GM enriched bone marrow of LETO rats. Both receptors were downregulated by proglumide using 10 mg/kg orally for 4 weeks. In OLETF rats with CCK-1R deficiency, we detected CCK-2R without CCK-1R.

We detected both CCK receptors in CFU-GM of LETO rats, and our results with CCK antagonists suggested that CCK-1R deficiency of obese OLETF rats might contribute at least partly to the increased sensitivity of bone marrow progenitors to carboplatin. It is interesting that the anorexigenic insulin and leptin have a role in maintaining lympho- and myelopoiesis, and the protective effect of insulin was proven in granulopoiesis in our previous experiments, too. Based on our results, cholecystokinin also may have a protective role in granulopoiesis.

The showed results suggested the role of CCK-1 receptors in granulopoiesis. Therapy failures when leptin or cholecystokinin agonists were tried to suppress appetite in exogenous obesity suggest that not only insulin resistance but also decreased sensitivities against other cytokines may develop in obesity. It is supported by the findings of Desai et al. who described that CCK-1 responsiveness varied in the population and was decreased in obese and morbidly obese patients. We found previously that insulin resistance covered progenitors in bone marrow and

rosiglitazone, an insulin sensitizer, had a protective effect against myelotoxicity caused by 5-fluorouracil.

Our recent findings supported the results of Miyamoto et al. about the protective role of cholecystokinin in granulopoiesis via CCK-1R during the production of normal bone marrow-derived macrophages. Cholecystokinin antagonists affected granulopoiesis and sensitized granulocyte–macrophage progenitors against carboplatin toxicity presumably by inhibition of the protective effect of cholecystokinin via the CCK-1 receptor, which is the real specific cholecystokinin receptor. Corresponding with publications about the immunomodulatory effects of cholecystokinin on mature macrophages, we proved that regulatory functions covered also their progenitors in bone marrow. Further investigations are warranted to understand the function and significance of CCK receptors in granulocyte–macrophage progenitor cells (CFU-GM). If the protective role of cholecystokinin is corroborated in various physiological and pathological processes, it might have value, not only when CCK antagonists are tried for use in malignancies, but also if they are used in other clinical indications in obesity or during immunosuppressive therapies.

6. SUMMARY

The aim of our research group was to investigate the role of pterostilbene, as well as cholecystokinin and its antagonists, in experimental rat models under different pathophysiological conditions. In our work, we examined processes in which the extent of cellular damage can be experimentally modulated by the administration of a natural compound and by the modification of endogenous regulatory systems.

In the first part of the dissertation, the effects of pterostilbene were analyzed in a rat model of retinal ischemia–reperfusion injury. Based on our results, pterostilbene favorably influenced retinal functional status and reduced the extent of ischemia–reperfusion–associated functional, structural, and molecular alterations.

In the second part of the dissertation, we investigated the sensitivity of bone marrow CFU-GM progenitors to carboplatin under conditions of cholecystokinin receptor antagonist treatment and CCK-1 receptor deficiency. According to the obtained results, both factors increased the sensitivity of progenitor cells to toxic insults, supporting the role of cholecystokinin in the regulation of granulopoiesis.

7. NOVEL FINDINGS OF THE DISSERTATION

In a rat model of retinal ischemia–reperfusion injury, pterostilbene treatment significantly improves the functional state of the retina, as demonstrated by the increased scotopic b-wave amplitudes measured by electroretinography compared to the vehicle-treated ischemic group.

Preservation of retinal structural integrity under pterostilbene treatment is detectable under both ischemic and non-ischemic conditions, as the treatment significantly attenuates ischemia–reperfusion-induced retinal thinning.

Reactive gliosis and the cellular stress response characteristic of retinal I/R injury are significantly reduced by pterostilbene treatment, as supported by the decreased expression of GFAP and HSP70 proteins.

Activation of inflammatory and apoptotic signaling pathways is markedly attenuated in retinal tissue following pterostilbene treatment, as confirmed by the reduced expression of NFκB and PARP1 proteins.

In CCK-1 receptor-deficient obese OLETF rats, CFU-GM sensitivity to carboplatin is significantly increased, as indicated by the leftward shift of the dose–response curve and the reduction of the IC₅₀ value.

Administration of CCK receptor antagonists in non-obese LETO rats significantly decreases the number of CFU-GM progenitor cells *in vivo*, reflecting impaired granulopoiesis.

The CCK-1R-selective antagonist lorglumide exerts a more pronounced CFU-GM-reducing effect even at low doses than the equimolar non-selective antagonist proglumide, supporting the prominent role of the CCK-1 receptor in the regulation of granulopoiesis.

Pretreatment with proglumide and lorglumide increases the sensitivity of CFU-GM progenitor cells to carboplatin, manifested by a dose-dependent reduction in colony-forming capacity.

In vitro bone marrow cell cultures demonstrated that both CCK antagonists exert a direct, dose-dependent inhibitory effect on CFU-GM progenitor cell proliferation, thereby excluding the primary role of indirect metabolic mechanisms.

Both CCK-1R and CCK-2R expression are detectable in CFU-GM progenitor cells, whereas only CCK-2R is present in CCK-1R-deficient OLETF rats. Our findings suggest that cholecystokinin exerts a protective effect during granulopoiesis, predominantly through CCK-1R-mediated mechanisms.

8. APPENDIX



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List of publications related to the dissertation

1. **Pelles-Taskó, B.**, Varga, A., Géresi, K., Juhász, B., Szilvássy, Z., Benkő, I.: CCK-1R-selective and non-selective cholecystokinin antagonists, lorglumide, and proglumide increased toxicity of carboplatin to granulocyte-macrophage progenitor cells (CFU-GM) of bone marrow of rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 399 (1), 129-141, 2026.
DOI: <http://dx.doi.org/10.1007/s00210-025-04391-6>
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2. **Pelles-Taskó, B.**, Szekeres, R., Takács, B., Szilágyi, A., Ujvárosy, D., Bombicz, M., Priks, D., Varga, B., Gesztelyi, R., Szabó, Z., Szilvássy, Z., Juhász, B.: From Nature to Treatment: The Impact of Pterostilbene on Mitigating Retinal Ischemia-Reperfusion Damage by Reducing Oxidative Stress, Inflammation, and Apoptosis. *Life (Basel)*. 14 (9), 1-13, 2024.
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3. Károlyi, P. K., Képes, Z., Pelyvás, B., Marosi, M., Juhász, B., Berényi, E., Englohner, A., Kocsi, E., **Pelles-Taskó, B.**, Trouvé-Carpena, A., De Santis, S., Canals, S., Trencsényi, G., Meszár, Z., Papp, T.: Multimodal preclinical study of long-term effects of prenatal ultrasound on the mouse central nervous system. *Sci. Rep. [Epub ahead of print]*, 2026.
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