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ORIGINAL ARTICLE

Effect of the Proline-Rich Polypeptide Complex/Colostrinin™ on the Enzymatic Antioxidant System

Agnieszka Zabłocka · Maria Janusz

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Abstract Proline-rich polypeptide complex (PRP) and its constituent nonapeptide (NP) possess immunoregulatory and procognitive properties. PRP in the form of sublingually administered tablets called Colostrinin™ improves the outcome of patients with Alzheimer's disease (AD). Free radical-induced oxidative stress has been implicated in the pathogenesis of AD. It has been previously shown that PRP and NP inhibit overproduction of reactive oxygen species, nitric oxide and proinflammatory cytokines induced by lipopolysaccharide or PMA. Antioxidant defense includes both low molecular weight components and enzymatic systems including dismutases, catalase, glutathione reductase (GSSGR) and glutathione peroxidase (GSHPx). An early event during the development of AD is lipid and protein peroxidation. PRP and NP showed no modulatory effect on lipid peroxidation. A protective effect on protein oxidation was found only when high doses of NP were used. We have previously shown, in a model of human peripheral blood mononuclear cells, that PRP/NP affects activities of superoxide dismutase and NF-κB. In the present study with the use of the same cell model and whole blood cells we observed an activatory effect of PRP/NP on GSHPx and GSSGR activity but not catalase. The observed effect suggests that PRP/NP can act as a modulatory agent of the "first line" of antioxidant defense. It can be assumed therefore that PRP/Colostrinin by regulation of the early phase of the redox system does not reduce but rather prevents oxidative damage. This effect may shed some light on the beneficial effect of PRP/Colostrinin in AD patients.

A. Zabłocka (✉) · M. Janusz
Department of Immunochemistry, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland
e-mail: zablocka@iitd.pan.wroc.pl

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Abbreviations

PRP	Proline-rich polypeptide complex
NP	Nonapeptide
AD	Alzheimer's disease
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SOD	Superoxide dismutase
CAT	Catalase
MDA	Malondialdehyde
4-HNE	4-Hydroxynonenal
GSHPx	Glutathione peroxidase
GSSGR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
PBMC	Peripheral blood mononuclear cells
PMA	Phorbol 12-myristate 13-acetate
LPS	Lipopolysaccharide
BHT	Butylated hydroxytoluene
H_2O_2	Hydrogen peroxide

Introduction

The generation of reactive oxygen species (ROS) in normal cells, including neurons, is under tight homeostatic control. Unchecked, excessive ROS generation may lead to the destruction of cellular components including lipids, proteins, and DNA, and ultimately cell death via apoptosis or necrosis (Klein and Ackerman 2003). It is widely accepted that the free radical-induced oxidative stress increases

brain aging. The brain, compared with other organs, is especially vulnerable to free radical damage because of its high oxygen consumption rate, abundant lipid content, and relative paucity of antioxidant enzymes compared with other organs. Free radical oxidative stress is implicated in the pathogenesis of a variety of human diseases, including Alzheimer's disease (AD). According to the free radical hypothesis, AD involves acceleration of the normal aging process in affected brain regions, which become progressively more damaged by free radicals generated in the metabolism. Oxidative stress may cause neuronal cell dysfunctions through oxidative modification of macromolecules, including proteins. Living organisms have evolved several mechanisms to protect themselves from attack by ROS and reactive nitrogen species (RNS). The antioxidant defense mechanisms include scavenging ROS/RNS and their precursors, binding catalytic metal ions needed for ROS formation, and generation and up-regulation of endogenous antioxidant defenses. The major groups of antioxidant defense systems are low molecular weight antioxidant compounds, e.g., vitamin C and E, lipoic acid, and the enzymatic antioxidant system including superoxide dismutases (SODs), catalase (CAT), glutathione peroxidase (GSHPx), and glutathione reductase (GSSGR), among others (Poon et al. 2004; Vijayalakshmi and Chandrasekhar 2008).

Elevated levels of the end products of lipid peroxidation due to oxidative stress, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), were found in the brain and in ventricular cerebrospinal fluids in AD patients (Butterfield et al. 2006; Chen et al. 2007; Markesbery and Carney 1999).

In redox signaling pathways (e.g., nitric oxide, H_2O_2) that modulate synaptic activity and other nerve cell functions, sulfhydryl groups could participate. Disruption of nitric oxide signaling and consequential synaptic dysfunction have a pathophysiological connection with molecular events that mediate neurodegenerative status in Parkinson and AD (LoPachin and Barber 2006). ROS are cytotoxic, and evidence of ROS-induced damage to cell membranes, proteins, and DNA in AD is overwhelming. We have previously shown in cultures of human peripheral blood mononuclear cells (PBMCs) that H_2O_2-induced activity of PMA was inhibited (40–60 %), in a dose-dependent manner, by proline-rich polypeptide complex (PRP) and its constituent nonapeptide (NP) component (Zabłocka et al. 2007).

PRP was first isolated from ovine colostrum and subsequently found in human and bovine colostrum. PRP is composed of peptides of molecular masses ranging from 500 to 3,000 Da. The polypeptide complex contains 25 % proline and 40 % hydrophobic amino acids. It was shown to possess immunoregulatory properties, including effects on the maturation and differentiation of murine thymocytes and humoral and cellular immune responses, both in vivo and in vitro. PRP seems to restore balance in cellular immune functions and is not species specific. NP fragment, Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro, isolated from chymotryptic digestion of PRP and subsequently obtained by chemical synthesis, showed immunoregulatory activities similar to PRP (Janusz and Zabłocka 2010). PRP induces and regulates secretion of Th1 (interferon and tumor necrosis factor α) and Th2 (interleukin 6 and 10) cytokines involved in cell-mediated and humoral immune responses, respectively. PRP is able to inhibit the production of hydrogen peroxide (H_2O_2) induced by PMA and the activity of SOD (Zabłocka et al. 2001, 2007). PRP, besides its immunoregulatory properties, affects learning, memory and lifespan, as was shown in studies on rats, chickens and mice (Boldogh et al. 2007; Popik et al. 1999; Stewart and Banks 2006). PRP and NP inhibit formation aggregates of amyloid β (Aβ) and dissociate the aggregates already formed (Janusz et al. 2009; Schuster et al. 2005). PRP/Colostrinin induces signaling pathway leading to the neurite outgrowth (Bacsi et al. 2005). Properties of PRP, its role in the development of the immune system, cognitive function as well as Aβ aggregation, suggested its potential use in therapy. Therefore, the complex was proposed for the treatment of AD. The efficacy and safety of PRP administered to patients in the form of sublingually administered tablets called Colostrinin^{TM} was first shown in a double-blind placebo-controlled trial (Leszek et al. 1999) and confirmed in a long-term (16- to 28-month) open-label study (Leszek et al. 2002) and in multicenter clinical trials (Bilikiewicz and Gaus 2004). Colostrinin has been shown to have a stabilizing effect on the cognitive function. Patients who were an early stage of the disease responded better than those in advanced stage. In the early stage of the disease an oxidative damage occurs in the brain of AD patients.

H_2O_2 generation could be controlled by glutathione (GSH) metabolism and activity of antioxidant enzymes. Therefore, we decided to study the effect of PRP and NP on the enzymatic antioxidant system including CAT, GSHPx and GSSGR. The effect of PRP/NP on the lipid and protein peroxidation was measured by determination of the level of MDA and total sulfhydryl groups. There is a link between the activation of immune cells on the periphery, microglia, and neuronal injury in the central nervous system, including AD (Kalman et al. 1997). The experiments performed on the whole blood cells and PBMC activated with lipopolysaccharide (LPS), used as a proinflammatory substance, may be helpful in elucidating the positive clinical effect of PRP/Colostrinin observed in AD patients.

Materials and Methods

Materials

RPMI 1640 medium was obtained from the Laboratory of Biopreparations of the Institute Immunology and Experimental Therapy (Wrocław, Poland). Tissue culture plates were from Costar (USA). Lymphoflot was purchased from Nobipharm. Bacterial LPS from *E. coli* (serotype 055:B5), leukoagglutinin, nitrate reductase, nicotinamide adenine dinucleotide phosphate (tetrasodium salt), L-glutamine, antibiotics (penicillin/streptomycin mixture) and 5,5′ dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma (USA). *N*-(1-naphthyl)-ethylenediamine was purchased from Serva Feinbiochemica (Germany). Sulfanilamide, sodium nitrite, and orthophosphoric acid were obtained from Polish Chemical Reagents. Butylated hydroxytoluene (BHT) was from Cayman Chemical Company (USA). Fetal bovine serum (FBS) was obtained from Gibco BRL (UK). Quantitative H_2O_2 assay, colorimetric assay for GSH level and lipid peroxidation assay were obtained from Oxis International (USA). The PRP was prepared from ovine colostrum according to the procedure of Janusz et al. (1981). The NP, Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro, was synthesized by a solid-phase method in the Laboratory of Chemistry and Stereochemistry of Peptides and Proteins, Faculty of Chemistry, University of Wrocław (Poland).

Blood samples and "buffy coats" were kindly provided by the Station of Blood Donation, Military Hospital (Wrocław, Poland).

Human PBMCs

PBMCs were obtained by density centrifugation of buffy coats of healthy blood donors over Lymphoflot: 10 ml of buffy coats were suspended in 20 ml of "RPMI for isolation" (RPMI 1640 supplemented with 3 % L-glutamine, heparin 10 U/ml and 1 ml of streptomycin/penicillin mixture). Fifteen milliliter of the cell suspension were loaded on 7.5 ml of Lymphoflot and centrifuged at 600×*g* for 35 min at room temperature. The cells were washed 3 times with 30 ml of the medium for isolation and suspended in "RPMI medium for culture" (RPMI 1640 for isolation supplemented with 2 % FBS). To lower the effect of stimulation of cells induced by the isolation procedure, the cells were first cultured alone in RPMI 1640 for 20 h at 37 °C and then used in experiments. Cell viability was evaluated using the trypan blue exclusion assay. The viability was always greater than 95 %.

Induction of Oxidative Stress

Whole Blood Samples

Venous blood samples were collected in EDTA-containing syringes. One ml samples of the whole blood were plated in 48-well culture plates and incubated for 1.5 h in the presence of LPS (8 μg/ml), PRP (10 or 100 μg/ml) or NP (10 or 100 μg/ml). A modulatory effect of PRP/NP added simultaneously with LPS was also examined. The whole blood samples were used for the determination of H_2O_2 and GSH level, CAT, GSHPx and reductase activities.

PBMC

100 μl samples of PBMC suspension (1×10^7/ml in RPMI phenol red-free) were plated in 96-well culture plates and cultured for 1.5 h in the presence of LPS (8 μg/ml), PRP (10 or 100 μg/ml) or NP (10 or 100 μg/ml). To examine the effect of PRP/NP on the induced oxidative stress, PBMC were treated with peptides (10 or 100 μg/ml) added simultaneously with LPS. After incubation, the plates were centrifuged at 200×*g* for 5 min at 4 °C. The cells were treated with 1 ml of cold PBS containing 10 μl of 0.5 M BHT (to prevent oxidation during the extraction procedure) and next sonicated for 30 min. The cell homogenates were centrifuged for 10 min at 12,000×*g*. The supernatants were collected and used for lipid and protein peroxidation assay performed on the same day.

Hydrogen Peroxide Determination

Hydrogen peroxide (H_2O_2) production was determined in the supernatants after induction of oxygen stress in the human whole blood cells, as described previously (Zabłocka et al. 2007).

Activity of GSHPx

GSHPx activity was determined in the whole blood cell lysates by monitoring oxidation of the reduced form of NADPH in the presence of H_2O_2 (Sinitsyna et al. 2006). The concentration of the reduced NADPH was measured in a UV–Vis spectrophotometer (Shimadzu UV-1202) at 340 nm in the presence of reduced GSH and GSSGR in 50 mM phosphate buffer (pH 7.1, 25 °C). The results were presented as U/ml of whole blood (1 U represents a number of micromoles of NADPH oxidized per 1 min).

Activity of GSSGR

GSSGR was determined according to the procedure of Glatzle (Goldberg and Spooner 1983). The reaction product (GSH formed in the presence of GSSGR) was measured spectrophotometrically at 340 nm (Shimadzu UV-1202). One unit of enzymatic activity is defined as a change in the absorbance value of 0.01/min.

GSH Concentration

In short, the protein was precipitated with 5 % metaphosphoric acid from the whole blood samples and diluted with a glass-distilled water. The samples were then centrifuged at 2,000×*g* for 5 min, GSH and GSSG concentrations were determined spectrophotometrically according to the procedure recommended by the manufacturer (Oxis Researchers™).

Determination of Lipid Peroxidation Products

Assay for MDA was performed using the PBMC hemolysates according to the procedure recommended by the producer of the assay kit (Oxis). 650 µl of activated 1-methyl-2-phenylindole and 200 µl of sample or standard (0–100 µM tetraethoxypropane) were added to 2 ml microcentrifuge tubes, vortex mixed, and treated with 150 µl of 37 % hydrochloric acid. The reaction mixture was incubated at 45 °C for 60 min, then placed on ice to stop the reaction. The reaction product was measured spectrophotometrically at 586 nm.

Determination of Protein Oxidation by the Ellman Method

Total content of [–SH] groups in hemolysates of PBMC was determined according to the DTNB method (Fuse et al. 2007). The absorbances of the samples were measured at 412 nm using a spectrophotometer (Shimadzu UV 1202). The absorbance values were converted into sulfhydryl group content (µM) using a regression equation. The regression equation was calculated from three independent standard experiments performed with increasing concentrations of GSH (range 2–200 µM).

Statistical Analysis

Each experiment was repeated at least ten times in duplicate. Data are presented as the median ± SD using nonparametric Wilcoxon test. Statistically significant differences between the experimental groups were determined by analysis of variance. The results were considered significant when $p \leq 0.05$.

Results

Effect of PRP and NP on Hydrogen Peroxide Generation

In the preliminary experiments we observed that the time needed to obtain maximal H_2O_2 release by human whole blood cells was 90 min. Non-stimulated whole blood cells released H_2O_2 in the range of concentration 4–5 µM. The secretion of H_2O_2 was elevated in the presence of LPS. PRP and NP did not change the amount of H_2O_2 constitutively released. When PRP or NP was applied to the cells 45 min after or 45 min before LPS administration, no inhibitory effects were observed. However, both PRP and NP in concentrations of 10 and 100 µg/ml, when applied to the cell cultures simultaneously with LPS, inhibited H_2O_2 release in a dose-dependent manner. The PRP complex shows much higher inhibitory activity compared with one of its constituent components, NP. In the presence of 100 µg PRP, the level of H_2O_2 released is markedly below the control level (Fig. 1a, b).

Effect of PRP and NP on GSHPx, GSSGR Activity and the Level of GSH

The protection against ROS mediated by GSH leads to GSH oxidation and formation of glutathione disulfide

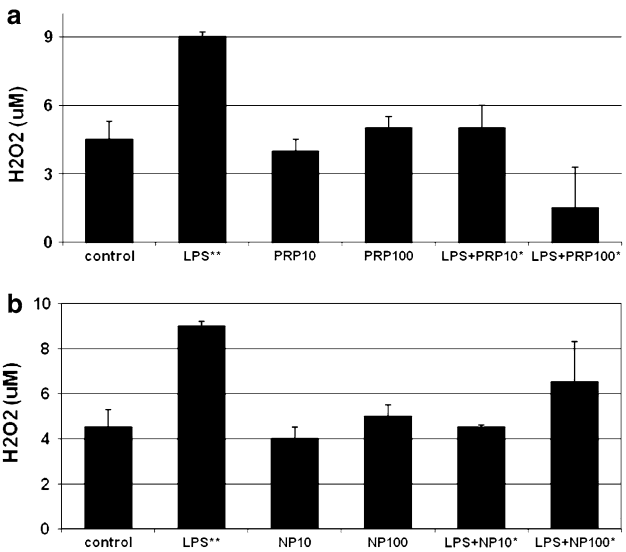

Fig. 1 Effect of PRP (**a**) and NP (**b**) on H_2O_2 release induced by LPS in human whole blood cells. Human whole blood cells were incubated for 90 min in the presence of LPS (8 µg/ml), PRP (10 and 100 µg/ml) (**a**) and NP (10 and 100 µg/ml) (**b**) or in the presence of LPS simultaneously applied with PRP or NP. H_2O_2 was determined in cell supernatants. Control cells were incubated in the absence of inducers. Concentration of H_2O_2 from at least ten independent experiments was performed in duplicate. *Each bar* represents the median ± SD. The difference between groups was analyzed using nonparametric Wilcoxon test. Statistically significant differences ($p \leq 0.05$): *sample versus LPS and **LPS versus control ($p \leq 0.05$)

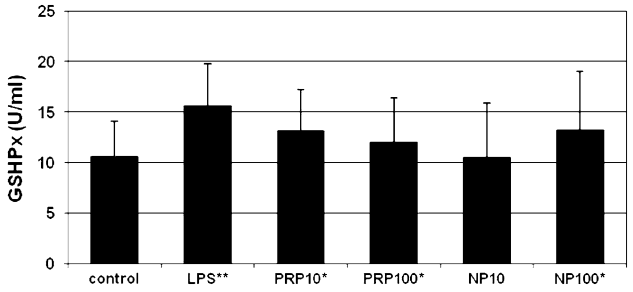

Fig. 2 Effect of PRP and NP on glutathione peroxidase (GSHPx) activity. Human whole blood cells were incubated for 90 min in the presence of LPS (8 μg/ml), PRP (10 and 100 μg/ml) and NP (10 and 100 μg/ml). Control cells were incubated in the absence of inducers. GSHPx activity was determined in the whole blood cell lysates by monitoring oxidation of the reduced form of NADPH in the presence of H_2O_2 and was expressed as units per ml of the whole blood (U/ml). *Each bar* represents the median ± SD. The difference between groups was analyzed using nonparametric Wilcoxon test. Statistically significant differences ($p \leq 0.05$): *sample versus control and **LPS versus control

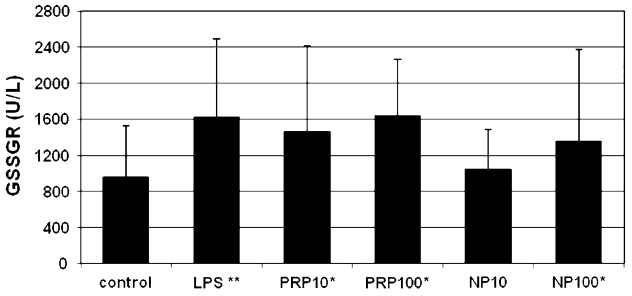

Fig. 3 Effect of PRP and NP on glutathione reductase (GSSGR) activity. Human whole blood cells were incubated for 90 min in the presence of LPS (8 μg/ml), PRP (10 and 100 μg/ml) and NP (10 and 100 μg/ml). Control cells were incubated in the absence of inducers. GSSGR activity was determined in cell hemolysates and was expressed as U/L of whole blood. *Each bar* represents the median ± SD. The difference between groups was analyzed using nonparametric Wilcoxon test. Statistically significant differences ($p \leq 0.05$): *sample versus control and **LPS versus control

(GSSG) in a reaction catalyzed by GSHPx. The reverse reaction, GSSG-GSH, is catalyzed by GSSGR. In the presence of PRP (10 and 100 μg/ml) and NP (100 μg/ml) statistically significant induction of GSHPx activity was observed (Fig. 2). Activity of GSSGR was elevated in the presence of both PRP (10 and 100 μg) and NP (100 μg/ml) (Fig. 3). The elevated GSSGR activity in the presence of PRP and high doses of NP prevent accumulation of harmful GSSG and is accompanied by reconstitution of the GSH pool (Fig. 4a, b). PRP and NP can regulate the oxidative status of cells at the level of GSH–GSSG transition, influencing the GSHPx and GSSGR activity (Table 1).

Effect of PRP and NP on Lipid Peroxidation

PRP and NP did not affect lipid peroxidation in the PBMC samples. The level of one oxidative stress marker, MDA, was significantly elevated in samples treated with LPS. However, no modulatory effect was observed in the presence of PRP and NP (data not shown).

Effect of PRP and NP on Protein Oxidation

Sulfur-containing amino acids are easily and reversibly oxidized under relatively mild conditions. Therefore, free [–SH] groups were estimated as a marker of the PRP/NP effect on protein oxidation. Under our experimental conditions differences in [–SH] group content were observed between LPS and LPS + PRP/LPS + NP-treated PBMC samples (Fig. 5).

Discussion

It is known that exposure to oxidative stress induces the transcription of a number of genes, as well as enzymes

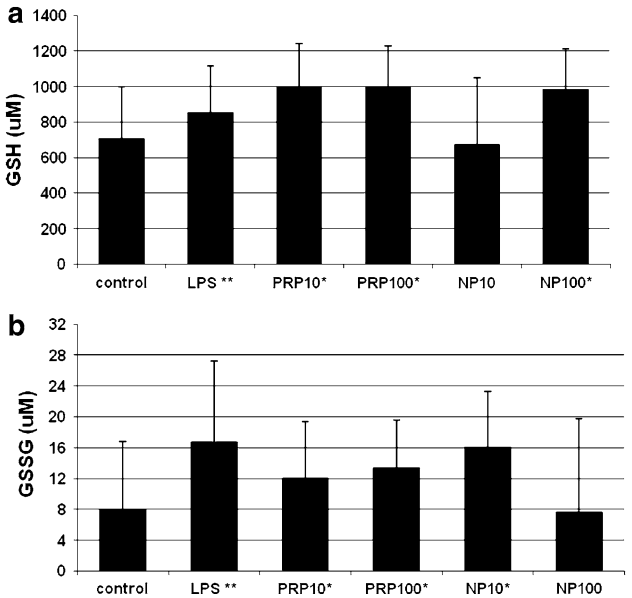

Fig. 4 Effect of PRP and NP on level of reduced (**a**) and oxidized (**b**) glutathione. Human whole blood cells were incubated for 90 min in the presence of LPS (8 μg/ml), PRP (10 and 100 μg/ml) and NP (10 and 100 μg/ml). Control cells were incubated in the absence of inducers. GSH level was determined in cell hemolysates. GSH/GSSG concentrations were expressed as μM. *Each bar* represents the median ± SD. The difference between groups was analyzed using nonparametric Wilcoxon test. Statistically significant differences ($p \leq 0.05$): *sample versus control and **LPS versus control

activity including SOD, CAT and GSHPx. We have previously shown that PRP and NP inhibited by about 50 % SOD activity and release of H_2O_2 induced by PMA in the human PBMCs (Zabłocka et al. 2007). This inhibition was not due to the death of the cells. Cell viability was evaluated using trypan blue. Neither PRP nor NP or LPS in concentrations used in experiments affected the cell viability. Properties of PRP/Colostrinin, especially with

Table 1 Effect of proline-rich polypeptide complex (PRP) and its nonapeptide (NP) fragment on GSH/GSSG ratio

Sample	GSH/GSSG ratio
Control	351
LPS (8 μg)	201
PRP (10 μg)	331
PRP (100 μg)	384
NP (10 μg)	323
NP (100 μg)	515

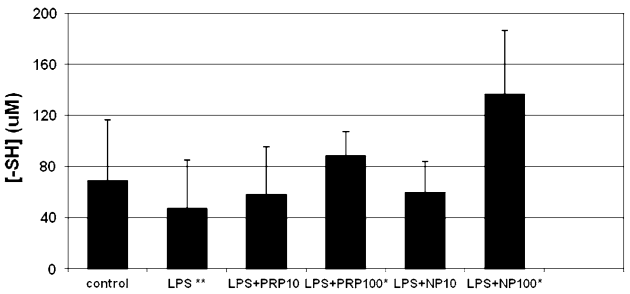

Fig. 5 Effect of PRP and NP on regulation of protein peroxidation. 1×10^7/ml of PBMC were incubated for 90 min in the presence of LPS (8 μg/ml), PRP (10 and 100 μg/ml) and NP (10 and 100 μg/ml) or in the presence of LPS simultaneously applied with PRP or NP. Control cells were incubated in the absence of inducers. Total [–SH] group content in hemolysates of PBMC was expressed as μM. *Each bar* represents the median ± SD. The difference between groups was analyzed using nonparametric Wilcoxon test. Statistically significant differences ($p \leq 0.05$): *sample versus LPS and **LPS versus control

respect to oxidative stress modulation, are exhaustively discussed by Boldogh and Kruzel (2008). It is known that the transcription of genes coding a wide variety of proteins which regulate the inflammatory processes is controlled by NF-κB. In our previous studies we showed that PRP inhibited NF-κB activity (Zabłocka et al. 2010).

No effect of PRP and NP on CAT activity was observed. Catalase, an enzyme which protects cells from accumulation of H_2O_2 by dismutation into water and oxygen, is one of the most potent catalysts known and is considered as an enzyme of the "second line of defense". Lack of any effect of PRP on CAT activity suggests that PRP and NP can modulate the "first line of defense". In the "first line", a toxic level of H_2O_2 is reduced with GSHPx and GSSGR. As shown in Figs. 2 and 3, both PRP and NP can elevate GSHPx and GSSGR activities. In this way, the reduced form of GSH is recovered and a cell can be protected against harmful accumulation of the oxidized form of glutathione (GSSG) without CAT intervention (Fig. 4a, b). Differences between PRP and NP activities, e.g., the effect on GSSGR activity (Fig. 3) and GSH level (Fig. 4a, b), suggest that also other constituent peptides of PRP complex, besides NP, play a role in the regulatory effect. The biphasic character of the relationship between the concentration of the PRP peptides and their activity indicate regulatory, not only inductive, function of these peptides (Wirkus-Romanowska et al. 2000; Zabłocka et al. 2001).

Padurariu et al. (2010), studying AD patients and age-matched control individuals, observed a significant decrease of GSHPx activity in the serum of AD patients. No change was observed in healthy individuals. Melo et al. (2009) showed that fresh or aggregated Aβ significantly increased the amount of ROS and lipoperoxidation. In Aβ-treated neurons, the depletion of reduced glutathione seems to be related to the decrease in GSHPx and GSSGR activities. These alterations in the GSH antioxidant system were prevented by galantamine. Similar prevention of PRP/NP activity was found in our studies using whole blood cells and PBMC models. Our results suggest that the inhibition of SOD shown in our previous studies (Zabłocka et al. 2007), and elevation of GSHPx activity by PRP/NP, might be involved in the beneficial effects of Colostrinin in AD patients. It is suggested that decrease in the intracellular level of ROS under influence of Colostrinin may delay the development of cellular aging (Basci et al. 2007).

During enhanced oxidative stress, cells' antioxidant mechanism is insufficient. In consequence, increased lipid peroxidation is observed. Alterations in membrane fluidity after peroxidation of membrane-bound lipids generate the neurodegenerative processes observed in the case of AD (Marcus et al. 1998). Elevated levels of the lipid peroxidation products MDA and 4-HNE were found in AD. HNE metabolites are recognized as potential biomarkers of disease progression (Chen et al. 2007; Moreira et al. 2010; Völkel et al. 2006). It was found that PRP/Colostrinin reduces 4-HNE-mediated cellular damage and suppresses 4-HNE-induced cellular signaling in PC12 cultured cells (Boldogh et al. 2003). In our experimental model, using human PBMCs, no effect of PRP and NP on lipid peroxidation measured as the level of MDA was found.

PRP/NP showed inhibitory effect on 4-HNE, SOD, inducible nitric oxide synthase and stimulating effects on the GSHPx and GSSGR activity. No effect on CAT activity determined in the whole blood cells hemolysates according to the method of Aebi (1984) was observed (data not shown). These results and the fact that PRP inhibited the NF-κB activity suggest that PRP and NP act as the modulatory agents of the "first line of defense in an organism". Because the oxidative damage begins early in the course of the disease, it represents a potential therapeutic target for slowing the onset and progression of AD. PRP activity as a "first line" modulatory agent was shown in our previous studies on thymocyte maturation/differentiation (Wieczorek et al. 1989). Also, efficacy of PRP/Colostrinin in AD treatment was found to be dependent on the stage of the disease. Patients at the beginning of the trial who were at

an early stage of the disease responded to the PRP treatment better than those with very advanced disease (Leszek et al. 1999). Our data also suggest the possibility of using one of the PRP complex constituent peptides, the NP Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro, which has a known structure and is easy to obtain by chemical synthesis, for selective modulation of the antioxidant system.

Taking into consideration our previous and present results, we can surmise that PRP/Colostrinin through its influence on the early phase of the redox system can prevent oxidative damage, e.g. to lipids or proteins, rather than reduce it. The present results can shed some light on the beneficial effect of PRP/Colostrinin in AD patients.

Conflict of interest All the authors who have taken part in this study declare that they have nothing to disclose regarding competing interests or funding from industry with respect to this manuscript.

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