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Lipoic Acid Decreases the Viability of Breast Cancer Cells and Activity of PTP1B and SHP2

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Abstract. *Background: Protein tyrosine phosphatases PTP1B and SHP2 are potential targets for anticancer therapy, because of the essential role they play in the development of tumors. PTP1B and SHP2 are overexpressed in breast cancer cells, thus inhibition of their activity can be potentially effective in breast cancer therapy. Lipoic acid has been previously reported to inhibit the proliferation of colon, breast and thyroid cancer cells. Materials and Methods: We investigated the effect of alpha-lipoic acid (ALA) and its reduced form of dihydrolipoic acid (DHLA) on the viability of MCF-7 cancer cells and on the enzymatic activity of PTP1B and SHP2 phosphatases. Results: ALA and DHLA decrease the activity of PTP1B and SHP2, and have inhibitory effects on the viability and proliferation of breast cancer cells. Conclusion: ALA and DHLA can be considered as potential agents for the adjunctive treatment of breast cancer.*

Breast cancer is one of the most common female tumors worldwide. Breast cancer therapy includes surgery, radiotherapy or adjuvant chemotherapy. The development of breast cancer is associated with numerous disorders of tyrosine phosphorylation pathways (1).

Protein tyrosine phosphatase PTP1B dephosphorylates tyrosine kinases essential for the induction of breast cancer, *i.e.* HER1/EGFR, Src, JAK and STAT. PTP1B phosphatase is overexpressed in breast cancer cells and triggers the growth of a tumor (2). PTP1B phosphatase inhibitors are promising compounds for treating metabolic diseases, *i.e.* type 2 diabetes, obesity and metabolic syndromes. Protein tyrosine phosphatase SHP2 is overexpressed in breast cancer cell lines, and usually provided by oncogenic signaling functions to promote growth factors and cytokines. Additionally SHP2 mutations have been found in breast cancer cells. Due to oncogenic implications of SHP2, inhibition of these phosphatases can produce a favorable effect in anticancer therapy (3). Due to the key part of protein tyrosine phosphatases in cancer biology, they might constitute promising targets for the development of new anticancer diagnostic and therapeutic strategies (4).

Previous studies have shown that fatty acids may be natural inhibitors of pro-oncogenic tyrosine phosphatase SHP2 (5). Important seems to be the fact that fatty acids have recently begun playing a role in the design of treatment of numerous diseases, with particular focus on cancer. Previous results confirmed that the selected carboxylic acids, *i.e.* octanoic acid, in comparison to hydrogen peroxide, possess significantly higher binding affinity to bind in the PTPs active site and can be strong inhibitors of PTPs (6).

Lipoic acid (LA) is a short-chain fatty acid, an organosulfur compound derived from octanoic acid. LA can be synthesized in the human body, but it can also be absorbed from the diet. Alpha-lipoic acid (ALA), also known as thioctic acid, can be reduced to dihydrolipoic acid (DHLA) (Figure 1). Both compounds, ALA and its reduced form DHLA, are powerful antioxidants (7). Recent studies suggested the possibility of lipoic acid as a potential anti-cancer agent and confirmed that LA can inhibit cell proliferation of, among others, breast, thyroid or colon cancer cells (8-10). Herein we present the studies on the effect of alpha-lipoic acid (ALA) and its reduced form of dihydrolipoic acid (DHLA) on the viability of MCF-7 breast cancer cells. We, also, compared the impact of ALA and DHLA on the enzymatic activity of PTP1B and SHP2 phosphatases.

Materials and Methods

Reagents. Phosphatases PTP1B (No. SRP0215) and SHP2 (No. SRP0217) were obtained from Sigma Aldrich, Schnelldorf, Germany. MCF-7 cell line was purchased from The European Collection of Cell Cultures (ECACC). Alpha-lipoic acid (No. T5626), dihydrolipoic acid (T8260), cell media, supplements and

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Key Words: Breast cancer, lipoic acid, ALA, DHLA, PTP1B, SHP2.

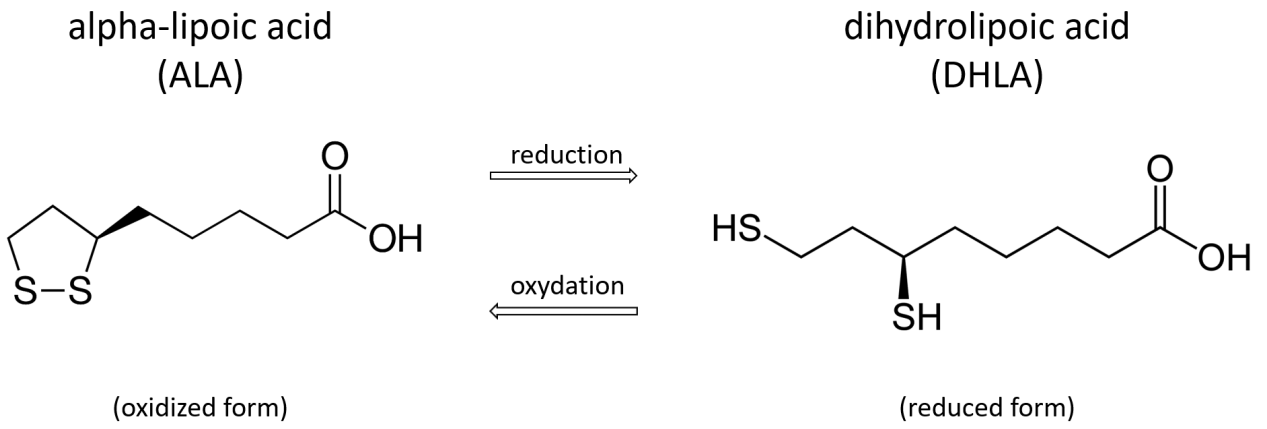

Figure 1. *Alpha-lipoic acid can be reduced to dihydrolipoic acid.*

other reagents were obtained from Sigma Aldrich. Cell proliferation assay kit was obtained from BioAssay Systems.

Cell culture. The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 μg/ml penicillin/streptomycin and 2 mM L-glutamine. The culture was maintained at 37˚C and in an atmosphere containing 5% CO_2. The cell culture density was kept to maximum 1×10^6 cells/ml. At least every two days the medium was replaced with the fresh one, and the cells were counted and reseeded to maintain the recommended density.

Cell viability test. The cells (1×10^6 cells/mL) untreated (control) or treated with solutions of ALA and DHLA after the appropriate incubation time were suspended in solution of 5 mg/ml MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) in DMEM without phenol red. The 100 μl samples were incubated for 3 to 4 h at 37˚C in 96-well plates. When the purple precipitate was clearly visible under the microscope, 100 μl of DMSO were added to each well and the plate with cover was left in the dark for 15 min. The absorbance at 590 nm was determined using a microplate reader.

Fluorescent assay for cell proliferation. The cells (1×10^6 cells/mL) untreated (control) or treated with solutions of ALA and DHLA after the appropriate incubation time were suspended in solution of CellQuantiBlue reagent of resazurin. The 200-μl samples were incubated for 1-5 h at 37˚C in 96-well plates. The fluorescence intensity of highly fluorescent product (resorufin) was measured using a fluorescence reader (excitation wavelength=530-570 nm, emission wavelength=590-620 nm).

PTP1B, SHP2 and CD45 activity assay. The solutions of the recombinant PTP1B and SHP2 phosphatase were prepared in 10 mM HEPES buffer pH 7.4. The final concentration of phosphatases in reaction samples was 1.5 μg/ml (3.3 nM). The enzymes were untreated (control) or treated with solutions of oxidized and reduced lipoic acids. The assay was performed in 96-well microplates, and the final volume of each sample was 200 μL. The enzymatic activity of phosphatases was measured at 37˚C at 405 nm on a microplate reader Jupiter (Biogenet) using DigiRead Communication Software (Asys Hitech GmbH), using 2 mM chromogenic substrate *para*-nitrophenyl phosphate (pNPP).

Statistical analysis. The experiments were performed at least three times. The data were applied and analyzed with GraphPad Prism (GraphPad Software, v.4, La Jolla, CA, USA). Statistical analyses were performed using ANOVA combined with Tukey's test or T test combined with Wilcoxon test. The data were expressed as means±SD. Differences between means were considered significant for $p<0.05$.

Results

ALA and DHLA effect on MCF-7 breast cancer cell viability and proliferation. To evaluate the effect of ALA and DHLA on the viability of breast cancer cells, we tested the MCF-7 cell line. We found that ALA and DHLA are able to decrease cell viability in concentration dependent manner. After 24 h of treatment, the viability of cells was significantly ($p<0.001$) decreased by 100, 500 μM ALA and DHLA (Figure 2). Fifty μM ALA have no impact on cell viability, whereas 50 μM DHLA has an inhibitory effect ($p<0.05$).

To evaluate the impact of ALA and DHLA on breast cancer cells after longer exposition we treated cells for 72 h with 100 μM, 1 mM ALA and DHLA. We observed that both compounds significantly ($p<0.001$) decreased the cell viability (Figure 3A). We measured the effect of ATA and DHLA on cell proliferation with fluorescence assay and we found that both compounds significantly ($p<0.001$) decreased the cell proliferation of MCF-7 cells (Figure 3B). After 72 h incubation time ALA has a slightly stronger inhibitory effect on MCF-7 cell proliferation than DHLA (Figure 3B).

Inhibitory effect of ALA and DHLA on enzymatic activity of PTP1B, SHP2 phosphatases. In the next step, we examined the effect of ALA and DHLA on enzymatic activity of

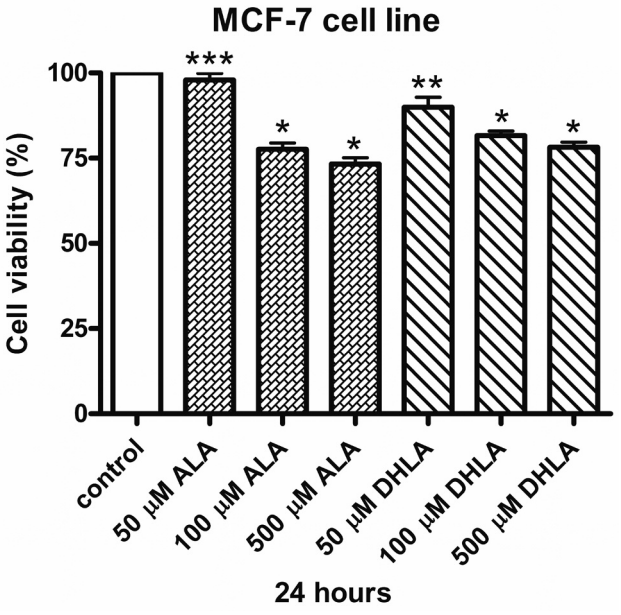

Figure 2. *Cell viability of MCF-7 breast cancer cell line after treatment for 24 h with 50, 100, 500 μM alpha-lipoic acid (ALA) and dihydrolipoic acid (DHLA) measured with MTT-based cell viability test. Data are presented as percent of the control viability (100%, cells not treated), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means significantly different from control (p<0.001). **Means significantly different from control (p<0.05). ***Means not significantly different from control (p<0.05).*

protein tyrosine phosphatases PTP1B and SHP2. In order to assess the inhibitory effect of ALA and DHLA on PTP1B and SHP2 phosphatases we performed an enzymatic activity assay of recombinant PTPs after treatment and 30 min incubation with tested compounds. We found that ALA and DHLA are able to decrease the enzymatic activity of PTP1B and SHP2 in concentrations in the micromolar range (Figure 4). The inhibition of phosphatases was concentration-dependent. 10-100 μM ALA and DHLA induce only a slight effect on enzymatic activity of PTP1B, while ALA and DHLA in concentrations more than 300 μM have a significant inhibitory impact (Figure 4A and B). 10 mM ALA and DHLA induced high inactivation of PTP1B.

Similar results we observed for recombinant SHP2 phosphatase (Figure 4C and D). ALA and DHLA in concentrations exceeding 300 μM caused significant inhibition of enzymatic activity of phosphatase SHP2 (Figure 4C and D).

Calculations of IC_{50} values of ALA and DHLA on PTP1B, SHP2 and CD45 phosphatases. To compare the influence of ALA and DHLA on various protein tyrosine phosphatases we calculated IC_{50} values of one-domain non-receptor PTP1B, SHP2 phosphatases and two-domain receptor CD45 (Table I).

Table I. *Inhibitory properties of alpha-lipoic acid (ALA) and dihydrolipoic acid (DHLA) to PTP1B, SHP2 and CD45 phosphatases presented as IC_{50} values. IC_{50} values were determined from a plot presenting inhibitor concentration versus percentage of the enzymatic activity measured as absorbance with pNPP substrate after 30 min incubation with inhibitors, at substrate concentration equal to Km value. Data are presented as an estimated concentration from the plot.*

	Calculated IC_{50} values	
Phosphatase	ALA	DHLA
PTP1B	1.4±0.07 mM	4.8±0.24 mM
SHP2	8.3±0.42 mM	6.5±0.32 mM
CD45	6.5±0.33 mM	5.9±0.29 mM

We used different concentrations of ALA an DHLA and 30 min incubation time of phosphatases with tested compounds. We calculated IC_{50} values based on a plot presenting ALA and DHLA concentration *versus* percentage of the enzymatic activity of recombinant PTP1B, SHP2 and CD45 measured as absorbance with pNPP substrate. The pNPP concentration for IC_{50} calculations was equal to Km value determined for PTP1B, SHP2 and CD45, where Km value is defined as substrate concentration at which enzyme activity is at half-maximal.

The obtained results indicated that ALA and DHLA are able to decrease the enzymatic activity of selected phosphatases and are effective in a concentration in a range of about 1-8 mM (Table I). We observed the tendency that alpha-lipoic acid was the most effective, and that PTP1B phosphatase was the most sensitive for ALA induced inhibition (Table I).

Discussion

Breast cancer is one of the most common female tumors and usually presents with complicated etiology and multiple organ metastasis. The number of breast cancer cases is still growing in recent years. Because of the genetic and environmental factors, breast cancer is more often becoming a problem of younger women (11).

Recent studies have shown that lipoic acid and its analogs may have a significant impact in cancer metabolism and can be considered in chemotherapy (12). Lipoic acid was found to increase apoptosis in, among others, breast, colon, lung or leukemic cancer cell lines (8, 13-15). Recent studies showed that anti-proliferative action of lipoic acid can be possibly due to the activation of pyruvate dehydrogenase, thus inducing the suppression of aerobic glycolysis and induction of cell death (16).

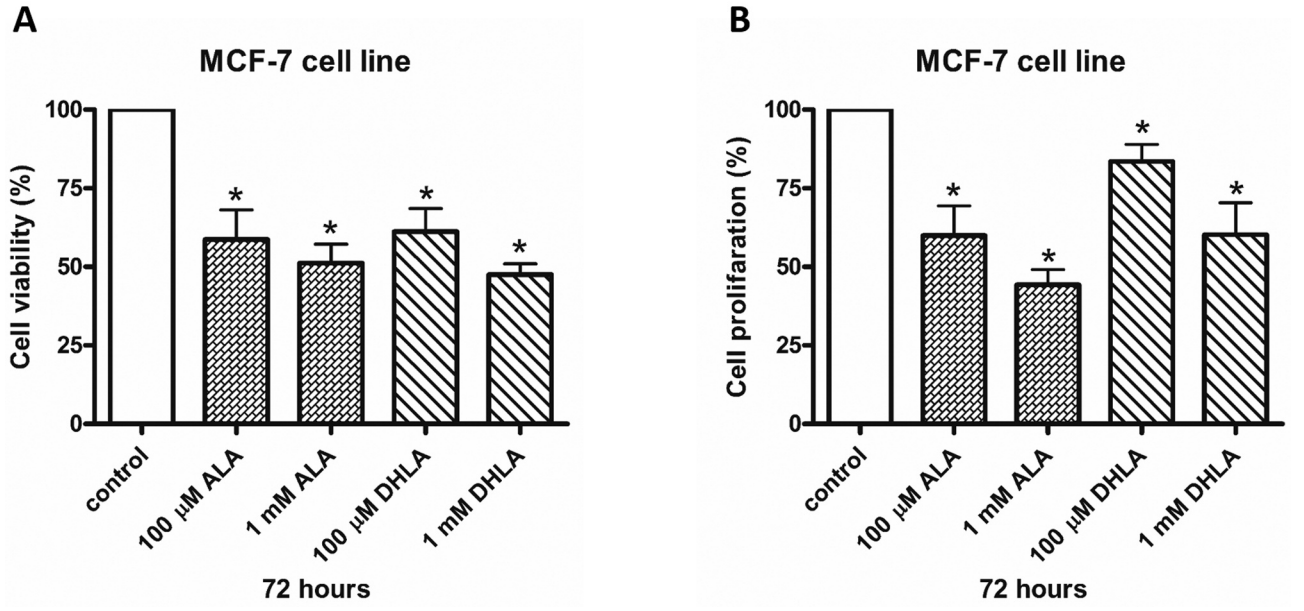

Figure 3. *(A) Cell viability of MCF-7 breast cancer cell line after treatment for 72 h with 1 mM, 100 μM alpha-lipoic acid (ALA) and dihydrolipoic acid (DHLA) measured with MTT-based viability test. Data presented as percent of the control viability (100%, cells not treated), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means were significantly different from control (p<0.001). (B) Cell proliferation of MCF-7 breast cancer cell line after treatment for 72 h with 1 mM 100 μM alpha-lipoic acid (ALA) and dihydrolipoic acid (DHLA) measured with resazurin-based fluorescent cell proliferation test. Data are presented as a percent of the control (100%, cells not treated), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means were significantly different from control (p<0.001).*

Rapidly-dividing cancer cells have a higher iron requirement than properly-dividing cells. Tumor cells are, thus, sensitive to decrease of iron (17). This enables the use of iron chelators as a new concept for the design of cancer treatments. Lipoic acid is known to possess metal-chelating properties, which could be important mechanism of action of LA in anticancer treatment (18).

In this study, we found an inhibitory effect of ALA and DHLA on cancer cell viability using the MCF-7 breast cancer cell line model. These findings indicate that lipoic acid can be considered a potential agent for breast cancer therapy. LA can be used combined with other drugs as an adjuvant therapy. Many anticancer drugs induce generation of reactive oxygen species (ROS) (19). Both ALA and DHLA have been reported to scavenge a variety of oxygen species (7) and, apart from inhibitory effect on tumor cells, they could scavenge free radicals and ROS produced by systemic anticancer agents.

There are evidences that ALA might be useful agent in chemoprevention of obesity-related cancers (20). ALA was already experimentally utilized in treatment of diabetic, as ALA induces reduction of oxidative stress and causes improvement in nerve function (21). It is important that, phosphatase PTP1B is involved in pathogenesis of type-2 diabetes and obesity. PTP1B has become a major target for pharmacological modulation in the therapy of these pathologies. It was found that mice lacking PTP1B phosphatase are healthy, and have enhanced sensitivity to insulin (22). Protein tyrosine phosphatases are implicated also in cancer development (4). PTP1B is considered as drug target in colon cancer (23) and, as well as SHP2 phosphatase, in breast cancer (1). Our studies showed that ALA and DHLA are able to inhibit protein tyrosine phosphatases PTP1B and SHP2.

In summary, we herein showed that ALA and DHLA decrease the viability of breast cancer cells and have inhibitory effects on enzymatic activity of potentially oncogenic phosphatases PTP1B and SHP2. Our studies indicate that ALA and DHLA can be potential agents for the treatment of breast cancer, possibly as an adjuvant therapy with other known systemic therapeutic agents. Recent studies have shown that lipoic acid used as a dietary supplement was well tolerated, with no significant toxicity or interaction (24). Lipoic acid can be considered as a supplement to systemic anticancer treatment to enhance the inhibitory effect on tumor cells and to protect from ROS generated by anti-cancer agents.

Conflicts of Interest

The Authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

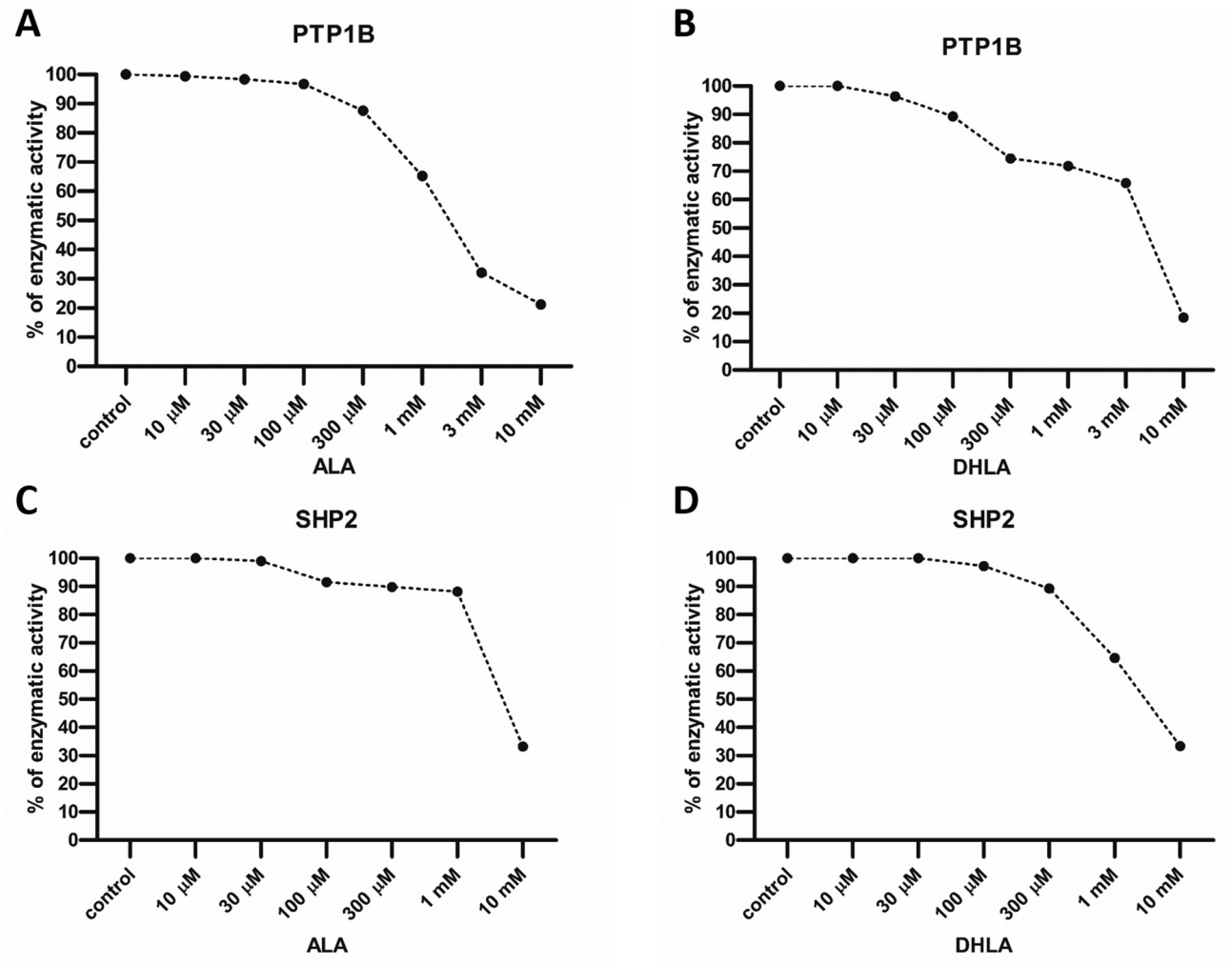

Figure 4. *(A) Enzymatic activity of PTP1B phosphatase after 30 min of treatment with different concentrations of alpha-lipoic acid (ALA). (B) Enzymatic activity of PTP1B phosphatase after 30 min of treatment with different concentrations of dihydrolipoic acid (DHLA). (C) Enzymatic activity of SHP2 phosphatase after 30 min of treatment with different concentrations of alpha-lipoic acid (ALA). (D) Enzymatic activity of SHP2 phosphatase after 30 min of treatment with different concentrations of dihydrolipoic acid (DHLA). Data are presented as percent of the control enzymatic activity (100%, cells not treated), mean±SD (n=3).*

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