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Curcumin and Cinnamaldehyde as PTP1B Inhibitors With Antidiabetic and Anticancer Potential

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Abstract. *Background/Aim: Protein tyrosine phosphatase (PTP1B) is a potential target for the treatment of type 2 diabetes and cancer. Curcumin and cinnamaldehyde have been previously reported to have antidiabetic and anticancer potentials. The aim of this study was to investigate the effect of curcumin in comparison to cinnamaldehyde on the enzymatic activity of PTP1B and the viability of MCF-7 cancer cells. Materials and Methods: Enzymatic activity and cell viability assays were utilized. Experiments were performed using the breast cancer MCF-7 cell line. Results: Curcumin and cinnamaldehyde decreased the activity of PTP1B, and had inhibitory effects on the viability of MCF-7 cancer cells. Curcumin had a significantly higher inhibitory effect than cinnamaldehyde. Conclusion: Curcumin can be considered a potential agent for the treatment of type-2 diabetes or cancer.*

Protein tyrosine phosphatases (PTPs) are potential therapeutic targets due to their involvement in numerous disease processes, such as type-2 diabetes and obesity or cancer development (1). PTP1B protein tyrosine phosphatase due to its role in the regulation of insulin signaling pathways has become a therapeutic target for the treatment of type-2 diabetes (2). The role of PTP1B in the formation and development of tumors has also been already documented (3, 4). Because of the contribution of protein tyrosine phosphatases in cancer biology, they may be considered as promising targets for the development of new anticancer diagnostic and therapeutic strategies (5).

Due to its participation in the regulation of insulin signaling, PTP1B phosphatase is related with the development of type-2 diabetes and obesity, which in turn predispose to the development of cancer (6, 7). The connection of type-2 diabetes, as well as obesity with the induction of tumors is associated primarily with insulin resistance resulting from obesity, or further with hyperinsulinemia as a consequence of insulin resistance (8, 9).

PTPs were shown to be implicated in the development of glioblastomas, colon, lung, breast, stomach and multiple myeloma cancers (10). Phosphatase PTP1B has a key role in breast cancer development and pathophysiology. Many research groups observed overexpression of PTP1B phosphatase and mutations in the *PTP1B* gene in breast cancer cell lines (11, 12). PTP1B dephosphorylates tyrosine kinases responsible for the induction of breast cancer, such as HER1/EGFR, Src, JAK and STAT and initiates tumor formation (13).

Recent studies indicated that selected natural dietary compounds can be considered as potential antidiabetic and anticancer agents (14, 15). Moreover, some of these compounds are able to reduce PTPs activity.

Curcumin (Figure 1A) is a natural phenol present in *Curcuma longa*, a member of the ginger family, *Zingiberaceae*. Recent studies indicate that the curcumin derivative alleviates the glucose intolerance caused by obesity, giving rise to further studies on the use of curcumin compounds in the design of antidiabetic agents (16). In addition, it was found that curcumin may have inhibitory properties against PTP1B (17). Importantly, there are studies supporting that curcumin has enormous potential in the prevention and therapy of cancer (18, 19).

Cinnamaldehyde (Figure 1B) is a flavonoid that naturally occurs in the bark of cinnamon trees and other species of the genus *Cinnamomum*. Half of the essential oils of cinnamon bark is cinnamaldehyde. Studies have shown a beneficial role of cinnamaldehyde in the treatment of diabetes mellitus and its complications, as well as they suggest that cinnamaldehyde can regulate PTP1B phosphatase activity (20). Cinnamaldehyde has also reviled potency as an anticancer and chemo-preventive agent (21-23). It has been shown that cinnamaldehyde increases intracellular reactive

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oxygen species (ROS) production that lead to cancer cell death (24).

This study aimed to evaluate the inhibitory effect of curcumin and cinnamaldehyde on the activity of PTP1B phosphatase. The impact of these compounds on breast cancer cell viability was also analyzed.

Materials and Methods

Reagents. Phosphatase PTP1B (No. SRP0215) was obtained from Sigma Aldrich (Schnelldorf, Germany). MCF-7 cell line was purchased from The European Collection of Cell Cultures (ECACC). Curcumin (C1386), cinnamaldehyde (W228613), cell media, supplements and other reagents were obtained from Sigma Aldrich.

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

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, in an atmosphere containing 5% CO_2. The cell culture density was kept to a maximum of 1×10^6 cells/ml. At least every two days the medium was replaced with fresh medium, and the cells were counted and reseeded to maintain the recommended density.

Cell viability test. Untreated cells (1×10^6 cells/ml) (control) or cells treated with solutions of curcumin and cinnamon aldehyde after the appropriate incubation time were suspended in a 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 was left in the dark for 15 min. The absorbance at 590 nm was determined using a microplate reader.

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

Curcumin and cinnamaldehyde decreased PTP1B enzymatic activity. An inhibitory activity assay of curcumin and cinnamaldehyde on recombinant PTP1B phosphatase was performed.

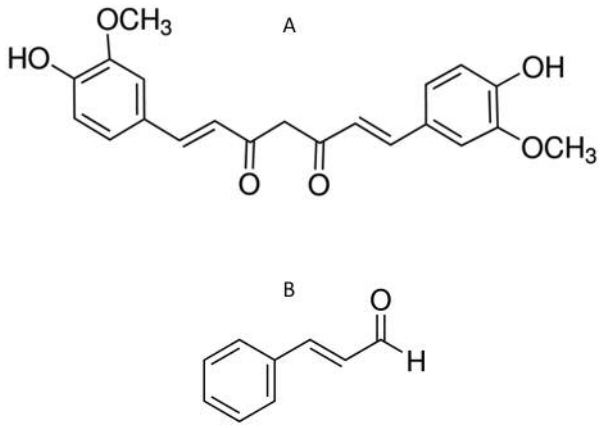

Figure 1. *Structures of (A) curcumin and (B) cinnamaldehyde*.

Curcumin as well as cinnamaldehyde were able to decrease the enzymatic activity of PTP1B phosphatase. However, curcumin was much more effective than cinnamaldehyde. Curcumin was able to inhibit PTP1B in concentrations starting from 1 μM, with an IC_{50} value around 100 μM (Figure 2). While 100 μM cinnamaldehyde was not effective against PTP1B. Cinnamaldehyde decreased PTP1B activity in concentrations starting form 500 μM, with an IC_{50} value around 1 mM (Figure 3).

Curcumin and cinnamaldehyde decreased breast cancer MCF-7 cells viability. To evaluate the effect of curcumin and cinnamaldehyde on the viability of breast cancer cells, MCF-7 cells were treated with either compound. Both curcumin and cinnamaldehyde were able to decrease cell viability in a concentration dependent manner. After 24 h of treatment with 100 or 500 μM curcumin, the viability of cells was significantly ($p<0.0001$) decreased (Figure 4). Treatment with 50 μM cinnamaldehyde had a slightly smaller effect in reducing cell viability relatively to treatment with 10 μM curcumin (Figures 4 and 5).

Discussion

PTP1B phosphatase, regulates insulin signaling which is related to the development of type 2 diabetes and obesity, which in turn predisposes to the development of cancer. PTP1B has become a therapeutic target in the treatment of type 2 diabetes, and its role in the formation and development of tumors has been documented (25-27).

Natural products have shown promise as potential antidiabetic agents. Moreover, the structural modifications of natural compounds could advance the generation of new clinical candidates to target PTP1B for the treatment of type-2 diabetes (28). Recent research indicates that curcumin

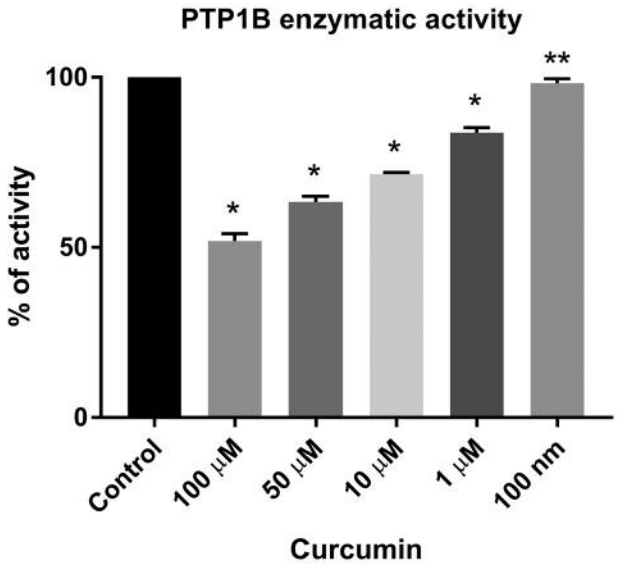

Figure 2. *Enzymatic activity of PTP1B phosphatase after 60 min of treatment with curcumin. Data are presented as percent of the control enzymatic activity (100%, phosphatase not treated), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means were significantly different from control (p<0.0001). **Means were not significantly different from control (p>0.05).*

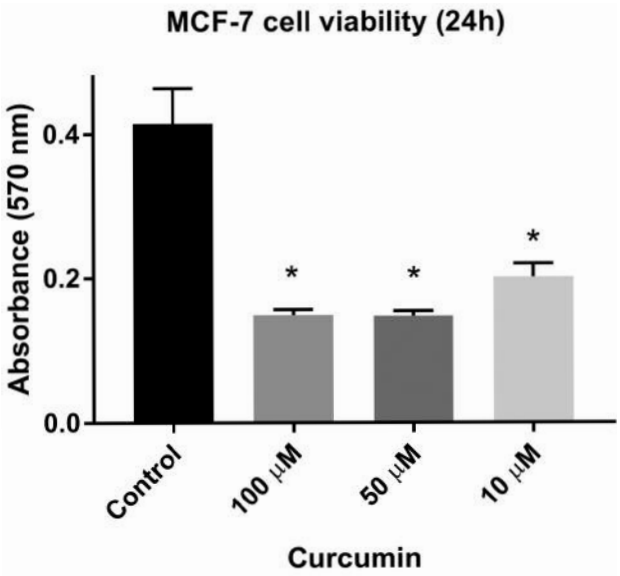

Figure 4. *Cell viability of MCF-7 breast cancer cell line after treatment for 24 h with 100, 50, 10 μM curcumin measured with MTT-based cell viability test. Data are presented as absorbance (570 nm), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means significantly different from control (p<0.0001).*

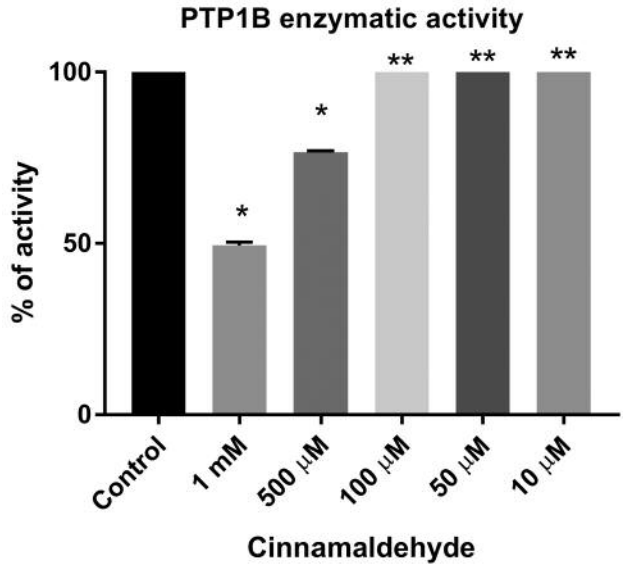

Figure 3. *Enzymatic activity of PTP1B phosphatase after 60 min of treatment with cinnamaldehyde. Data are presented as percent of the control enzymatic activity (100%, phosphatase not treated), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means were significantly different from control (p<0.0001). **Means were not significantly different from control (p>0.05).*

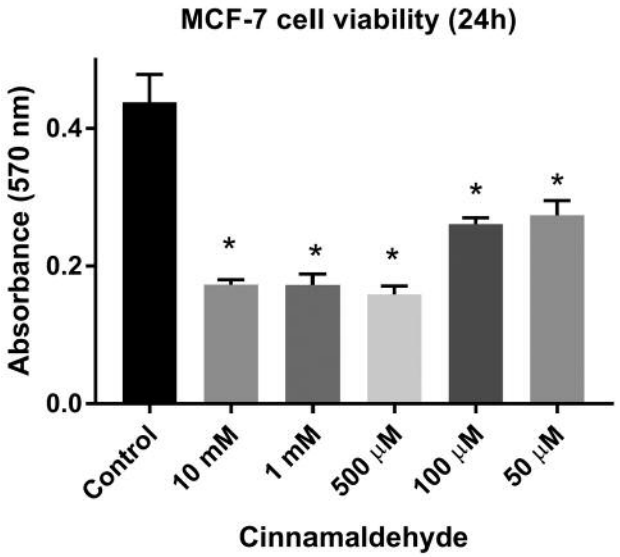

Figure 5. *Cell viability of MCF-7 breast cancer cell line after treatment for 24 h with cinnamaldehyde measured with MTT-based cell viability test. Data are presented as absorbance (570 nm), mean±SD (n=3). One-way Anova test combined with Tukey test. *Means significantly different from control (p<0.0001).*

derivatives alleviate the glucose intolerance caused by obesity, giving rise to further studies on the use of curcumin molecule in the design of antidiabetic agents (16). In addition, it was found that curcumin may have PTP1B phosphatase inhibitory properties (17). Curcumin induces apoptosis and autophagy, and also exhibits iron chelating activity in prostate cancer cells (29). Other studies have shown that curcumin also suppresses the constitutive IκBα phosphorylation by inhibiting IκB kinase activity (30). Epidemiological evidence suggests that phenolic antioxidants, including curcumin, protect against heart disease and cancer (31). Studies carried out so far showed a beneficial role of cinnamaldehyde in the treatment of diabetes mellitus and its complications, as well as suggest that cinnamaldehyde can regulate PTP1B phosphatase activity (20) and possess anticancer properties (22). This evidence inspires the search for other utilizations of curcumin and cinnamaldehyde compounds.

In the present study, we analyzed the inhibitory properties of curcumin and cinnamaldehyde molecules against PTP1B phosphatase and MCF-7 breast cancer cell viability. Both compounds reduced the enzymatic activity of PTP1B phosphatase, as well as MCF-7 cell viability and provide the basis for further research on their use in the design of antidiabetic and anticancer therapies. Comparing the effects of both tested compounds, we can conclude that curcumin is more effective than cinnamaldehyde and can be considered as a more potent PTP1B inhibitor.

The significance of the type-2 diabetes global public health problem in present times is underlined by the worldwide increasing childhood obesity cases, which have more than doubled since 1980 (32). Type-2 diabetes increases the risk for cardio-metabolic diseases and cancer development in adulthood. Recent data have shown a strong association between higher body mass index and increased risk for several malignancies such as leukemia, colorectal or breast cancer (33, 34). As PTP1B phosphatase is implicated in both obesity and type-2 diabetes, as well as cancer development, targeting its enzymatic activity is a promising strategy in the design of treatments for these pathologies. Moreover, knowledge about the preventive effects of natural products could be utilized in choosing a diet for people with these disorders.

In conclusion, the key contribution of protein tyrosine phosphatases in cancer biology indicates that they can be promising targets for the development of new therapeutics. Due to the inhibitory properties of curcumin and cinnamaldehyde molecules against protein tyrosine phosphatases implicated in diabetes and cancer development, they can be utilized to design potential antidiabetic and anticancer therapies.

Conflicts of Interest

The Authors declare that they have no conflict of interest regarding this study.

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