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RESEARCH ARTICLE

In vitro effects of cinnamic acid derivatives on protein tyrosine phosphatase 1B

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Abstract

Protein Tyrosine Phosphatase 1B (PTP1B) is a major negative regulator of insulin signaling pathways. Finding selective PTP1B inhibitors from natural sources has been widely recognized as a potential drug target for the treatment of diabetes mellitus and obesity. In the present study, we evaluated the inhibitory activity of cinnamic acid derivatives against PTP1B *in vitro*. Among 14 cinnamic acid derivatives and related compounds, the most potent inhibitor PTP1Bs were *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid, which had IC_{50} values of 137.67 ± 13.37 and 181.60 ± 9.34 μM, respectively. The kinetics analysis revealed that PTP1B was inhibited by *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid in a non-competitive manner. *o*-Hydroxycinnamic acid (25 μM) and *p*-hydroxycinnamic acid (25 μM), in combination with sodium orthovanadate (0.0125 μM), demonstrated a synergistic effect to inhibit PTP1B activity. In conclusion, the findings provide a new insight into naturally occurring PTP1B inhibitors that could be useful for treatment of diabetes and obesity.

Keywords: Cinnamic acid derivatives, protein tyrosine phosphatase 1B, kinetic inhibition, synergism

Introduction

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin signaling pathways by rapid dephosphorylating at phosphotyrosine residues of the activated insulin receptor kinase[1]. The consequence of its action inactivates the insulin receptor and thereby terminates the insulin signaling. Current scientific evidence clearly indicates that PTP1B activity is directly associated with the insulin resistance and type 2 diabetes[2]. Interestingly, it has been demonstrated that the inhibition of PTP1B can enhance insulin signaling, primarily through prolonged activation of the insulin receptor, resulting in increased insulin sensitivity of skeletal muscle and adipose tissue[3]. Nowadays, PTP1B inhibitors have emerged as an attractive novel target, in particular for the treatment of type 2 diabetes and other associated metabolic syndromes[2]. Recent studies have shown that administration of PTP1B inhibitors can enhance insulin sensitivity and improve glucose tolerance in diabetic rats[4,5].

The phytochemical compounds from dietary plants and fruits are tremendous sources of lead compounds in the search for new types of inhibitors of PTP1B. In particular, cinnamic acid derivatives refer to one of the most numerous and widely distributed groups of phytochemicals in plant-based foods[6]. Recently, cinnamic acid derivatives have been reported to possess various pharmacological properties including antioxidative[7], hepatoprotective[8], and antityrosinase[9], as

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well as antidiabetic activities[10]. Several mechanisms have been proposed for the antidiabetic effect of cinnamic acid derivatives, such as the inhibition of α-glucosidase[11,12], the stimulatory insulin secretion[13,14], and the increased glucose uptake in skeletal muscle[15,16]. Furthermore, cinnamic acid derivatives have been reported to inhibit fructose-mediated protein glycation *in vitro*[17]. Although there have been many studies demonstrating the antidiabetic mechanisms of cinnamic acid derivatives, the inhibition of PTP1B is yet to be investigated. Therefore, it would be interesting to examine the inhibitory effect of cinnamic acid derivatives on PTP1B activity. In the present study, 14 cinnamic acid derivatives and related compounds were investigated for inhibition of PTP1B *in vitro*. Furthermore, the study was conducted to evaluate the types of kinetic inhibition on PTP1B. Finally, the combined effects of cinnamic acid derivatives and sodium othorvanadate (SOV), a PTP1B inhibitor, were also studied.

Materials and methods

Chemicals

Protein tyrosine phosphatase 1B (human recombinant) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). *p*-Nitrophenol phosphate (*p*-NPP), cinnamaldehyde, cinnamyl alcohol, caffeic acid (3,4-dihydroxycinnamic acid), 3,4-dimethoxycinnamic acid, and methyl cinnamate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cinnamic acid, *o*-hydroxycinnamic acid, *m*-hydroxycinnamic acid, and *p*-hydroxycinnamic acid were purchased from Fluka (St. Louis, MO, USA.). *o*-Methoxycinnamic acid, *m*-methoxycinnamic acid, and *p*-methoxycinnamic acid were purchased from ACROS (Pittsburgh, PA, USA). Ferulic acid (3-methoxy-4-hydroxycinnamic acid) and isoferulic acid (4-methoxy-3-hydroxycinnamic acid) were purchased from Chromadex (Laguna Hills, CA, USA). Sodium orthovanadate was obtained from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

Assay for the PTP1B activity

The assessment of the PTP1B activity was slightly modified based on a previous method[18]. PTP1B was diluted before the experiment to 1.2 μg/mL in Tris buffer, pH 7.6 (10 mM Tris, 1.0 mM EDTA, 3.0 mM DTT, 0.01% w/v NaN_3). The tested compounds were dissolved in DMSO. The enzyme reaction buffer was Tris buffer, pH 7.5 (50 mM Tris, 0.15 M NaCl, 3 mM DTT). A typical 10 μL of tested compounds was added to the enzyme solution (50 μL), and the resulting mixture was preincubated at 37°C for 30 min. The enzyme reaction was initiated by the addition of 8 mM *p*-NPP (40 μL). The concentration of *p*-nitrophenol from the reaction mixtures was recorded at 405 nm, 15-min intervals for 120 min. SOV was used as a positive control in this study.

Measurements of the kinetics constant

In order to investigate the type of inhibition, the enzyme kinetic analysis was performed according to the above reaction. Maintaining the quantity of PTP1B constant at 1.2 μg/mL of PTP1B, the test compounds were measured in final concentrations of *p*-NPP (0.8-6.4 mM). The types of inhibition were calculated on the base of Lineweaver–Burk by reciprocally plotted data (substrate concentration on horizontal axis and velocity on vertical axis).

The combined effect of tested compounds and sodium orthovanadate

SOV was combined with or without the tested compounds. The reaction was performed according to the above assay. Results were expressed as the percentage inhibition of the corresponding control values.

Data analysis

Data was expressed as mean ± SEM ($n = 3$). The IC_{50} values were determined from plots of log concentration of inhibitor concentration versus percentage inhibition curves. The types of inhibition and constants were calculated on the base of Lineweaver-Burk and its replot of slopes as the non-linear regression. Statistical analysis was performed by Student's *t*-test ($P < 0.01$).

Results and discussion

As shown in Figure 1, we investigated 14 promising compounds of cinnamic acid and its derivatives and related compounds for PTP1B inhibition. The result in Figure 2 shows the percentage inhibition of the compounds at a concentration of 100 μM. In course of screening for PTP1B inhibitors, all compounds were found to inhibit PTP1B activity by approximately 12.50–39.40%. Cinnamic acid showed a percentage inhibition of 16.0 ± 0.01, whereas the percentage inhibition of cinnamyl alcohol, cinnamaldehyde and methyl cinnamate inhibited PTP1B activity ranged from 12.5 to 18.0%. The findings suggest that replacing the carboxylic acid group of cinnamic acid with alcohol, aldehyde, and methyl ester groups had no effect to increase the percentage of PTP1B inhibition.

The addition of a hydroxyl residue to the cinnamic acid at the *ortho*- or *para*-positions resulted in a significant increase in the percentage of inhibition. We found that *o*-hydroxycinnamic acid (39.4 ± 2.52%) and *p*-hydroxycinnamic acid (35.9 ± 5.65%) displayed the highest percentage of PTP1B inhibition among all tested compounds. However, the presence of a hydroxyl residue at the *meta*-position showed a slight increase in the percentage inhibition (20.9 ± 0.76%). Replacing a hydroxyl residue, substituted in the cinnamic acid by a methoxyl residue, markedly decreased the PTP1B inhibitory activity. The findings revealed that the presence of a hydroxy substituent at the *ortho*- or *para*- position was more active than that of a methoxy substituent.

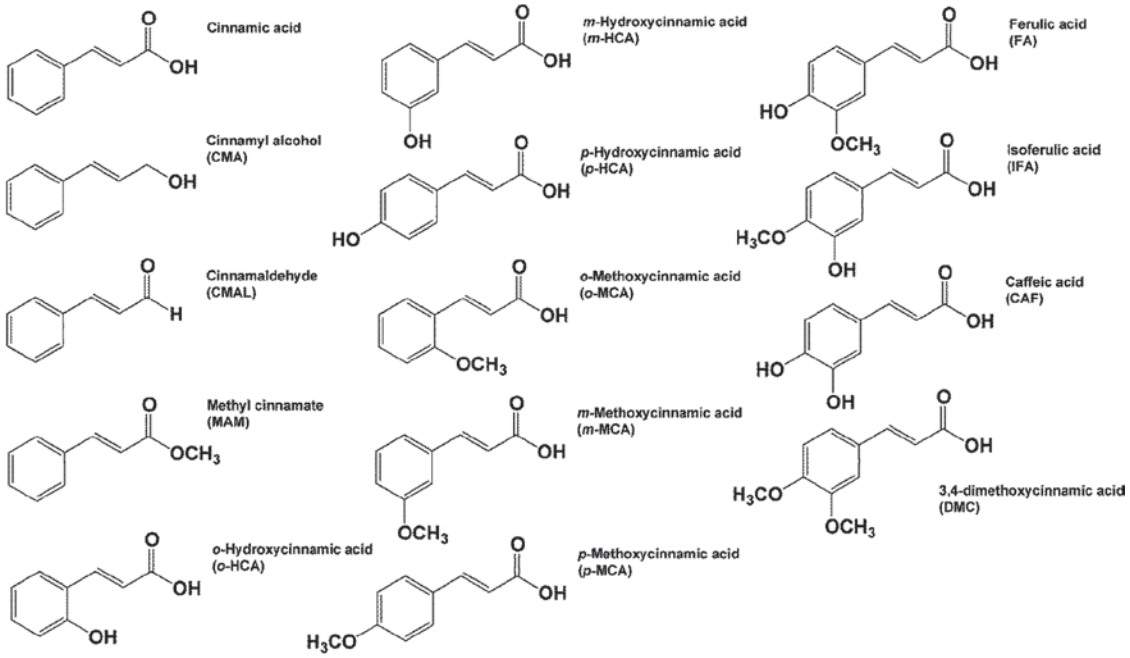

Figure 1. The chemical structure of cinnamic acid derivatives and related compounds.

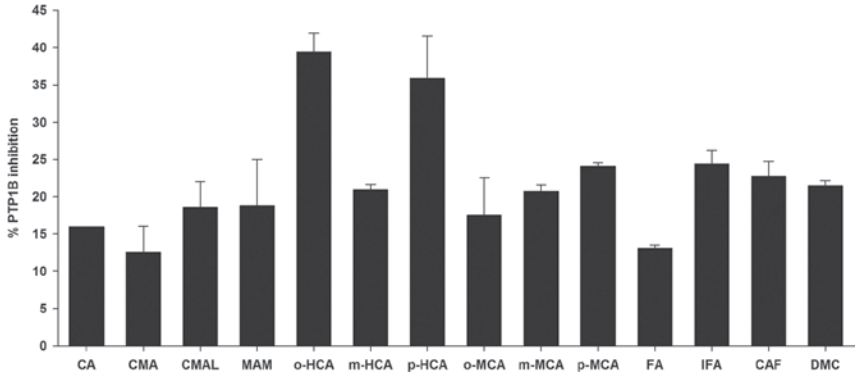

Figure 2. The percentage PTP1B inhibition of cinnamic acid derivatives and related compounds (100 μM). Data are expressed as mean ± SEM, $n = 3$.

Interestingly, the introduction of two moieties in cinnamic acid markedly exerted a higher percentage of inhibition than cinnamic acid (21.5–24.4%), except when the substituent was at the *meta*-methoxy and *para*-hydroxy positions (ferulic acid). Considering the previous studies regarding the structure–activity relationship of cinnamic acid derivatives, it indicates that ferulic acid is the most potent intestinal α-glucosidase inhibitor and insulin secreting agent among cinnamic acid derivatives[11,12]. This is surprising, given the differences from the present study that ferulic acid slightly inhibited PTP1b activity. The interaction phenomena between cinnamic acid derivatives and PTP1B remain unknown. We assume that cinnamic acid derivatives may form hydrogen bonds with the polar groups of amino acid residues in the active site of PTP1B by covalent and/or non-covalent interaction. The differences in chemical structure of cinnamic acid derivatives may result in the difference in ability to bind the active site. The explanation for this hypothesis requires clarification through further investigation, using computer modeling to evaluate the binding activity of these compounds on the enzyme.

Based on the screening results, we further investigated the IC_{50} values of *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid. The results showed that they inhibited the hydrolysis of the *p*-NPP catalyzed by PTP1B in a dose-dependent manner (data not shown) with IC_{50} values of 137.74 ± 13.20 and 168.40 ± 17.69 μM, respectively. A known PTP1B inhibitor, SOV was employed as a positive control that had the IC_{50} value of 25.39 ± 0.65 nM. It was suggested that *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid were less potent than SOV on the PTP1B inhibition. Hydroxycinnamic acids are commonly found in fruits and in different parts of plants[19]. The highest content of hydroxycinnamic acids is commonly found in fruits such as blueberries, kiwis, plums, cherries, and apples, which contain 0.5–2 g hydroxycinnamic acids/kg fresh fruits[20]. It has been shown in a recent investigation that daily intake

of hydroxycinnamic acids alone is estimated at 211 mg/day and can reach up to 800 mg/day[21,22]. It is expected that consumption of plant-enriched hydroxycinnamic acids may reduce the risk of diabetes and its complications. Recent studies have reported the mechanisms underlying the antidiabetic action of hydroxycinnamic acids on the inhibition of α-glucosidase[11], the stimulating pancreatic insulin release[12], the activation of glucose uptake[23], the stimulating adiponectin release[24], and the inhibition of protein glycation[16]. Considering the data obtained from this investigation, we provide a novel mechanism for the antidiabetic activity of cinnamic acid derivatives *via* the inhibition of PTP1B. It is possible that hydroxycinnamic acids may enhance the plasma glucose-lowering effect in diabetic animals associated with the PTP1B inhibitory activity.

To further explore the inhibitory characteristics of cinnamic acid derivatives, the kinetic assay was performed using Lineweaver-Burk double reciprocal plots. The inhibitory mechanisms on PTP1B by *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid are shown in Figure 3.

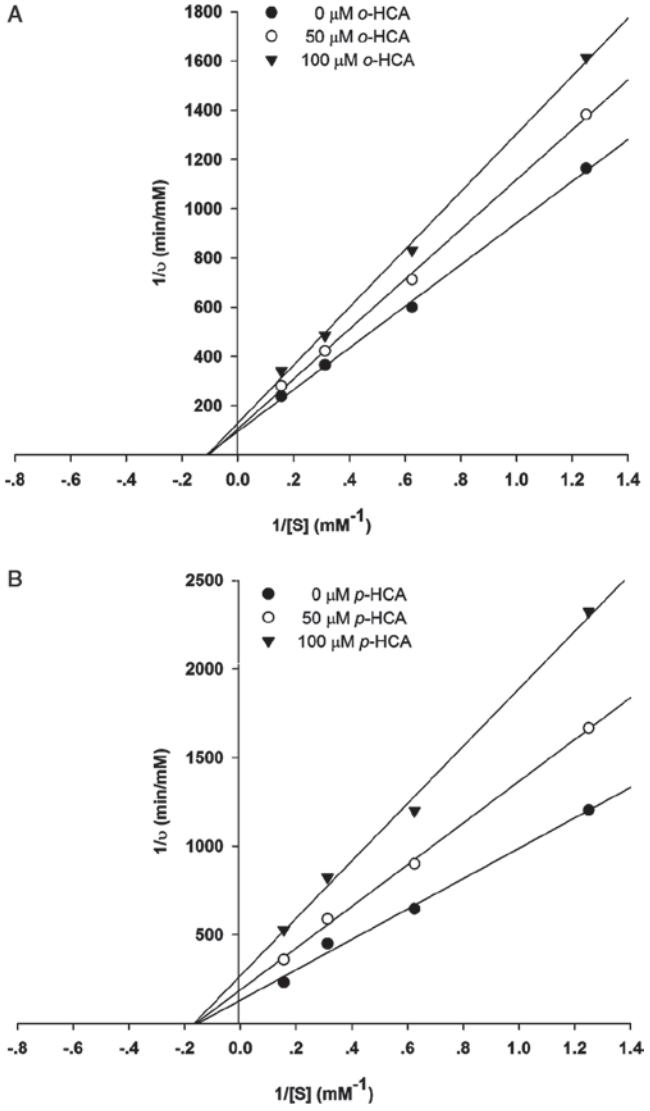

Figure 3. Lineweaver-Burk plots for inhibitory activity of (A) *o*-hydroxycinnamic and (B) *p*-hydroxycinnamic on PTP1B.

A Lineweaver-Burk plot of *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid generated straight lines that had the same intersection on the *X*-axis, indicating that their inhibitions were of a non-competitive type. It has been reported that the identification of non-competitive inhibitors targeting the allosteric site in PTP1B may present in the alternative approaches to the development of selective PTP1B inhibitors[2,25–27]. The results of the present study suggest that *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid are non-competitive inhibitors that can be developed into highly selective inhibitors of PTP1B.

It was of interest to establish whether cinnamic acid derivatives and SOV might interact synergistically or additively on PTP1B. The assay was then performed in the solutions containing SOV alone or in mixture with cinnamic acid derivatives. The combined effects of compound *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid with SOV on PTP1B inhibition are shown in Figure 4. With the addition of *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid to the assay system with SOV (0.012 μM), the percentage inhibition was significantly increased when compared with SOV or the compounds alone. The percentage inhibition of mixtures was greater than the summing effect of SOV and those compounds. The findings indicate that *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid, together with SOV, synergistically inhibits the PTP1B activity. SOV exhibits a wide variety of insulin-like effects both *in vitro* and *in vivo*[28]. It has been recently reported that SOV inhibits protein tyrosine phosphatase activity and this in turn enhances the activity of protein tyrosine kinases, thus resulting in an enhancement of insulin sensitivity and a prolongation of insulin biological response[29,30]. However, it has shown some short- and long-term toxicity problems in diabetic animals, such as the loss of body weight, dehydration,

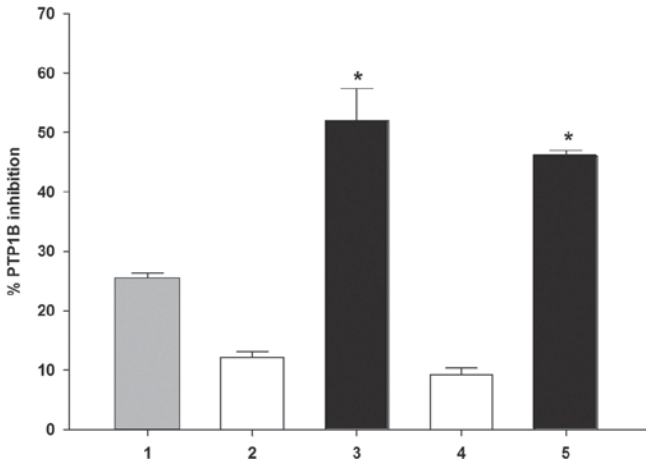

Figure 4. The percentages of enzyme inhibition of SOV and its combination with *o*-hydroxycinnamic and *p*-hydroxycinnamic on PTP1B. (1): 0.012 μM SOV;(2): 25 μM *o*-hydroxycinnamic;(3): 25 μM *o*-hydroxycinnamic + 0.012 μM SOV;(4): 25 μM *p*-hydroxycinnamic; (5): 25 μM *p*-hydroxycinnamic + 0.012 μM SOV. Data are expressed as mean ±S.E.M., n =3. * $P < 0.01$, compared to SOV alone.

hematological changes, biochemical alterations, and nephrotoxicity[31,32]. At this point, the researchers trying to exploit its toxicity are attempting to develop the complex-forming capability of vanadium compounds with organic compounds[33]. Moreover, there have been several attempts to reduce the toxicity without compromising their biological effects by reducing the dose of vanadate and combining the treatment with herbal medicines[34,35]. Our findings suggest that *o*-hydroxycinnamic acid and *p*-hydroxycinnamic acid may enable a lower dose of SOV to be used, leading to diminishment of the adverse effect. It is advantageous to apply SOV and hydroxycinnamic acids as a mixture for an inhibitory effect, as the coadministration can reduce the effective doses of these compounds for the same inhibitory effect on PTP1B activity.

Although cinnamic acid derivatives have been investigated regarding the potential mechanisms for glucose-lowering effect, the present study also demonstrates a new mechanism of action *via* the inhibition of PTP1B activity. On the basis of the above studies, cinnamic acid derivatives can be developed as a PTP1B inhibitor for the treatment of diabetes.

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Declaration of interest

The authors report no conflicts of interest.

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