Feasibility of Sijunzi Tang (Chinese medicine) to enhance protein disulfide isomerase activities for reactivating malate dehydrogenase deactivated by polycyclic aromatic hydrocarbons

By: Ho Man Leung, Francis Siu Lai Kwok, Wing Yin Mo, Kwai Chung Cheung, Yik Kit Yue, Yee Keung Wong, Chi Kin Au, Martin Tsz-Ki Tsui, and Ken Kin Lam Yung

Leung, Ho Man; Kwok, Francis Siu Lai; Mo, Wing Yin; Cheung, Kwai Chung; Yue, Yik Kit; Wong, Yee Keung; Au, Chi Kin; Tsui, Martin Tsz Ki; Yung, Ken Kin Lam. Feasibility of Sijunzi Tang (Chinese medicine) to enhance protein disulfide isomerase activities for reactivating malate dehydrogenase deactivated by polycyclic aromatic hydrocarbons. *Environmental Science and Pollution Research*. (2018). https://doi.org/10.1007/s11356-018-3230-5

Abstract:

The objective of this research is to investigate the enzymatic activities between protein disulfide isomerase (PDI) found in animals and plants and the properties found in a commonly used Chinese medicine called *Sijunzi Tang*. During the investigation, PDI, which is a monomer with a molecular mass of 57.0 kDa, was used to reactivate malate dehydrogenase (MDH). However, with the interference of polycyclic aromatic hydrocarbons (PAHs), evidence indicates that such chemicals are carcinogenic, mutagenic, and toxic to humans. The enzymatic activity of PDI found in animal's liver and plant was 1657 folds of purification; 0.284 unit/mg of enzyme activity, and 5694.4 folds of purification; 1.00 unit/mg of enzyme activity, respectively. PDI extracted in treated animal and plant tissue revealed 2.40% and 80.44% of regaining MDH enzymatic activity, respectively. Although in its initial phase of investigation, it is assumed that the properties found in *Sijunzi Tang* can help regain enzymatic activity in those affected by xenobiotic substances, thus, making it a potential ingredient in assisting with PDI functions.

Keywords: Protein disulfide isomerase (PDI) | Malate dehydrogenase (MDH) | *Sijunzi Tang* | Glutathione reductase assay | Polycyclic aromatic hydrocarbons (PAHs)

Article:

Introduction

Similar to those of climate change and global warming (as a result of greenhouse gases), human activity within the past century has allowed an influx of environmental toxicants and pollutants into ecosystems on a global scale, such as heavy metals and persistent organic pollutants (POPs). An important subset among these are polycyclic aromatic hydrocarbons (PAHs), which are present in a range of environmental sources and can enter the body through various routes. PAHs are commonly incorporated into living organisms (Rengarajan et al. 2015). Human exposure to PAHs can occur both passively, through skin absorption, and actively through food and aerosols, interfering with the function of the cellular membrane and the enzymatic system (Haritash and Kaushik 2009). In addition, Oleszczuk and Baran (2005) have revealed PAHs to be a priority pollutant, containing carcinogenic and mutagenic properties, and are therefore toxic to the human body. Furthermore, PAHs were found in various abiotic factors such as water, soil, and sediments (Baran et al. 2002; Baran et al. 2004; Oleszczuk and Baran 2005). Thus, such pollutants are easily accumulated in the body (Calafat et al. 2005; Calafat et al. 2008; He et al. 2009) through the mechanisms of bioaccumulation and biomagnification in the food chain.

Protein disulfide isomerase (PDI) is widely distributed in eukaryotic cells and several researches were conducted by Gilbert (1998). It is an abundant protein in animals (0.4% *w*/*w* of total cellular protein) and plants (Kimura et al. 2015) and its concentration in the lumen of the endoplasmic retriculum (ER) can approach millimolar levels (Hillson et al. 1984) in humans. Such enzymes are distributed widely in organs and tissues, and similar molecule structures were found among all species (Hillson et al. 1984). It is a member of the thioredoxin superfamily, consisting of two regions (carboxyl and amino terminus) that are homologous to the redox active protein thioredoxin. Each thioredoxin domain contains two of the cysteines in the sequence WCGHCK and catalyzes various thiol-disulfide exchange reactions with a molecular mass of approximately 55,000 Da. Other than its enzymatic property that catalyzes in vitro isomerization of intramolecular disulfide bridges, PDI also acts as a molecular chaperone, being able to bind unfolded or misfolded proteins, and reactivate proteins in the bodies of those suffering from various diseases such as Alzheimer's disease (Honjo et al. 2010), Creutzfeldt-Jacob disease (Mossuto 2013), ischemia (Tanaka et al. 2000), and Parkinson's disease (Uehara et al. 2006).

Recently, PDI has been identified to bind various kinds of organic pollutants (Hashimoto et al. 2012a; Okumura et al. 2014). Hashimoto et al. (2012b) found that K_D values (the equilibrium dissociation constant between an antibody and its antigen) decreased to 1000-fold molar concentration with an addition of PDI. In addition, in the presence of disulfide isomerase-like protein AtPDIL1–2 in plants, trichlorophenol (TCP), a type of POPs, does not only wrap up the TCP but also increases plant resistance to the toxin through its disulfide isomerase function (Peng et al. 2017). However, there are limited studies to show the effects of PDI with *Sijunzi Tang*. Thus, it was hypothesized that the organisms that were fed *Sijunzi Tang* had a stronger PDI activity in defending against toxic pollutants compared to the control group.

Research provided within thousands of studies demonstrate the significance of Chinese herbal medicines as treatment for proteins that have been denatured by cancer cells (Si et al. 2016; Wu et al. 2016; Kim et al. 2000; King et al. 2016; Son et al. 2016; Wang et al. 2016; Chen et al. 2016; Zhao et al. 2016; Auyeung et al. 2016; Wong et al. 2016); however, scarce information could be found on the effect of the Chinese medicine on enzyme structures, nor its characteristics which enhance PDI's ability to reach protein inactivated by environmental toxicants. Henceforth,

the objective of this research is to investigate the action of PDI induced by *Sijunzi Tang*, a famous Chinese medicine, to reactivate the functions of MDH deactivated by PAHs**.**

Materials and methods

Materials for PDI extraction and purification

Di-sodium hydrogen sulfate, sodium dihydrogen sulfate, sodium citrate, citric acid ammonium sulfate, and EDTA were purchased from Univar. CM cellulose and DEAE cellulose were purchased from Whatman; glutathione (reduced form), bovin insulin, glycine, SDS, glutathione reductase, guanidine hydrochloride, triton X-100, OAA, NADH, and NADPH were purchased from Sigma. See-blue marker and acrylamide/bisacrylamide were purchased from BioRad. Mini gel system was purchased from BioRad. High-performance liquid chromatography was purchased from Waters. Avanti J25I High Performance Centrifuge, JA10, and JA20 rotor were purchased from Beckman.

Chinese medicine pellet preparation

The dried materials for *Sijunzi Tang* (*Radix Codonopsis*:*Atractylodes macrocephala*:*Wolfiporia extensa*:*Radix Glycyrrhizae* = 2:2:2:1) were purchased from Lui Seng Chun (Hong Kong Baptist University). One kilogram of the dried materials was cooked with water for 6 h to obtain water extract and concentrated into 15 l. The solution was mixed with fruit peel and vegetables. After drying for a day, the dried food was pulverized and mixed with starch (acting as a coagulating agent) to make Chinese medicine pellets. Prepared diets were then stored in a dry room indoors for further use.

Animal and plant sample preparation

Ten intact male pigs per group (with and without treatment) with similar body weight fed on the Chinese medicine pellets and dried herbs according to their body weight continuously for 3 months, respectively. Pigs were fed the basal diet (Table 1) supplementation with Chinese medicine decoction with a dose of 0.20 g/kg/BW/day. All pigs were allowed to consume both feed and water ad libitum. Body weights and feed intakes were measured at the beginning and end of trial. The average body weight gained was between 8.02 ± 0.24 kg and 10.89 ± 0.65 kg for the control groups and 7.65 ± 0.17 kg to 11.32 ± 0.11 kg for the treatment groups in 3 months.

Seeds of bread wheat (*Triticum aestivum*) were sterilized by immersing into 10% peroxide solution. Four seeds were placed into the pot and one of the seedlings with the largest biomass was chosen for further experimentation. All treated and non-treated seedlings were added with nutrients to each compartment: 300 mg nitrate (2 ml of 0.1 M calcium nitrate), 50 mg phosphorus (0.3 ml of 0.1 M calcium hydrogen phosphate), and 200 mg potassium (1.3 ml of 0.1 M potassium sulfate) as a basal application. The soil in the treatment group was mixed with Chinese medicine pellets (0.20 g/kg/week). Ten replicates were randomly placed with natural light illustration in a glasshouse for 3 months. The average plant biomass gained was between 3.21 ± 1.85 g and 90.9 ± 2.55 g for the control groups and 4.50 ± 0.17 g and 120.88 ± 3.56 g for the treatment groups in 3 months.

Table 1. Compositions of experiment diets (as feed basis)

Ingredients	Percent
Cone	60.3
Soybean meal	11.8
Fish meal	14.5
Wheat	9.94
Calcium sulfate	1.80
Salt	0.76
Methionine	0.15
Vitamin-mineral mix[a]	0.75
Total	100

[a]Vitamins and minerals were included to provide the following amounts per kilogram of diet: 150 mg Zn; 110 mg Fe; 100 mg Cu; 30 mg Mn; 0.1 mg Co; 6000 IU vitamin A; 720 IU vitamin D3; 30 IU vitamin E; 3.8 mg vitamin K3; 2.1 mg vitamin B1; 6.0 mg vitamin B2; 14 mg D-pantothenic acid; 3.8 mg vitamin B6; 0.95 mg folic acid; 50 μg biotin; 25 mg vitamin B12

PDI extraction

The animals were given electric shocks using a stun gun (bolt pistol) and exsanguinated. Livers were collected immediately and rinsed with sterile cold saline solution free of RNase contamination. The plants were harvested and rinsed with deionized water. Both animal and plant samples were blended with a precooled blender containing 800 ml homogenizing buffer (0.1 M sodium phosphate, 5 mM EDTA, and 4 g Triton X-100) at 18,000×*g* for 30 min with precooled JA–20 rotors in a Beckman Avanti J25I High Performance Centrifuge. The pellet was discarded. The supernatant was filtered through glass wool to remove the residue. The supernatant was placed in a 60 °C water bath with constant stirring for 15 min. Then, the extract was placed on an ice bath and cooled to < 10 °C. The extract was centrifuged at 18,000×*g* for 40 min and the supernatant was transferred to a beaker on ice and performed 55–85% ammonium sulfate fractionation immediately. The pellet was obtained and dissolved again in 50 ml of 25 mM citrate buffer at pH 6. The extract was dialyzed with the buffer for 3 h and subsequently changed to 5 l of 25 mM citrate buffer overnight. The supernatant was further purified by cation-exchange chromatography (CM cellulose) which had been pre-equilibrated with 25 mM citrate buffer pH 6. Peak fractions containing PDI activity were polled and passed through anion-exchange chromatography (DEAE cellulose) immediately which had been pre-equilibrated with 25 mM sodium phosphate buffer pH 7. The column was then washed with sodium phosphate buffer continuously until no protein was found in the washtrough. The bounded materials were eluted with a linear gradient of 0–0.7 M sodium chloride in 25 mM sodium phosphate buffer at pH 7. Fractions containing PDI activity were pooled. The supernatant was then loaded on a HPLC gel filtration column, which had been previously equilibrated with 0.1 filtered and degassed sodium phosphate buffer pH 7.0. The enzyme was eluted with the same buffer at a flow rate of 0.25 ml/min. Fractions containing protein disulfide isomerase activity were collected and pooled together. The purified protein disulfide isomerase was obtained and stored at − 20 °C. Native molecular mass determination was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) technique (Laemmli 1970). Protein quantification was performed by Lowry method (Lowry et al. 1951). Homogenous fractions in HPLC were pooled and concentrated for further characterization.

Study of the purified PDI activity in different pH and temperatures

Further steps were required in the study of the enzymatic activity of PDI (with and without treatment of the Chinese medicine) due to the numerous chemical compounds found in the herbal formulae, with possible effects on enzyme reusability, stability, and separation. As most enzymatic activity varies significantly with temperature and to a lesser extent pH (Pinsonneault et al. 2016), tests were carried out to determine PDI's thermostability. Twenty-five millimolars of sodium phosphate buffer pH 7 subsequently performed at 30–90 °C was tested. Afterwards, both enzymes were tested with sodium phosphate buffer in incremental pH values (i.e., 0–10) and inoculated for 30 min at the temperature of highest enzyme activity obtained from a previous experiment. Both enzyme activities were measured by an assay mentioned below.

Determination of enzyme activity in PDI by assay

In each purification step, PDI activity was determined by glutathione reductase assay. This PDI could convert reduced glutathione (GSH) into oxidized glutathione (GSSH) as a major product in the initial phase, as well as being converted back into GSH, which represents both PDI activity by the following equation: 30 μM insulin, 3.7 mM GSH, 0.12 mM NADPH, and 16 units of glutathione reductase (GR) were added and well mixed. Assay buffer (0.2 M sodium phosphate and 5 mM EDTA (ethylenediaminetetraacetic acid) at pH 7.5) was used to make the solution reach 1 ml. The PDI activity was measured at 340 nm for 1 min by UV spectrophotometer at room temperature. Such value was expressed as the nanomoles of $NADP^+$ formed per 1 min and such assay for PDI (one of the enzymes in thioredoxin superfamily) (Lorimer and Baldwin 1998):

$$\text{Insulin} - (\text{SS}) + 2\text{GSH} \xrightarrow{\text{PDI}} \text{Insulin}(\text{SH})_2 + \text{GSSG}$$

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{Glutathione reductase}} 2\text{GSH} + \text{NADP}^+$$

The total enzyme activity of PDI was calculated as concentration of $NADP^+$ formed: [(Initial absorbance in 340 nm − Final absorbance in 340 nm)/6.23] mM/min divided by volume of sample used for assay (in μl) and multiplied by volume of sample at the step (in μl). The activity of PDI was calculated by total activity of PDI divided by weight of total protein in the sample (in mg) where 1 absorbance unit at 280 nm was assumed to be equal to 1 mg of protein per milliliter.

Study of MDH reactivation by PDI extracted from plant and animal samples

In this research, PAHs were used instead of guanidine chloride to deactivate enzymes, due to its recognized role within anthropogenic polluting sources and its effect on enzyme activities (Gianfreda et al. 2005). In 100 mM Tris-HCl pH 7.5, 5 mg/ml of MDH (purchased from Sigma Aldrich) was prepared, containing 3 μg/kg of PAHs and allowed to denature at 25 °C for 1 h. The mixture was then dialyzed against 100 ml of reactivation buffer (100 mM Tris-HCl pH 7.5, 1 mM of dithiothreitol, and 0.1 M potassium chloride) overnight. One hundred microliters of the solution mixture was withdrawn for MDH activity assay and its protein concentration was measured at 340 nm before and after reactivation. Reactivation of MDH was initiated by measuring MDH concentration at 280 nm. The same concentration of MDH was divided into

two portions: 50 µl of PDI (with adjusted to 0.1 unit/mg in each treatment) and 50 µl of assay buffer was added to MDH solution and incubated for 3 h at 25 °C. Both mixtures were withdrawn after 1 h at room temperature and assayed for MDH activity by the following equation. The assay of MDH was conducted by 100 mM Tris-HCl buffer pH 7.5, 0.5 mM oxaloacetate, and 0.2 mM NADH. Fifty microliters of the sample (both with and without PDI) was added and incubated at room temperature. The activity of MDH was expressed as the nanomoles of nicotinamide adenine dinucleotide (NAD^+) formed per minute.

$$\text{Oxaloacetate} + \text{NADH}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

The total enzyme activity of MDH was calculated as concentration of NAD^+ formed: [(Initial absorbance in 340 nm−Final absorbance in 340 nm)/6.23] mM/min divided by volume of sample used for assay (in µl) and multiplied it by volume of sample at the step (in µl). The specific activity of MDH was calculated by total activity of MDH^+ divided by weight of total protein in the sample (in mg) where 1 absorbance unit at 280 nm was assumed to be equal to 1 mg of protein per milliliter.

Results and discussion

Purification of PDI

Table 2. The purification table of PDI extracted from treatment and non-treatment animal groups

Item	Treatment	Total weight of protein (mg)	Total enzyme activity (units)	Specific activity (units/mg)	Fold of purification	Yield (%)
Treated group						
1	Homogenate	1,168,000	199.4	1.71×10^{-4}	1.00	100.00
2	Heat treated	11,920	153.6	0.12×10^{-3}	75.48	77.03
3	Ammonium sulfate precipitation	2305	46.33	2.01×10^{-2}	117.74	23.23
4	Dialysis	2237	45.51	2.03×10^{-2}	119.17	22.82
5	Cation-exchange chromatography	838.4	33.95	4.05×10^{-2}	237.20	17.03
6	Anion-exchange chromatography	235.2	28.43	1.21×10^{-1}	708.04	14.26
7	After HPLC	7.451	2.109	2.83×10^{-1}	1657.98	1.06
Control group						
1	Homogenate	1,674,530	192.3	1.11×10^{-4}	1.00	100.00
2	Heat treated	10,547	150.2	0.14×10^{-3}	124.01	78.11
3	Ammonium sulfate precipitation	2561	48.23	1.88×10^{-2}	163.99	25.08
4	Dialysis	2310	42.15	1.82×10^{-2}	158.89	21.92
5	Cation-exchange chromatography	894.2	30.25	3.38×10^{-2}	294.58	15.73
6	Anion-exchange chromatography	195.7	24.85	1.26×10^{-1}	1105.73	12.92
7	After HPLC	9.568	1.564	1.63×10^{-1}	1423.41	0.81

PDI extracted from both organisms was purified by homogenization, heat treatment, ammonium sulfate precipitation, dialysis against citrate buffer, and subsequent chromatography on CM-cellulose, DEAD-cellulose, and gel filtration HPLC. As PDI carries net negative charge in DEAE cellulose column, DEAE lock negatively charged PDI into the matrix. A substantial amount of impurities was removed by HPLC due to higher sensitivity and rapid turnover rate (Coskun 2016) compared to the previous procedure. Therefore, the number of folds of purification by PDI in the livers of the pigs in the treated and non-treated groups was 1657 and 1423, respectively, and the final yield of the enzyme was 1.05% and 0.81% (Table 2), respectively. The number of folds of purification by PDI in the plants in the treated and non-treated groups was 8510 and 282, respectively, and the final yield of the enzyme was 0.51% and 0.26% (Table 3), respectively. Both purified PDIs in the animals (as the form of ERp57) and plants treated by Chinese medicine pellets were shown as a single band with 57.0 kDa molecular weight on SDS–PAGE gel (Figs. 1 and 2).

Table 3. The purification table of PDI extracted from treatment and non-treatment animal groups

Item	Treatment	Total weight of protein (mg)	Total enzyme activity (units)	Specific activity (units/mg)	Fold of purification	Yield (%)
Treated group						
1	Homogenate	1,382,111	162.4	1.17×10^{-4}	1.00	100.00
2	Heat treated	10,532	138.2	1.31×10^{-2}	111.67	85.10
3	Ammonium sulfate precipitation	2402	38.10	1.58×10^{-2}	134.99	23.46
4	Dialysis	2102	35.20	1.67×10^{-2}	142.52	21.67
5	Cation-exchange chromatography	602.1	29.40	4.88×10^{-2}	415.56	18.10
6	Anion-exchange chromatography	102.3	23.20	0.22×10^{-1}	1930.06	14.29
7	After HPLC	0.123	0.123	1.00	56,944.68	0.51
Control group						
1	Homogenate	1,024,189	185.2	1.80×10^{-4}	1.00	100.00
2	Heat treated	10,001	145.3	1.45×10^{-2}	80.35	78.46
3	Ammonium sulfate precipitation	2548	40.23	1.57×10^{-2}	87.32	21.72
4	Dialysis	2301	38.5	1.67×10^{-2}	92.53	20.79
5	Cation-exchange chromatography	994	30.2	3.03×10^{-2}	168.02	16.31
6	Anion-exchange chromatography	302.2	22.4	7.41×10^{-2}	409.91	12.10
7	After HPLC	9.547	0.487	5.10×10^{-2}	282.10	0.26

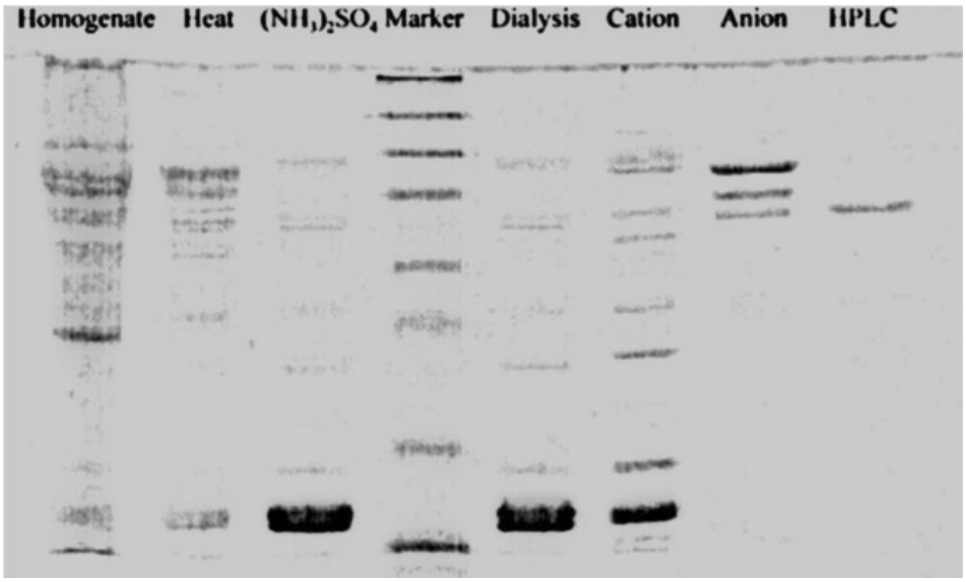

Figure 1. Gel electrophoresis of PDI purification from animal feed with pellet containing *Sijunzi Tang* extracts

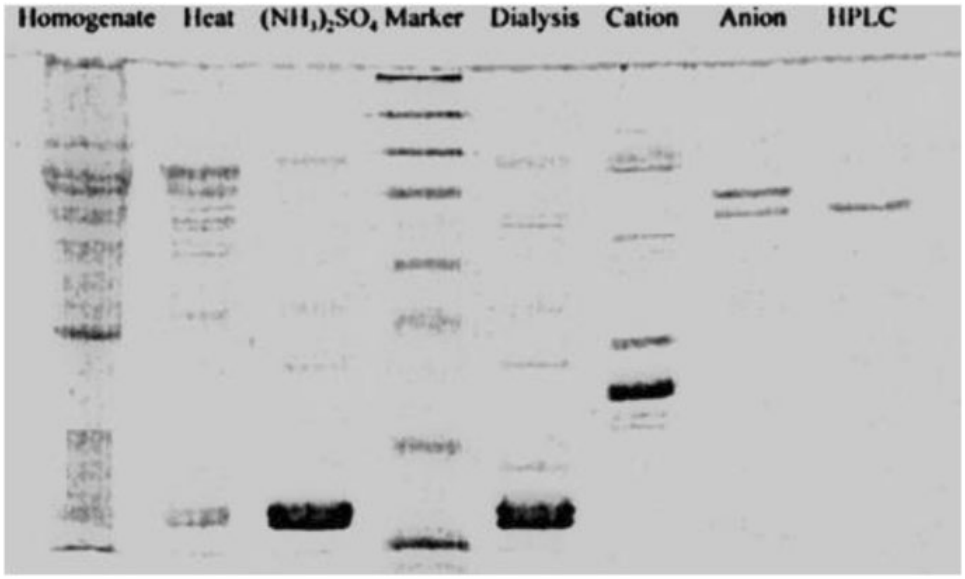

Figure 2. Gel electrophoresis of PDI purification from plant cultivated with pellet containing *Sijunzi Tang* extracts

Characterization of the PDI activity in different temperatures and pH

As shown in Figs. 3 and 5, the enzyme activity extracted from the animals and plants dramatically increased from 30 to 70 °C and then decreased after 70 °C and the enzyme was found to be thermostable. Higher enzyme activity was found at pH 6 than at the other pH values (Figs. 4 and 6), suggesting PDI was adapted to slightly acidic condition.

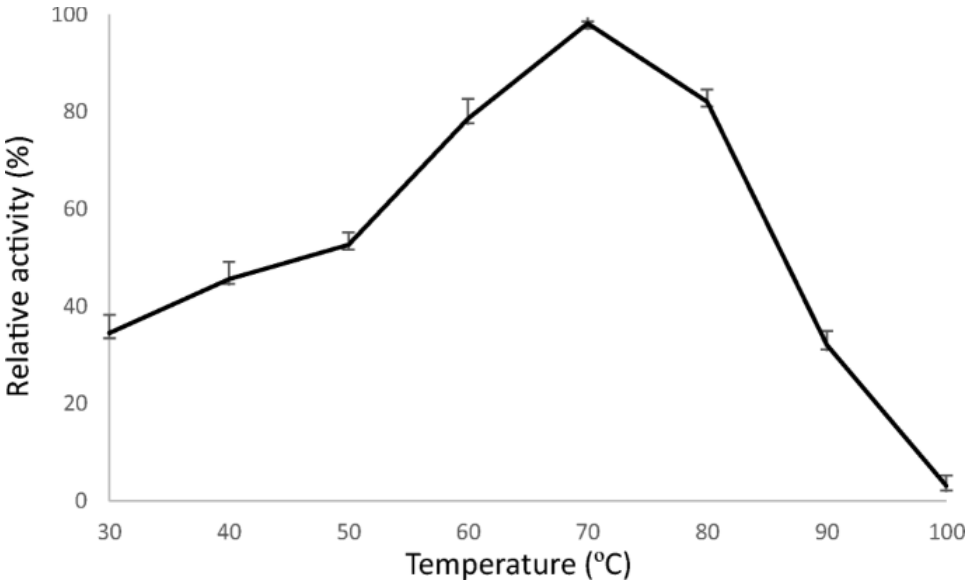

Figure 3. Enzyme activity of PDI extracted from animal in different temperature environments. PDI was incubated for 30 min in sodium phosphate buffer

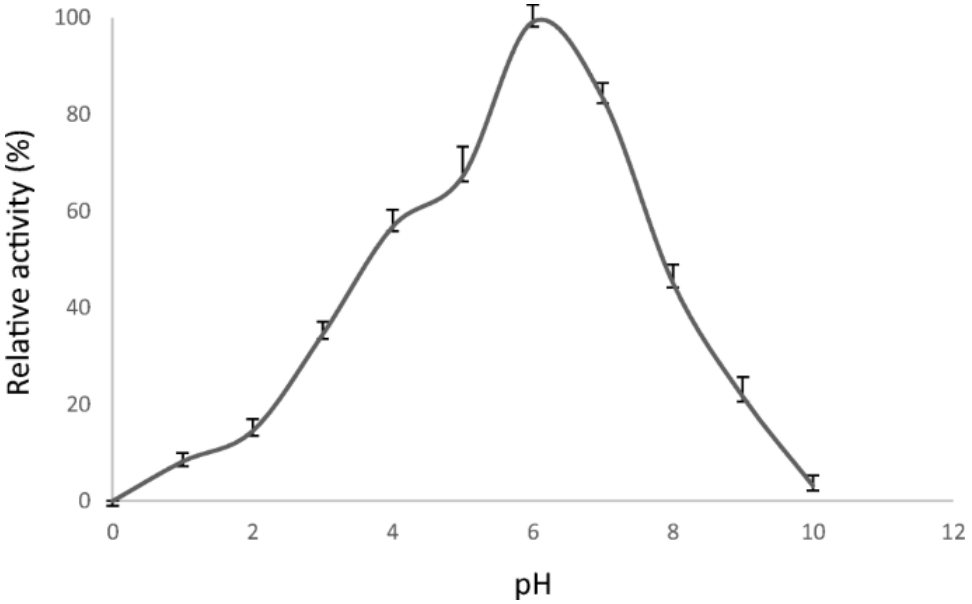

Figure 4. Enzyme activity of PDI extracted from animals in different pH environments. Optimum pH was determined in various buffers at 70 °C

Reactivation study of MDH by PDI

From the results, it was found that the PDIs in both treated or untreated groups were capable of enhancing the reactivation of enzymes affected by PAHs (Figs. 4, 5, and 6). A significantly higher percentage of MDH activity was regained with the aid of PDI extracted in treated (80.44%) and untreated (39.55%) groups (Table 4). However, in plants, only 2.40% and 1.53% of inactivated MDH regained its activity in both treated or untreated groups (Table 5). There was a lower resumption power of PDI extracted from the plant than that in the animal samples that could be attributed to environmental variables for plant propagation such as temperature, water,

and fertilizer, which influence the rate and duration of wheat grain development, protein accumulation, and starch deposition in unique ways. Therefore, the effects of the environment are imposed on the intrinsic temporal patterns of gene expression during grain development and hence its resumption power (Dhanapal and Porceddu 2013).

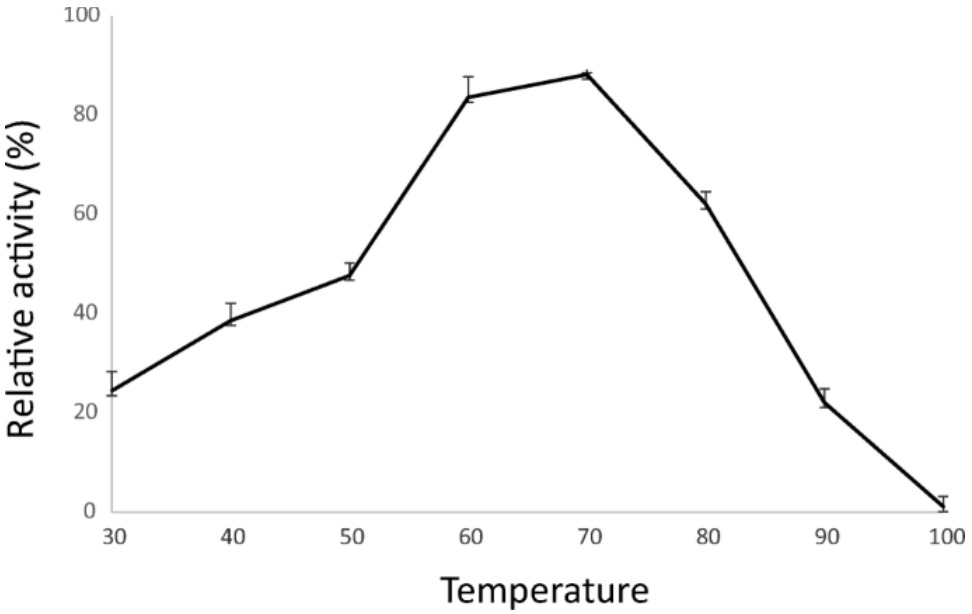

Figure 5. Enzyme activity of PDI extracted from plants in different temperature environments. PDI was incubated for 30 min in sodium phosphate buffer

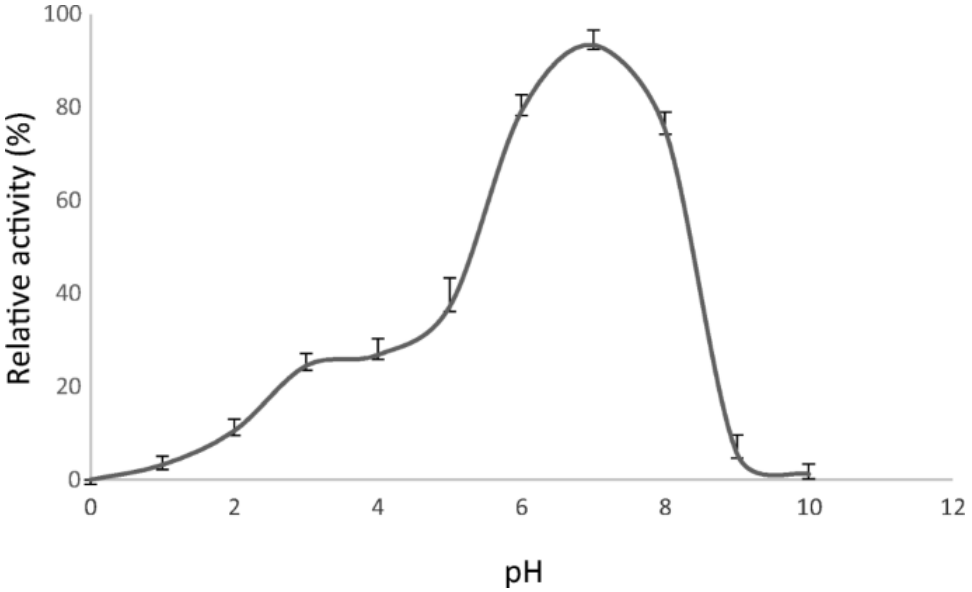

Figure 6. Enzyme activity of PDI extracted from plant in different pH environments. Optimum pH was determined in various buffers at 70 °C

Thus, the experiment exhibited that MDH reactivation was especially dependent on PDI assistance. In addition, regardless of the source of PDI extracted from plants and animals, the higher percentage of MDH recovery found in the treated groups compared to the untreated

groups may be subject to PDI extracted in the treated group that could bind toxicant strongly (Šribar et al. 2005) than PDI extracted in the untreated group, hence preventing PAHs competing with NAD binding to MDH.

Table 4. The activity of MDH in the treatment and non-treatment animal groups ($n = 10$)

Treatment	MDH activity (units)	Percentage (%) recovery of MDH
Native MDH	12.99 ± 0.06	–
Inactivated MDH	0	–
Inactivated MDH + PDI extracted in the treatment group	10.45 ± 0.45	80.44 ± 0.15[a]
Inactivated MDH + PDI extracted in the control group	5.137 ± 0.89	39.55 ± 0.28[b]

Within the column, means with the same letter are not significantly different according to Duncan's multiple range test at 0.5% level

Table 5. The activity of MDH in the treatment and non-treatment plant groups ($n = 10$)

Treatment	MDH activity (units)	Percentage (%) recovery of MDH
Native MDH	15.41 ± 0.87	–
Inactivated MDH	0	–
Inactivated MDH + PDI extracted in the treatment group	0.36 ± 0.08	2.40 ± 0.06[a]
Inactivated MDH + PDI extracted in the control group	0.23 ± 0.05	1.53 ± 0.04[a]

Within the column, means with the same letter are not significantly different according to Duncan's multiple range test at 0.5% level

Moreover, the action of PDI was affected by external conditions such as interspecific and intraspecific competition with xenobiotic molecules. The high percentage of MDH reactivation found in the treated group minimized the formation of insoluble aggregates by Chinese medicine and facilitated the refolding activity in MDH. In addition, the finding was in line with that of Woo et al. (2014) that an organism exposed to PAHs triggers the activation of PDI as a defense mechanism.

However, as PDI is located in the endoplasmic reticulum and MDH is a component of the Krebs cycle, the findings suggest that PDI assisting in reactivating MDH does not necessarily mean that PDI plays a role during MDH deactivation in vivo. Xenobiotic substances such as quercetin may be present in the extract of Chinese medicine to prevent thrombosis and thus inhibit PDI activity (Galinski et al. 2016). In addition, an eventual PDI-assisted activity recovery of deactivated MDH is far from completion. Although some thioredoxin proteins have a low specificity as far as the target protein is concerned, they are not entirely indiscriminate in their action, and are so to different extents; such is also the case for the refolding of different proteins by the same thioredoxin proteins (Bucher et al. 1992).

Conclusion

Based on the findings, it has been speculated that PDI extracted from animals that were fed with Chinese medicine pellets can interfere with the binding ability of PAHs thus increasing the chance of MDH to interact with NAD^+. Furthermore, the formation of insoluble aggregates represents a significant step in understanding how the recovery of inactivated MDH might occur in its intracellular environment. Further analysis of the amino acid sequence of PDI extracted

from animal feed containing *Sijunzi Tang* in response to mutagenic stress is necessary to understand the stability of PDI in adverse conditions.

References

Auyeung KK, Han QB, Ko JH (2016) Astragalus membranaceus: a review of its protection against inflammation and gastrointestinal cancers. Am J Chin Med 44:1–22 Article Google Scholar

Baran S, Oleszczuk P, Lesiuk A, Baranowska E (2002) Trace metals and polycyclic aromatic hydrocarbons in the surface sediment samples from the river Narew (Poland). Pol J Environ Stud 11:299–305 Google Scholar

Baran S, Bielin'ska EJ, Oleszczuk P (2004) Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons (PAH). Geoderma 118:221–232 Article Google Scholar

Bucher J, Brinkmann U, Pastan I (1992) Renaturation of a singlechain immunotoxin facilitated by chaperones and protein disulfide isomerase. Bio/Technology 10:682–685 Google Scholar

Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL (2005) Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect 113:391–395 Article Google Scholar

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL (2008) Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ Health and Perspect 116:39–44 Article Google Scholar

Chen F, Qi W, Jiang J, Li HL, Tan YF, Li YH, Zeng NK (2016) Could the gut microbiota reconcile the oral bioavailability conundrum of traditional herbs? J Ethnopharmacol 179:253–264 Article Google Scholar

Coskun O (2016) Separation techniques: chromatography. North Clin İstanb 3:156–160 Google Scholar

Dhanapal AP, Porceddu E (2013) Functional and evolutionary genomics of protein disulphide isomerase (PDI) gene family in wheat and its wild relatives: a review. Annu Rev Res Biol 3:935–958 Google Scholar

Galinski CN, Zwicker JI, Kennedy DR (2016) Revisiting the mechanistic basis of the French paradox: red wine inhibits the activity of protein disulfide isomerase in vitro. Thromb Res 137:1690173 Article Google Scholar

Gianfreda L, Antonietta RM, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Soil Total Environment 341:265–279 Article Google Scholar

Gilbert HF (1998) Protein disulphide isomerase. In: Methods in enzymology, vol 290. Academic Press/Elsevier, San Diego, CA, pp 26–50 Google Scholar

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15 Article Google Scholar

Hashimoto S, Shiomoto K, Okada K, Imaoka S (2012a) The binding site of bisphenol a to protein disulphide isomerase. J Biochem 151:35–45 Article Google Scholar

Hashimoto S, Yoshimura H, Okada K, Uramaru N, Sugihara K, Kitamura S, Imaoka S (2012b) Effects of polybrominated diphenyl ethers (PBDEs) and their derivatives on protein disulfide isomerase activity and growth hormone release of GH3 cells. Chem Res Toxicol 25:656–663 Article Google Scholar

He P, Wang AG, Xia T, Gao P, Niu Q, Guo LJ, Xu BY, Chen XM (2009) Mechanism of the neurotoxic effect of PBDE-47 and interaction of PBDE-47 and PCB153 in enhancing toxicity in SH-SY5Y cells. NeutroToxicology 30:10–15 Article Google Scholar

Hillson DA, Lambert N, Freedman RB (1984) Formation and isomerization of disulfide bonds in proteins: protein disulfide-isomerase. Methods Enzymol 107:281–294 Article Google Scholar

Honjo Y, Ito H, Horibe T, Takahashi R, Kawakami K (2010) Protein disulfide isomerase-immunopositive inclusions in patients with Alzheimer disease. Brain Res 1349:90–96 Article Google Scholar

Kim HT, Russell RL, Raina AK, Harris PL, Siedlak SL, Zhu X, Petersen RB, Shimohama S, Smith MA, Perry G (2000) Protein disulfide isomerase in Alzheimer disease. Antioxid Redox Signal 2:485–489 Article Google Scholar

Kimura S, Higashino Y, Kitao Y, Masuda T, Urade R (2015) Expression and characterization of protein disulfide isomerase family proteins in bread wheat. BMC Plant Biol 15:73 Article Google Scholar

King YA, Chiu YJ, Chen HP, Kuo DH, Lu CC, Yang JS (2016) Endoplasmic reticulum stress contributes to arsenic trioxide-induced intrinsic apoptosis in human umbilical and bone marrow mesenchymal stem cells. Environ Toxicol 31:314–328 Article Google Scholar

Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685 Article Google Scholar

Lorimer GH, Baldwin TO (1998) Molecular chaperones. Methods Enzymol 290:32–33

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265 Google Scholar

Mossuto MF (2013) Disulfide bonding in neurodegenerative misfolding diseases. Int J Cell Biol 2013:318–319 Article Google Scholar

Okumura M, Kadokura H, Hashimoto S, Yutani K, Kanemura S, Hikima T, Hidaka Y, Ito L, Shiba K, Masui S (2014) Inhibition of the functional interplay between endoplasmic reticulum (ER) oxidoreductin-1 alpha (Ero1 alpha) and protein-disulfide isomerase (PDI) by the endocrine disruptor bisphenol A. J Biol Chem 289:27004–27018 Article Google Scholar

Oleszczuk P, Baran S (2005) The concentration of polycyclic aromatic hydrocarbons in sewage sludge-amended soil. Commun Soil Sci Plant Anal 36:1083–1097 Article Google Scholar

Peng RH, Qiu J, Tian YS, Gao J, Han HJ, Fu XY, Zhu B, Xu J, Wang B, Li ZJ, Wang LJ, Yao QH (2017) Disulfide isomerase-like protein AtPDIL1–2 is a good candidate for trichlorophenol phytodetoxification. Nat Sci Rep 7:40130 Article Google Scholar

Pinsonneault AJ, Moore TR, Roulet NT (2016) Temperature the dominant control on the enzyme-latch across a range of temperate peatland types. Soil Biol Biochem 97:121–130 Article Google Scholar

Rengarajan T, Rajendran P, Nandakumar N, Lokeshkumar B, Rajendran P, Nishigaki I (2015) Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac J Trop Biomed 5:182–189 Article Google Scholar

Si LL, Zheng LL, Xu LN, Yin LH, Han X, Qi Y, Xu YW, Wang CY, Peng JY (2016) Dioscin suppresses human laryngeal cancer cells growth via induction of cell-cycle arrest and MAPK-mediated mitochondrial-derived apoptosis and inhibition of tumor invasion. Eur J Pharmacol 772:105–117 Article Google Scholar

Son B, Jun SY, Seo HJ, Youn H, Yang HJ, Kim W, Kim HK, Kang C, Youn B (2016) Inhibitory effect of traditional oriental medicine-derived monoamine oxidase B inhibitor on radioresistance of non-small cell lung cancer. Sci Rep 6:21986 Article Google Scholar

Šribar J, Anderluh G, Fox JW, Križaj I (2005) Protein disulphide isomerase binds ammodytoxin strongly: possible implications for toxin trafficking. Biochem Biophys Res Commun 329:733–737 Article Google Scholar

Tanaka S, Uehara T, Nomura Y (2000) Up-regulation of protein-disulfide isomerase in response to hypoxia/brain ischemia and its protective effect against apoptotic cell death. J Biol Chem 275:10388–10393 Article Google Scholar

Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA (2006) S-Nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441:513–517 Article Google Scholar

Wang X, Sankarapandian K, Cheng YH, Woo SO, Kwon HW, Perumalsamy H, Ahn YJ (2016) Relationship between total phenolic contents and biological properties of propolis from 20 different regions in South Korea. BMC Complement Altern Med 16:65 Article Google Scholar

Wong HY, Liu KD, Liu YH, Yang SM, Chen TW, Cherng J, Chou KS, Chang CM, Yao BT, Cherng JM (2016) Cinnamomum verum component 2-methoxycinnamaldehyde: a novel anticancer agent with both anti-topoisomerase I and II activities in human lung adenocarcinoma A549 cells in vitro and in vivo. Phytother Res 30:331–340 Article Google Scholar

Woo S, Lee A, Denis V, Chen CA, Yum S (2014) Transcript response of soft coral (*Scleronephthya gracillimum*) on exposure to polycyclic aromatic hydrocarbons. Environ Sci Pollut Res 21:901–910 Article Google Scholar

Wu M, Li TT, Chen LL, Peng SG, Liao W, Bai RL, Zhao X, Yang H, Wu CH, Zeng HJ, Liu YY (2016) Essential oils from *Inula japonica* and *Angelicae dahuricae* enhance sensitivity of MCF-7/ADR breast cancer cells to doxorubicin via multiple mechanisms. J Ethnopharmacol 180:18–27 Article Google Scholar

Zhao LJ, Wen Q, Yang G, Huang ZQ, Shen T, Li HZ, Ren DM (2016) Apoptosis induction of dehydrobruceine B on two kinds of human lung cancer cell lines through mitochondrial-dependent pathway. Phytomedicine 23:114–122 Article Google Scholar