Antioxidative Mechanisms at a Molecular Level

PADMA 28

PADMA –

For the benefit

of our health.

Summary
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The present results and earlier clinical studies support the rational application of PADMA 28 in cases of:

- arteriosclerosis-related circulatory dysfunction
- inflammatory reactions in vessels
- chronic-inflammatory disease
- extreme physical stress

Brief overview over the results.

The interactions between the Tibetan herbal formulation, PADMA 28, and the major reactive oxygen and nitrogen species were investigated in this study. PADMA 28 was found to prevent oxidative stress effects on proteins and DNA. This protective effect is related to the reductive potential of PADMA 28 and/or its ability to chelate (bind) heavy metal ions, such as iron and copper. Because of these mechanisms PADMA 28 is very effective in the management of functional disorders that are related to oxidative stress.

Calculations showed that the uptake of one tablet of PADMA 28 provides the same reductive potential to an individual of 70 kg body weight as the recommended daily dose of vitamin C (1.8 mg/kg or 125 mg total). However, it shall be emphasized in this context that the informative value of in vitro models in general is limited.

PADMA 28 was successfully used in the management of intermittent claudication (Charcot's syndrome), arteriosclerosis, and disturbances of peripheral circulation (Sallon'98, Saller'97, Smulski'95, Drabaek'93) and as an immune system modulator (Winther'94, Gladysz'93, Korwin-Piotrowska'92, Jankowski'91). This clinical experience has been confirmed in in vitro studies of macrophage and neutrophil functions (Ginsburg'99, Matzner'95).

Introduction

The role of oxygen in life

The mitochondrial respiratory chain of aerobic organisms continually produces highly reactive molecules needed as energy substrates. Energy rich molecules are the basis of all life processes. As a side product of these processes, reactive oxygen radicals are produced in quantities equivalent to 1 –2% of the oxygen turnover (Chance'79). This corresponds to approx. $1 - 2 \times 10^{20}$ oxygen radicals per minute.

Natural Systems Providing Protection from Free Radicals

In order to protect the organism from these highly reactive molecules, several antioxidative defence mechanisms have developed during evolution, including

- antioxidative molecules (plant flavonoids and polyphenols, α-tocopherol, ascorbate, glutathione),
- metal ion-binding proteins,
- enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase).

These protective mechanisms aim to scavenge free radicals before they can damage cellular structures. Moreover, these mechanisms must be sufficiently specific as to not disturb the vital processes that are ongoing in the cells in order to ultimately maintain a crucial balance within the organism (Cottier'95).

Oxidative Stress is a fundamental pathogenetic process

Oxidative stress arises unless the free radicals are scavenged and detoxified before they cause greater damage. Oxidative stress may occur

- if the stock of antioxidants is exhausted (e.g. due to malnutrition, chronic disease),
- if the acute radical production is high and exceeds the body's defensive capabilities (e.g. under extreme stress, (extreme) physical exertion, exposure to radiation and environmental poisons, etc.).

These conditions may lead to defects in the cells with far-reaching consequences, such as peroxidation of membrane lipids, oxidation of proteins or the genetic substance, DNA, with ensuing severe metabolic dysfunctions, eventually leading to lipid peroxidation, inflammatory reactions in peripheral vessels, arteriosclerosis, asthma, and a number of other ailments (Hogg '98,Gutteridge '93).

Results

Preliminary remark: PADMA 28 was investigated as the intact mixture of substances. For this purpose, an extract was generated by mixing 50 mg of PADMA 28 powder with 1 ml of water. In the experiments, a defined volume v of the extract was added to the test solution with a volum V (quantified as: «Vol» = v/V or» unit volume of extract per volume of test solution»). Thus, the dosage of the PADMA 28 extract is comparable between experiments.

1.

PADMA 28 is an electron donor, I: PADMA 28 reduces cytochrome c

Cytochrom c, a major electron transport protein of the respiratory chain, was used as a model protein to investigate one of the reaction mechanisms of PADMA 28: PADMA 28 is a potent reducing agent for the active heme group of cytochrome c. PADMA 28 reduced oxidized cytochrome c by up to 85% in a concentration-dependent manner (fig.1.).

The cytochrome c model was then used to compare the **reductive capacity** of PADMA 28 with that of the antioxidant vitamin C: in an individual of 70 kg body weight 1 tablet of PADMA 28 delivers the same reductive capacity as the recommended daily dose of vitamin C (125 mg). (The recommended daily dose of PADMA 28 is 2 to 6 tablets).

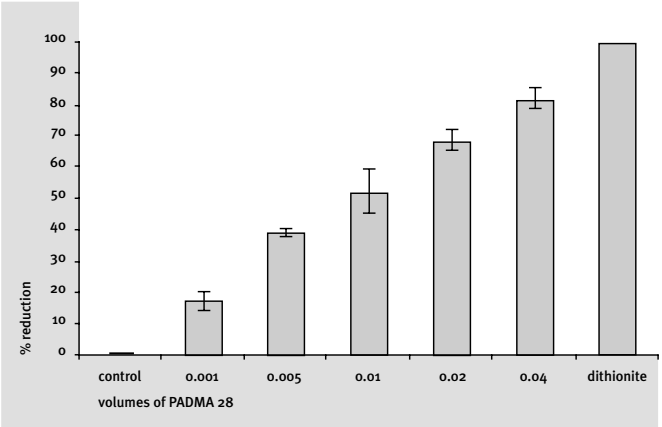

Fig.1. *Reduction of cytochrome c by PADMA 28. Cytochrome c (10 µM) in oxygensaturated phosphate buffer (0.1 mM, pH 7) was incubated at room temperature with increasing volumes of PADMA 28 extract. Reduction was followed at 550 nm. Complete reduction (100%) was determined after the addition of dithionite (a strong reducing agent).*

2.

PADMA 28 is an electron donor, II: PADMA 28 transfers electrons to enzymes (e.g. horseradish peroxidase)

Peroxidases are enzymes that transfer electrons to hydrogen peroxide (H_2O_2) and thereby degrade this precursor of reactive radicals. Therefore, peroxidases are also important protective systems for the cells of the immune system keeping the extent of self-inflicted damage during inflammatory processes in check. By means of its electron-transferring mechanism, PADMA 28 effectively acts as a **regenerating substrate for this protective system** (Fig.2.). It was shown in a control experiment that PADMA 28 alone does not directly transfer electrons to H_2O_2, i.e. it truly recycles the enzyme.

This finding is in agreement with earlier studies (Ginsburg'99, Matzner'95, and Winther'94), in which strong inhibition of the «oxidative burst» in macrophages and neutrophils by PADMA 28 was noted.

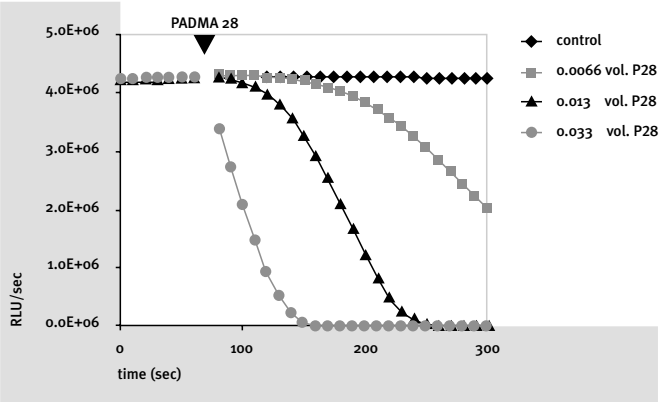

Fig.2. *PADMA 28 as a horseradish peroxidase substrate. The curve shows the time course of degradation of 8 mM hydrogen peroxide (H_2O_2) in the presence of horse-radish peroxidase (100 µg/ml) and PADMA 28 measured as the chemoluminescence of the dye, luminol, which emits light when exposed to H_2O_2. Luminol (62.5 µM) in glycine buffer (0.9 M; pH 9.4). The arrow indicates the time of addition of various dilutions of PADMA 28. RLU = relative luminescence units.*

3.A.

PADMA 28 binds (chelates) iron ions

Free transition metal ions, such as iron, act as catalysts in redox reactions and thus may contribute substantially to oxidative stress (Fenton reaction, Fig.3.b.). However, if bound in chelate complexes, these ions may loose their oxidative capacity, essentially by «being removed from circulation». It was shown in this experiment that the aqueous PADMA 28 extract also chelated iron ions (Fig.3.a.).

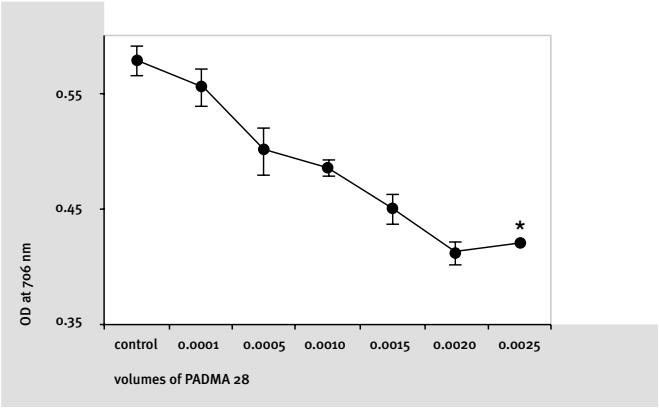

Fig.3.a. *Chelation of Fe^{2+} by PADMA 28. Competition experiment in water in the presence of the complexing agent, potassium hexacyanoferrate complex $K_3[Fe(CN)_6]$ (100 µM), various dilutions of PADMA 28, and $FeCl_2$ (100 µM). The decrease in absorbance at 706 nm (i.e. at the absorption maximum of the potassium hexa-cyanoferrate complex) shows that PADMA 28 binds iron(II) ions in a concentration-dependent manner. *Because of its inherent absorption the PADMA 28 extract could not be used in the experiment at levels exceeding 0.002 vol.*

The significance of iron chelation has recently been documented (Van Acker'98). Removal of iron is currently under discussion as a promising strategy to mitigate or prevent oxidation-induced diseases (Polla'99).

3.B.

PADMA 28 reduces iron(III) to iron(II)

This result was obtained in a control of the experiment described in Fig.3.a.

It is self-evident that the strong reductive power of PADMA 28 may also affect other molecules and ions, especially certain metals, such as Fe^{2+} and Cu^{+}. If they undergo the Fenton reaction, these reduced metals may form highly reactive OH • radicals (Hippeli'99; see diagram in Fig.3.b.). Fortunately, the protective mechanism of chelation prevents this process and selectively binds this pro-oxidative potential (see experiments 3.a. and 4.). This type of multiple activity is characteristic of mixtures consisting of a variety of substances keeping in mind that PADMA 28 alone does not directly transfer electrons to H_2O_2, as was shown in the control of experiment 2.

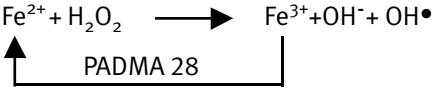

Fig.3.b. *General diagram showing the Fenton reaction for iron*

4.

Reactive oxygen and nitrogen species may damage the genetic substance, DNA. Hydroxyl radicals (OH•) and nitrogen monoxide radicals (NO•) as well as related compounds may cause the DNA-strand to break or modify DNA-bases of the genetic substance (Beckman '99, Burney'99). It has been estimated that the DNA of a single human cell is damaged by oxidation up to 10,000 times per day. This damage must subsequently be repaired or, preferably, prevented before it occurs (Ames '93).

Combining the results of the series of experiments 3., it can be concluded that PADMA 28 also exerts a protective effect under the reaction conditions of the Fenton reaction. This conclusion was confirmed in the following experiment.

4.A.

PADMA 28 prevents DNA damage,I

This experiment was designed to investigate the effect of PADMA 28 on DNA strand breaks and oxidative base modification in a plasmid DNA model under the conditions of the Fenton reaction (addition of Cu^{+} und H_2O_2), under which hydroxyl radicals (OH •) are generated. At very low PADMA 28 concentrations, the experiment showed the substance to have an initial prooxidative effect that is related to the recycling of Cu^{2+} ions by PADMA 28-mediated reduction to Cu^{+} (as in 3.b.).

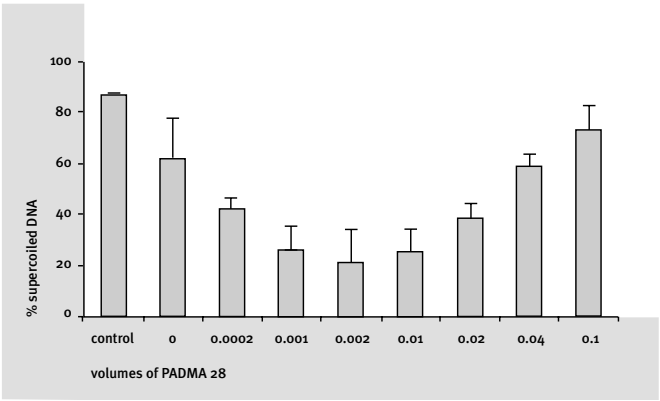

Fig.4.a. *Interaction of PADMA 28 with DNA under the conditions of the Fenton reaction. Supercoiled plasmid DNA (pBR322 1.25 µg/ml) was oxidized with $CuCl_2$ (40 µM)and H_2O_2 (0.8 mM) in the presence of the specified volumes of PADMA 28 extract. (The normal in vivo serum concentration of copper is 12-20 µM (Kruse-Jarres'93)). The first bar shows unmodified plasmid DNA, whereas the second shows slightly oxidized DNA. The results are expressed as the percentage of super-coiled DNA.*

However, with increasing PADMA 28 concentration this behavior changes and PADMA 28 begins to exert its antioxidative effect protecting the DNA from further damage. Several action mechanisms of PADMA 28 play a role in this effect: (i) PADMA 28 contributes to the chelation of metal ions and thus effectively blocks the Fenton reaction (experiment 3.a.), (ii) PADMA 28 directly scavenges the radicals or (iii) the substance acts by means of its reducing potential (electron-transfer).

The same effect has been observed by other authors investigating 8-OHdG (8-hydroxydeoxyguanosine), a well-known marker for oxidative base modification.

Due to the initially unexpected finding of concentration-dependence in their experiment, the researchers proceeded to replace PADMA 28 by vitamin C (ascorbate), which is also known to be an electron donor for transition metals (Halliwell'96). With increasing dosage, vitamin C caused substantial strand breaks, albeit without the benefit of a protective effect at elevated dosages. This is to be expected, since vitamin C, like many other antioxidants and isolated flavonoids and polyphenols, possesses no metal-chelating properties (Morel '98).

4.B.

PADMA 28 prevents DNA damage, II

At elevated concentrations, the PADMA 28 extract largely prevented strand breaks in the plasmid DNA induced by peroxynitrite ($ONOO^{-}$), one of the most potent oxidizing agents. The reductive potential of PADMA 28 made the substance play an important role in this reaction as both an antioxidant and radical scavenger.

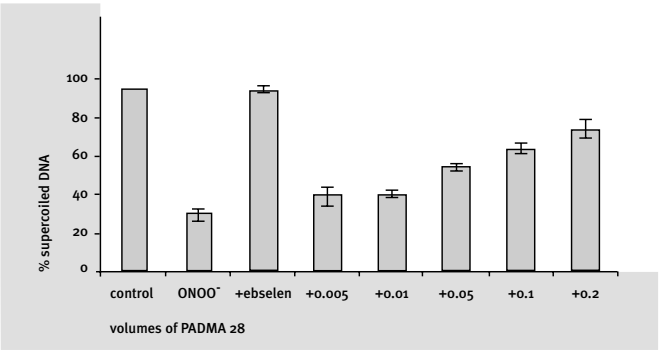

Fig.4.b. *Prevention of peroxynitrite-induced DNA damage. Plasmid DNA (pBR322, 1.25 µg/ml) was oxidized with 0.1 mM peroxynitrite in 0.1 M phosphate buffer, pH 7 (second bar). This process can be inhibited with Ebselen, an established peroxynitrite radical scavenger (1 mM) (third bar). The results are expressed as the percentage of supercoiled DNA.*

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For additional references and all studies mentioned please contact PADMA AG.

This study demonstrates the action mechanisms of PADMA 28 as a

reducing agent/electron donor

iron and copper chelating agent

antioxidant

radical scavenger

protecting DNA and other important biomolecules from oxidative stress-related damage

PADMA 28

Composition: Aegle sepiar fructus 20 mg, Amomi fructus 25 mg, Aquilegiae vulgaris herba 15 mg, Calcii sulfas pulv. 20 mg, Calendulae flos 5 mg, Cardamomi fructus 30 mg, Caryophylli flos 12 mg, Costi amari radix 40 mg, Dextrocamphora 4 mg, Hedychii rhizoma 10 mg, Lactucae sativae folium 6 mg, Lichen islandicus 40 mg, Liquiritiae radix 15 mg, Meliae tousend fructus 35 mg, Myrobalani fructus 30 mg, Plantaginis herba 15 mg, Polygoni herba 15 mg, Potentillae aureae herba 15 mg, Santali rubri lignum 30 mg, Sidae cordifoliae herba 10 mg, Aconiti tuber 1 mg, Valerianae radix 10 mg, Excip. pro compr.

Indications: Tingling sensation, formication, heaviness und tenseness in arms and legs, numbness of hands and feet, cramps in the calf (systremma).

Administration/Dosage: Initially, ingest 3 x 2 tablets 1/2 – 1 hours before meals. Depending on the patient's condition, the dosage may subsequently be reduced to 1 – 2 tablets daily.

Side effects: Occasionally gastrointestinal symptoms may occur. Gastric discomfort can be remedied by ingesting plenty of fluid (1 – 2 glasses of fluid) or by taking the tablets during the meals. In a few isolated cases, palpitations and slight restlessness have been observed in predisposed individuals.

Information: Comprehensive information regarding this product can be found in the «Arzneimittelkompendium der Schweiz – Publikumsausgabe».

Production and distribution: PADMA AG, Wiesenstrasse 5, CH-8603 Schwerzenbach, Switzerland, Tel. +41-1-887 00 00, Fax +41-1-887 00 99, mail@padma.ch, www.padma.ch