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Curcumin as a modulator of oxidative stress during storage: A study on plasma

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ABSTRACT

Background: Plasma reflects the situations of oxidative stress in blood. Curcumin as an additive in storage solutions is not explored.
Materials and methods: Blood was drawn from male *Wistar* rats (4 months old) and stored over a period of 20 days at 4 °C in CPDA-1. Plasma was isolated from stored blood at regular intervals. Oxidative stress markers were analyzed in controls and experimental samples (with curcumin 10 mM, 30 mM and 60 mM).
Results: Antioxidant enzymes and sulfhydryls ameliorated, while TBARS and protein carbonyls reduced in curcumin samples. However, curcumin could not protect the susceptible groups in proteins thereby leading to dityrosine linkages (AOPP).
Conclusion: Curcumin was beneficial at higher concentrations as it reduced oxidative stress, therefore paving way for the further studies on curcumin as a component in storage solutions.

1. Introduction

The "storage lesion" can be defined as a constellation of changes occurring in the blood which eventually results in irreversible damage and reduced post-transfusion survival. The progressive damage ultimately limits the storage period. Most of the irreversible events are favoured by the prolonged oxidative stress in the non-physiological conditions of storage [1]. Generation of free radicals during storage, overwhelms the antioxidant capacity, resulting in oxidative stress (OS) [2].

Endogenous and exogenous antioxidants can reduce the OS and curcumin is a well known antioxidant. Curcumin (1,7-*bis*(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) also known as diferuloylmethane, is a component of turmeric (*Curcuma longa*). Curcumin has been shown to have antioxidant activity, superoxide and hydroxyl radical scavenging activity [3] and pro-oxidant activity [4].

The interaction of curcumin nanoformulations with human plasma proteins and erythrocytes was studied by Yallapu et al. and these studies suggest that curcumin nanoformulations are a necessary component for harnessing and implementing improved *in vivo* effects of curcumin [5].

Whole blood transfusion is still a common practice when compared to component therapy. Therefore studies on whole blood storage, play a major role in understanding the modulations in stored blood. There are various studies on plasma storage, but minimal studies on plasma isolated from stored blood. Studies on curcumin as a component of storage solution have not been reported.

Hence, this study attempts to examine two aspects of blood storage through plasma, as plasma reflects the changes occurring in the blood: firstly, to evaluate the gradual oxidative changes occurring throughout storage period and secondly, to determine the role of curcumin in storage solution. The OS markers such as antioxidant

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enzymes, lipid peroxidation and protein oxidation products were assessed towards the above objectives.

2. Materials and methods

Animal care and maintenance was in accordance with the ethical committee regulations (841/b/04/CPCSEA).

2.1. Chemicals

Curcumin, Epinephrine, Thiobarbituric acid (TBA) and Bovine Serum Albumin (BSA) were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). All other chemicals used were of reagent grade and organic solvents were of spectral grade.

2.2. Blood sampling

Animals were lightly anaesthetized with ether and restrained in dorsal recumbancy as described earlier [6]. In brief, the syringe needle was inserted just below the xyphoid cartilage and slightly to the left of the midline. 4–5 ml of blood was carefully aspirated from the heart into 5 ml plastic collection tubes with CPDA-1 (Citrate Phosphate Dextrose Adenine).

2.3. Experimental design

Blood was drawn from male *Wistar* rats (4 months old) and stored over a period of 20 days at 4 °C in CPDA-1. Blood samples were divided into two groups: controls and experimentals. The experimentals were added with curcumin of varying concentrations – 10 mM, 30 mM and 60 mM. Plasma was isolated from these samples at regular intervals (every 5 days) and all the previously mentioned parameters were analyzed.

2.4. Plasma separation

Plasma was isolated in micro-centrifuge tubes by centrifuging in a fixed angle rotor for 20 min at 2000*g* and mixed with isotonic phosphate buffer [7].

2.5. Antioxidant enzymes

2.5.1. Superoxide dismutase [SOD, EC 1.15.1.1]

SOD was measured by the method of Misra and Fridovich [8]. Plasma was added to carbonate buffer (0.05 M). Epinephrine was added to the mixture and measured spectrophotometrically at 480 nm. SOD activity was expressed as the amount of enzyme that inhibits oxidation of epinephrine by 50%.

2.5.2. Catalase [CAT, EC 1.11.1.6]

CAT was determined by the method of Aebi [9]. Briefly, plasma with absolute alcohol was incubated at 0 °C. An aliquot was taken up with 6.6 mM H_2O_2 and decrease in absorbance was measured at 240 nm. An extinction coefficient of 43.6 M cm^{-1} was used to determine enzyme activity.

2.6. Lipid Peroxidation – Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was determined by the method of Bar-Or et al. [10]. Plasma with 0.9% NaCl was incubated at 37 °C for 20 min. 0.8 M HCl containing 12.5% TCA and 1% TBA was added and kept in boiling water bath for 20 min and cooled at 4 °C. Centrifugation was carried out at 1500*g* and absorbance was measured at 532 nm. TBARS was calculated by using the extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

2.7. Protein oxidation

2.7.1. Protein Carbonyls (PrC)

PrC was determined by the method of Reznick and Packer [11]. Protein carbonyl content was measured by forming labeled protein hydrazones derivative, using 2,4-dinitrophenyl hydrazine (DNPH), which were then quantified spectrophotometrically. Briefly after precipitation of protein with equal volume of 1% TCA, the pellet was resuspended in 10 mM DNPH. Samples were kept in dark for 1 h. An equal volume of 20% TCA was added and left in ice for 10 min, centrifuged at 3000*g* and pellet was washed with ethanol–ethylacetate mixture (1:1) to remove the free DNPH and lipid contaminants. Final pellet was dissolved in 6 M guanidine HCl in 133 mM tris and absorbance was measured at 370 nm. PrC was calculated by using the extinction coefficient of 22,000 M^{-1} cm^{-1}.

2.7.2. Advanced Oxidation Protein Products (AOPPs)

Spectrophotometric determination of AOPP levels were assayed as an index of dityrosine containing cross-linked protein products by Witko's method [12]. The sample was diluted in phosphate buffer saline and 1.16 mol L^{-1} potassium iodide was added, followed by the addition of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm. AOPP was calculated by using the extinction coefficient of 26 mM^{-1} cm^{-1}.

2.7.3. Protein Sulfhydryls (P-SH)

The concentration of P-SH in the proteins was measured as described by Habeeb [13]. In brief, 0.08 mol/L sodium phosphate buffer containing 0.5 mg/ml of Na_2-EDTA, and 2% SDS were added to each assay tube. 0.1 ml of 5,5′ DTNB was added and the solution was vortexed. Color was allowed to develop at room temperature and absorbance was measured at 412 nm. P-SH was calculated by using the extinction coefficient of 13,600 M^{-1} L^{-1} cm^{-1}.

2.8. Protein determination

Protein was determined in the plasma by the method of Lowry et al. [14], using bovine serum albumin as the standard.

2.9. Statistical analyses

Results are represented as Mean ± SE. Values between the groups were analyzed by two-way ANOVA and was considered significant at $p < 0.05$. Bonferroni Post Test was performed using GraphPad Prism 6 software.