The Protective Effect of Epigallocatechin-3-gallate on Paraquat-induced Haemolysis of Erythrocyte Membrane

K Moses, D Pepple, P Singh

ABSTRACT

Epigallocatechin-3-gallate (EGCG) is a major ingredient present in green tea, which has a high antioxidant activity. In this study, the effect of EGCG was investigated on paraquat-induced haemolysis of erythrocyte membrane. Erythrocytes were incubated in 0.03, 0.3, 3.0 and 30 mg/mL EGCG, respectively and exposed to 30 mg/mL of paraquat for 10 minutes. The effect of paraquat was determined by an analysis of the osmotic fragility of the erythrocytes. The results showed that EGCG (30 mg/mL) significantly ($p < 0.05$*) reduced the haemolysis of erythrocytes exposed to paraquat (5.0 mg/mL). This suggests that EGCG may have a protective effect on paraquat-induced erythrocyte membrane haemolysis and that consumption of green tea, with high EGCG concentration, could ameliorate the deleterious effect of paraquat toxicity on the haemolysis of erythrocyte membrane.*

Keywords: Epigallocatechin-3-gallate, free radicals, haemolysis, hypoxia, paraquat

Efecto Protector de la Epigalocatequina-3-galata en la Hemolisis de la Membrana del Eritrocito Inducida por Paraquat

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RESUMEN

La Epigalocatequina-3-galata (EGCG) es uno de los ingredientes principales del té verde, que tiene una elevada actividad antioxidante. En este estudio se investigó el efecto de EGCG en la hemolisis de la membrana del eritrocito inducida por paraquat. Los eritrocitos fueron incubados en 0.03, 0.3, 3.0, y 30 mg/mL de EGCG respectivamente, y expuestos a 30 mg/mL de paraquat durante 10 minutos. El efecto del paraquat fue determinado mediante análisis de la fragilidad osmótica de los eritrocitos. Los resultados mostraron que la EGCG (30 mg/mL) redujo significativamente ($p < 0.05$*) la hemolisis de los eritrocitos expuestos al paraquat (5.0 mg/mL). Esto sugiere que EGCG puede tener un efecto protector sobre la hemólisis de la membrana del eritrocito inducida por paraquat, y que el consumo de té verde, con alta concentración de EGCG, podría mejorar el efecto deletéreo de la toxicidad del paraquat sobre la hemolisis de la membrana del eritrocito.*

Palabras claves: Epigalocatequina-3-galata, radicales libres, hemolisis, hipoxia, paraquat

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INTRODUCTION

Paraquat (1,1-dimethly-4,4-bipyridyl) is used experimentally as a redox indicator as well as an herbicide in agriculture. Human toxicity results from accidental and intentional ingestion of the 20–40% paraquat dichloride formula (1). While the prime indicator of toxicity is extensive lung damage due to selective uptake of paraquat by this organ, overall toxicity is based on oxidative stress caused by free radicals formed in paraquat metabolism. Free radicals have been reported to cause haemolysis of erythrocyte membrane (2). Paraquat acts as a catalyst for the formation of the superoxide anion and subsequently hydroxyl free radical and hydrogen peroxide (3). Due to the lack of a pharmacological antagonist or a chelating agent for paraquat, treatment of its toxicity focusses on preventing accumulation by targeting the toxicokinetics, particu-

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larly by decreasing absorption with Fuller's earth and increasing elimination (4, 5).

Epigallocatechin-3-gallate (EGCG; (−)-cis-3,3′,4′,5,5′,7-Hexahydroxy-flavane-3-gallate, (−)-cis-2-(3,4,5-Trihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol 3-gallate), a major polyphenol naturally occurring in green tea (*Camelia sinensis*), is widely known for its antioxidant properties (6). One of the likely methods to ameliorate the deleterious consequences of paraquat poisoning could be the consumption of green tea because of its antioxidant effect. The present study was therefore designed to investigate the protective effect of EGCG on paraquat-induced haemolysis of erythrocyte membrane using the osmotic fragility test technique. The osmotic fragility test is used to assess membrane stability and functionality (7). The test is based on the principle of movement of water down its concentration gradient into erythrocytes placed in hypotonic saline causing lysis and liberation of haemoglobin.

SUBJECTS AND METHODS

Epigallocatechin-3-gallate (CAS: 989-51-5) of analytical grade (> 95%) was obtained from Sigma Aldrich Company (St Louis, MO, USA). Gramoxone® (paraquat dichloride) was obtained from Syngenta (Basel, Switzerland). Working paraquat solutions were prepared from a paraquat dichloride stock solution of 200 g/L by diluting in phosphate buffered saline (PBS) to give incubating concentrations of 0.5, 5.0 and 10.0 mg/mL, respectively. A stock solution of EGCG (72.26 mg/mL) was prepared in PBS to give incubating concentrations of 0.03, 0.3, 3.0 and 30.0 mg/mL, respectively.

Ten healthy adult female student volunteers were selected from The University of the West Indies, Mona campus, using the convenience sampling method. The subjects were recruited after obtaining their informed consent and confidentiality was maintained by assigning a code to each subject. Persons having blood transfusions within the last three months, with diseases known to affect osmotic fragility such as sickle cell disease or on medication were excluded from the study.

The study was approved by the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences Ethics Committee. The experiments were performed in accordance with International guidelines of the Helsinki declaration.

Blood (3 mL) was drawn from the antecubital vein of each subject using a 21-gauge needle into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. Samples were processed within one hour of collection.

Percentage haemolysis with different paraquat concentrations

Whole blood (50 µl) was incubated in 5 mL of PBS with paraquat concentrations of 0.5, 5.0 and 10 mg/mL, respectively for 10 minutes. An equal volume of blood in distilled water served as the control for each sample. Reagent blanks of paraquat in PBS were prepared for each concentration. The paraquat concentration causing approximately 100% haemolysis was determined. All tests were done in duplicates and the average taken.

Percentage haemolysis of different concentrations of EGCG on paraquat-induced haemolysis

Whole blood (50 µl) was incubated in 5 mL of PBS with EGCG concentrations of 0.03, 0.3, 3.0 and 30 mg/mL, respectively for 10 minutes. After this, 0.5 mL of 5.0 mg/mL paraquat was added to each sample followed by another 10-minute incubation period. An equal volume of blood in distilled water served as the control for each sample. The percentage haemolysis was calculated using the modified method of Anaba *et al* (8). Absorbance was measured at a wavelength of 540 nm using the Cecil CE 9050 UV spectrophotometer version R0039. The percentage haemolysis was calculated from the equation below:

% Haemolysis = [(Absorbance of sample – Absorbance of control)/ Absorbance of control] x 100

Statistical analysis

Data were represented as the mean ± standard error of the mean and analysed using SPSS version 12 for Student's *t*-test and analysis of variance (ANOVA). Statistical significance was taken at $p < 0.05$.

RESULTS

The effect of different concentrations of paraquat on erythrocyte haemolysis

A haemolysis of -11% was observed at 0.5 mg/mL paraquat which increased significantly ($p < 0.05$) to 119.62% at 5 mg/mL of paraquat. At 10 mg/mL paraquat, a further significant increase ($p < 0.01$) of 403.05% haemolysis was observed (Fig. 1). This very high level of haemolysis was deemed unsuitable for our study and was therefore not used. The concentration of 5 mg/mL of paraquat was chosen for further studies as it was close to 100%.

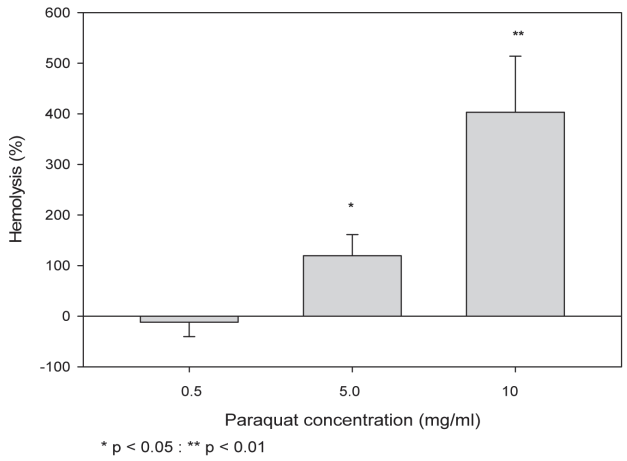

Fig. 1: The effect of various concentrations of paraquat on erythrocyte haemolysis.

The effect of different concentrations of EGCG on paraquat-induced haemolysis

The addition of 0.03, 0.3 and 3.0 mg/mL of EGCG, respectively to blood samples incubated with 5 mg/mL paraquat showed positive haemolysis values. However, a significant ($p < 0.05$) negative decrease in haemolysis was observed with the 30 mg/mL EGCG (Fig. 2).

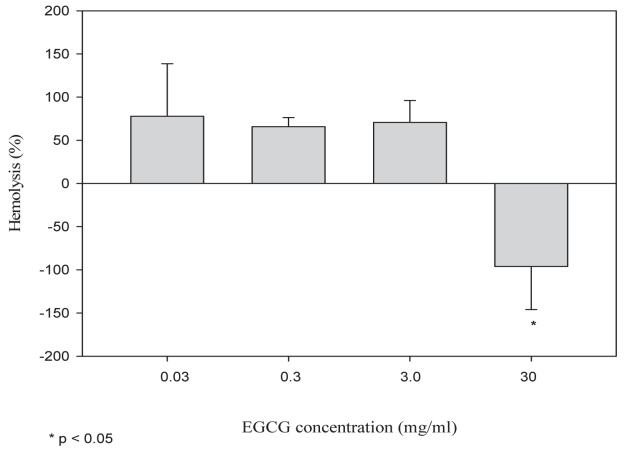

Fig. 2: The protective effect of different concentrations of epigallocatechin-3-gallate (EGCG) on paraquat-induced haemolysis.

DISCUSSION

The results of the present study showed that EGCG (30 mg/mL) had a protective effect on the haemolysis of erythrocytes incubated in 5 mg/mL of paraquat. This is in agreement with previous reports which showed that EGCG has a protective effect against oxidant stress induced by free radicals on erythrocyte membrane (9, 10). Higuchi *et al* (11) had proposed that EGCG reduces the effect of paraquat-induced oxidative stress by chelation of ferrous iron and radical scavenging. In a related study, guinea pigs given EGCG (30 mg/kg/day for 28 days) showed an improvement in age-induced haemorheological abnormalities (12).

Paraquat-induced haemolysis could also explain the hypoxia observed in paraquat poisoning. The release of haemoglobin and its oxidation by free radicals reduces the amount of oxygen delivered to tissues. The critical aspect of paraquat toxicity is death resulting from respiratory distress and fibrosis of the lung (3). The initial treatment for respiratory distress is the administration of oxygen. However, this can further aggravate the condition due to the generation of additional free radicals. Increased green tea consumption resulting in elevated blood levels of EGCG could be advantageous in offering some protection against haemolysis that could result in hypoxia due to paraquat poisoning; hence, its usefulness in ameliorating the hypoxic effects of paraquat in cases of intentional or accidental ingestion.

In the present study, only females were used to eliminate the bias due to the likely presence of glucose-6-phosphate dehydrogenase (G6PD) deficiency. This is an X-linked enzyme which could affect the ability of the erythrocyte to withstand lysis (13).

In conclusion, this study showed that erythrocytes incubated in 30 mg/mL EGCG exhibited significantly reduced haemolysis when challenged with 5 mg/mL of paraquat. The implication of this is that EGCG could be useful in ameliorating the haemolytic effect of paraquat toxicity.

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