Current Topics in Biophysics 2000, 24(2), 241–244

INHIBITION BY GENISTEIN-8-C-GLYCOSIDE OF SOME OXIDATIVE PROCESSES IN LIVER MICROSOMES AND ERYTHROCYTES

LEU B. ZAVODNIK*, ALINA P. SHKODICH, VLADIMIR A. OVCHINNIKOV, VYACHESLAV U. BUKO

Laboratory of Hepatology, Institute of Biochemistry, Academy of Sciences of Belarus, BLK-50, 230017, Grodno, Belarus

The purpose of this investigation was to reveal biochemical and antioxidant activity of genistein-8-C-glycoside (G8CG) a flavonoid isolated from flowers of *Lupinus luteus*. Oxidative stress in human red blood cells (RBC) and liver microsomal membranes has been induced by *in vitro* treatment with *tert*-butyl hydroperoxide (*t*BHP) or hypochlorous acid. G8CG develops a clear-cut antioxidant effect in liver homogenates and microsomes, preventing the destruction of cytochrome P-450 and its conversion to an inactive form cytochrome P-420. The pretreatment of RBC with G8CG (30 minutes before tBHP addition; 1, 3 and 5 mM antioxidant) decreased the level of TBARS by 37, 44 and 49% respectively. G8CG in the concentration range of 0.5–2 mM effectively inhibited HOCl-induced haemolysis to the same extent as did well known HOCl scavengers, taurine and reduced glutathione, but was less effective in the protection of intracellular GSH.

INTRODUCTION

Flavonoids are found in plants, vegetables, and flowers in a bewildering display of biosynthetic prowess, while isoflavones such as genistein was found in just a few botanical families. As a result, isoflavones are part of modern diet (Jones, Prise & Fenwick, 1989). Genistein and these glycoside conjugates are present at high concentrations (up to 3 mg/g) in soybeans (Coward, Barnes, Setchell & Barnes, 1993). A number of studies show a protective effect of flavonoids against cancer, toxic liver injury and cardiovascular diseases (Hollman & Katan, 1999). Being a phytoestrogen, genistein has recently generated interest as a potential anticancer and antiatherogenic agent (Adlercreutz, 1990). Several flavonoids are known as antioxidants and scavengers of free oxygen radicals (Bors, Heller, Michel & Saran, 1990). The antiatherogenic effect of isoflavons is associated with inhibition of myeloperoxidase in phagocytic cells producing hypochlorite, a highly reactive chlorinated species (Adlercreutz, 1998).

The purpose of this work was to investigate the glycosylated derivative of genistein, genistein-8-C-glycoside (G8CG), as a potential antioxidant and a scavenger of active chlorine. We also tried to evaluate a possible effect of G8CG on the oxidative damage of human red blood cells (RBC) caused by *t*-butyl hydroperoxide (*t*BHP) or hypochloric acid (HClO).

MATERIALS AND METHODS

G8CG was isolated from flowers of lupine (*Lupinus luteus L.*) according to the method developed by Laman and Volynetz (1974). Isoflavon was subsequently extracted wiht methanol, ethyl acetate, *n*-butanol and purified using a chromatographic column (3 × 80 cm) packed with polyamine. G8CG was eluted in ethanol-water gradient.

Blood from healthy donors was purchased from the Central Blood Bank of Grodno. Blood was taken into 3% sodium citrate. After removing plasma and the leukocyte layer, erythrocytes were washed three times with cold (4°C) phosphate buffered saline (PBS: 0.15 M NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4). Erythrocytes were used immediately after isolation. Rat liver homogenates were prepared in 1.15% KCl

*Author for correspondence: Zavodnik L. B. Laboratory of Hepatology, Institute of Biochemistry, Academy of Sciences of Belarus, BLK-50, 230017, Grodno, Belarus; e-mail: val@biochem.unibel.by

(1:3; v/v). The rat liver microsomal fraction was separated by differential centrifugation at 105 000 *g* using a VAC-602 centrifuge (Germany).

Lipid peroxidation in RBC, liver homogenates and microsomes was induced by 2 mM *t*BHP.

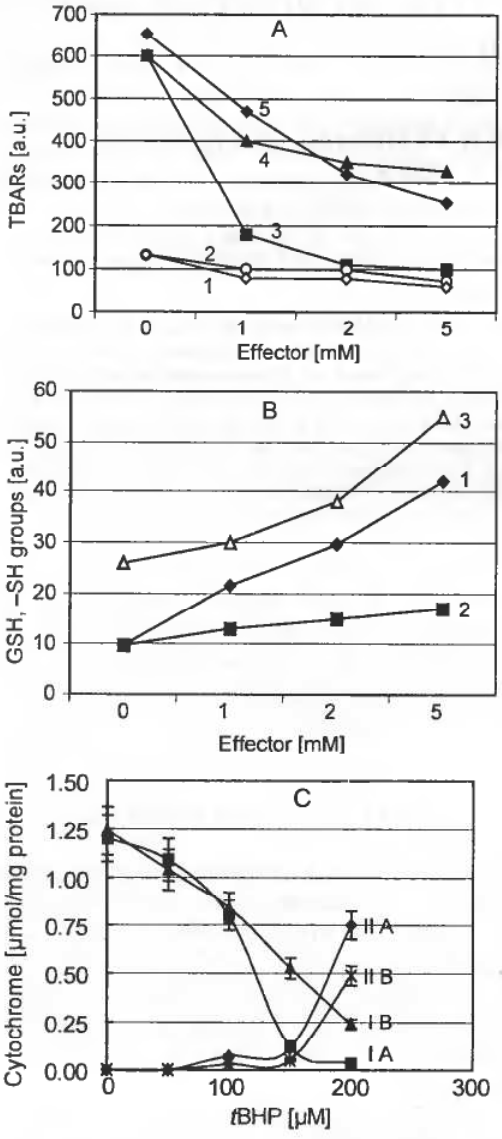

Fig. 1. A. Effects of G8CG and quercetin on TBARS formation induced by *t*BHP (2 mM): 1 – G8CG, 2 – quercetin, liver homogenates; 3 – quercetin; 4 – G8CG, RBC; 5 – G8CG, microsomes. B. Effects of G8CG (1) and quercetin (2) on GSH content in RBC treated with 2 mM *t*BHP and effect of G8CG on microsomal protein SH-groups after microsomal membrane treatment with 2 mM *t*BHP (3). C. Inactivation of CYP-450 by microsomal membrane treatment with *t*BHP in the absence (IA and IIA) and in the presence of 125 µM G8CG (IB and IIB). IA and IIB – the level of CYP-450; IIA and IIB – the level of CYP-420

Suspensions of RBC in PBS (haematocrit 10 %) were treated with different concentrations of hypochlorous acid at 22°C for 10 min. Then the cells were washed 3 times with excess of cold PBS and resuspended in PBS (haematocrit 10 %). HOCl was added as 25 mM stock solution of NaOCl in PBS to the cell suspension. The concentration of OCl^- was determined spectrophotometrically (Vissers & Winterbourn, 1995) using an absorbance coefficient of 350 $M^{-1}cm^{-1}$ (292 nm) at pH 9.0.

The susceptibility of erythrocytes to oxidative damage was measured in terms of the apparent rate constant of cell haemolysis (posthaemolysis), the accumulation of thiobarbituric-reactive species (TBARS), and the oxidation of intracellular oxyhaemoglobin (oxyHb) and GSH. The process of haemolysis of erythrocytes treated with oxidants was monitored by haemoglobin (Hb) release. Cytochrome P-450 (CYP-450) content and cytochrome P-420 (CYP-420) formation in liver microsomes was determined according to Omura and Sato (1964). TBARS were determined in the acid-soluble fraction of RBC suspension (Stocks & Dormandy, 1971). GSH concentration was measured by the method of Ellman using the absorbance coefficient of 13.6 $mM^{-1}cm^{-1}$ (412 nm) (Ellman, 1959).

G8CG was added at concentrations of 0.5, 1.0, 2.0 and 5.0 mM, and samples were preincubated at 22°C for 30 min. After the preincubation with G8CG either *t*BHP or HOCl were added and the measurements were carried out after 20 min. Flavonoid quercetin in the experiment with *t*BHP was used as comparative agents at the same concentrations.

RESULTS AND DISCUSSION

Effect of G8CG on oxidative damage caused by tBHP

As illustrated in Fig. 1A and 1B, when 2 mM *t*BHP was incubated in RBC suspension, TBARS formation was dramatically increased nearly 20-fold and GSH completely disappeared. In the oresence of the oxidant, 69% of Hb was oxidized to metHb. The flavonoids, quercetin and G8CG, inhibited dose-dependently TBARS production after preincubation with RBC. In this situation the effect of quercetin was especially pronounced. Neither G8CG nor quercetin developed a protective action against the Hb oxidation and GSH depletion.

The incubation of 2 mM *t*BHP in liver homogenates led to an increase of TBARS by 30%

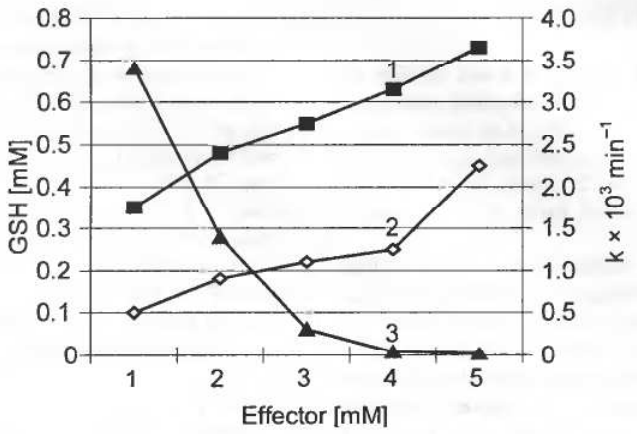

Fig. 2. Effect of G8CG on GSH content in RBC incubated with 0.5 mM (1) and 1.0 mM (2) of NaOCl and on the rate constant of RBC haemolysis, k (3) induced by 1 mM NaOCl

and the decrease of GSH content by 90% (Fig. 1A, 1B). Both quercetin and G8CG prevented lipid peroxidation, the effect of quercetin being especially marked. The protective effect of G8CG on the GSH depletion was dose-dependent and more pronounced compared to quercetin.

Similar effects of the oxidant and G8CG were observed in rat liver microsomes. The addition of 2 mM *t*BHP caused a more than 5-fold increase in the TBARS content and the amount of free protein SH-groups was lowered by 74%. G8CG was more effective in preventing GSH depletion, whereas its TBARS-decreasing effect was less pronounced (Fig. 1A, 1B).

The treatment of rat liver microsomes with 150 –200 μM *t*BHP caused a decrease of CYP-450 content with a simultaneous appearance of the inactive form, CYP-420 (Fig. 1C). The pretreatment with 125 μM G8CG prevented the inactivation of CYP-450 caused by the oxidant.

RBC damage induced by HOCl and its prevention by G8CG

RBC treated with HOCl underwent time-dependent lysis developing both in the presence of the oxidant (haemolysis) and after its removing (posthaemolysis). The apparent rate constant of posthaemolysis was a linear function of the oxidant concentration but the dependence of the apparent rate constant of haemolysis on the HOCl concentration was nonlinear. G8CG at concentrations of 0.5–2 mM effectively inhibited HOCl-induced RBC haemolysis (Fig. 2) and at concentrations of 1–2 mM completely protected RBC against the lytic effect of HOCl. The formation of chloramines was observed at the concentrations of HOCl of 0.2 mM and higher. The correlation between the membrane-bound chloramine contents and the rate constant of cell haemolysis suggests a key role of the formed chloramines in the HOCl-induced RBC damage. The inhibition of this damage by G8CG allows us to propose that the isoflavon acts as a scavenger of active chlorine forms, such as chloramines.

HOCl dramatically decreased the concentration of intracellular GSH (Fig. 2). However, we did not found an effect of the oxidant on the oxyHb oxidation and lipid peroxidation evaluated as TBARS formation. G8CG quite effectively prevented GSH depletion caused by the oxidant.

In conclusion, the data obtained suggest the efficiency of G8CG to support the antioxidant defense system in different tissues (blood, liver) via its protective effect against GSH depletion. However, the inhibition of lipid peroxidation by G8CG was not so significant. We believe that the protection by G8CG against the RBC oxidative damage caused by HOCl is connected not only with the prevention of GSH depletion, but is also due to trapping of the active form of chlorine.

As a result, G8CG corrects the RBC and microsomal membrane damage induced by HOCl and *t*BHP. The observed antioxidative effect of G8CG in these model systems allows to suggest it to be a cell protector in pathologies accompanied by activation of lipid peroxidation and some other oxidative processes.

Acknowledgements

This work was supported by the Belarussian Foundation of Basic Research (grant B98-141).

REFERENCES

Adlercreutz H. (1990). Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. *Scand. J. Clin.. Lab. Invest.*, **201**, 3.

Adlercreutz H. (1998). Lignans and phytoestrogens. Possible prevention role in cancer, [In:] *Frontiers of Gastrointestinal Research.* Rosen P. (ed.) Karger S., Basel.

Bors W., Heller W., Michel C. & Saran M. (1990). Flavonoids and antioxidant: determination of radical-scavanging efficiensis. *Methods Enzymol.*, **180**, 343.

Coward L., Barnes N. C., Setchell K. D. R. & Barnes S. (1993). Genietein, daidzein and their glicoside conjugates: antitumor isoflavones in soybean foods from american and asian diets. *J. Agric. Food. Chem.*, **41**, 1961–1967.

Ellman G. L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, **82**, 70.

Jones A. E., Prise K. R. & Fenwick G. R. (1989). Development of a high performanse liquid chromatographic method for the analysis of phytoestrogens. *J. Sci. Food Agric.*, **46**, 357–364.

Hollman P. C. H. & Katan M. B. (1999). Health effect and bioavailability of dietary flavonols. *Free Radic. Res.*, **31**, 575.

Laman N. A. & Volynets A. P. (1974). Flavonoids in ontogenesis of *Lupinus Luteus* L. *Plant Physiol.*, **21**, 737 (in Russian).

Omura T. & Sato R. (1964). The carbon monoxide binding pigment in liver microsomes. II. Solubilization, purification and properties. *J. Biol. Chem.*, **239**, 2379.

Stocks J. & Dormandy T. L. (1971). The autooxidation of human red cell lipids by hydrogen peroxide. *Br. J. Haematol.*, **20**, 95.

Vissers M. C. M. & Winterbourn C. C. (1995). Oxidation of intracellular glutathione after exposure of human red blood cells to hypochlorous acid. *Biochem. J.*, **307**, 57.