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A comparative study on the radioprotective potential of the polyphenolic glycoconjugates from medicinal plants of *Rosaceae* and *Asteraceae* families versus their aglycones

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ABSTRACT

Radioprotective potential of the polyphenolic glycoconjugates, isolated from flowers of *Sanguisorba officinalis* L. (So) and *Erigeron canadensis* L. (Ec), and from leaves of *Fragaria vesca* L. (Fv) and *Rubus plicatus* Whe. Et N. E. (Rp) as well as their aglycones (SoA, EcA, FvA and RpA, respectively), against γ-radiation-induced lipid peroxidation in human plasma and DNA damage in lymphocytes, were investigated in vitro. These properties were assessed by measuring the concentration of thiobarbituric acid reactive substances (TBARS) and using the alkaline comet assay, and were compared to the protective effects of rutin (R) and quercetin (Q). Cytotoxicity of the glycoconjugates/aglycones towards L929 mouse fibroblasts and human lymphocytes were also measured. Plant products from *S. officinalis*, similar to Q, were able to reduce the most radiation-induced lipid peroxidation as well as DNA damage and extent of oxidative damage to the DNA basis. Contrary to the pure flavonoids, where Q was shown to be significantly more effective than its glycoside R, the results did not show more benefit with application of SoA/EcA over So/Ec in terms of lipid peroxidation inhibition. Moreover, glycoconjugates Ec and So showed much higher capacity in protecting lymphocytes against radiation-induced genotoxicity which may suggest that between the polyphenolic and polysaccharide parts exist some synergistic effects. There were no significant differences between Fv versus FvA or Rp versus RpA in terms of the provided radioprotection. Summarizing, plant glycoconjugates isolated by the multi-step method offered sufficient radioprotection. In addition, they possess many advantages, compared to the synthetic polyphenolic compounds or the plant extracts, such as water-solubility and minor toxicity.

1. Introduction

Ionizing radiation (IR) mediates its toxic effects through the direct energy deposition to the target cellular molecule (DNA, proteins, lipids, saccharides) and through the interaction of the produced reactive oxygen and nitrogen species (ROS/RNS) with these biological molecules. Cell death can occur in consequence of the DNA and membrane damage. Plant phenolic compounds, such as anthocyanins, flavonoids, stilbenes, phenolic acids, tannins, etc., can greatly alleviate the effects of IR at the molecular, cellular and tissue levels [1,2].

Flavonoids exert strong antioxidant and radioprotective properties due to the structure of the polyphenol rings and the presence of several hydroxyl (−OH) groups [3]. In plant tissues flavonoids can occur both in the form of the glycosylated derivatives and as the aglycones containing no carbohydrate moieties. The glycon part may contain monosaccharides such as glucose, rhamnose, galactose, apiose or arabinose that might be linked with the aglycone part by the *O*-glycoside bond, usually at C3, but also at the positions of C7, C5, C3′, C4′ and C5′ of the flavonoid structure, blocking the available hydroxyl groups. Also known are *C*-glycoside derivatives with saccharide residues at the positions of C8 or C2 and C6. Flavonoids are reported to scavenge numerous free radicals including these generated by IR [1,3]. In the radical scavenging reaction a hydrogen atom is donated from the flavonoid to the unstable radical, which results in formation of more

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stable flavonoid-O· radical. Several in vitro studies have indicated that aglycones owing the increased accessibility of free −OH groups seem to be better antioxidants than their glycosides [3,4], but the opposite data as well as the pro-oxidative action of the aglycones at high concentrations have also been reported [5].

We have recently found that the polyphenolic-polysaccharide conjugates isolated from the selected medicinal plants of *Rosaceae* family, such as *Sanguisorba officinalis* L. (from flower), *Rubus plicatus* Whe. et N. E. (from leaf) and *Fragaria vesca* L. (from leaf), as well as of *Asteraceae* family - from *Erigeron canadensis* L. (from flower), had the ability to ameliorate γ-radiation-induced protein and lipid damage in human plasma [6]. All the compounds received from the four different plant materials were previously characterized from the chemical point of view [7]. These glycoconjugates were isolated from the dried plant materials according to the previously described multi-step method [7], with 1–2wt% yield and they were found to be the macromolecular compounds. The glycoconjugates are composed of the pectin-like polysaccharide parts and of the branched polyphenolic macromolecules, built of different polyphenolic monomers, mostly linked by C−C bonds very difficult to be hydrolyzed [7]. These polysaccharide parts are responsible for the solubility of the glycoconjugates in water conditions and for their polyelectrolyte properties because of the presence of hexuronic acids. The polyphenolic parts are believed to be responsible for the antioxidant and radioprotective potential of the glycoconjugates [6]. However, it has never been verified whether the polysaccharide fragments may also contribute to the beneficial effects of the whole glycoconjugates against γ-rays rather than the advantage of water-solubility results in a diminished radioprotective activity.

The aim of the study was to evaluate the radioprotective potential of the polyphenolic-polysaccharide conjugates as well as their aglycones, isolated from four selected medicinal plants: *E. canadensis*, *S. officinalis*, *R. plicatus* and *F. vesca*, against γ-radiation-induced lipid peroxidation and DNA damage in human plasma and lymphocytes, respectively. These properties were assessed by the methods based on measuring the concentration of thiobarbituric acid reactive substances (TBARS) in plasma and using the alkaline comet assay, and were compared to the protective effects of rutin (**R**) and quercetin (**Q**). In addition, the ABTS radical cation scavenging ability and cytotoxicity of the conjugates and aglycones towards L929 mouse fibroblasts and human lymphocytes were measured.

2. Materials and Methods

2.1. Chemicals and Reagents

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), quercetin (> 98% purity; HPLC), rutin trihydrate (> 95% purity; HPLC), dimethylsulfoxide (DMSO), potassium persulfate, Histopaque-1077, RPMI 1640 medium without glutamine, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), trifluoroacetic acid (TFA), low-melting-point (LMP) and normal-melting-point (NMP) agarose, ethylenediaminetetraacetic acid (EDTA), Triton X-100 and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Trypan blue, Dulbecco's modified Eagle Medium (DMEM) with low glucose and with phenol red, DMEM with low glucose and without phenol red, heat inactivated fetal bovine serum (FBS), antibiotics Pen-Strep (10,000 U/mL penicillin and 10,000 μg/mL streptomycin) and L-glutamine were obtained from Life Technologies (Waltham, MA USA). Human 8-oxoguanine DNA glycosylase (hOGG1), purified BSA 100 × and NEBuffer were from New England Biolabs® (UK)·The LDH Cytotoxicity Detection Kit (TaKaRa) was from TaKaRa-Clontech (Mountain View, CA USA). Other chemicals, all of the analytical grade, were obtained from Chempur, Piekary Slaskie, Poland.

2.2. Plant Materials and Isolation of the Plant Glycoconjugates/Aglycones

Dry flowering parts of *Sanguisorba officinalis* L. (voucher No. 004990) and *Erigeron canadensis* (L.) Cronq. (synonymus *Conyza canadensis* or *Leptilon canadense*) (voucher No. 019361) were collected from natural positions in Lower Silesia, Poland. Leaves of *Fragaria vesca* L. (voucher No. 004954) and *Rubus plicatus* Whe. et N. E. (voucher No. 013914) were purchased from the local market (Wrocław, Poland; the producer Plant Herb KAWON - HURT Nowak sp.j., Gostyn, Poland). The identity of the plants was certified by Prof. Krystyna D. Kromer and MSc Jolanta Kochanowska from Botanical Garden of Wrocław University, Wrocław, Poland, and the herbarium voucher specimens have been deposited in the Botanical Garden of Wrocław, Wrocław University, Wrocław, Poland.

The polyphenolic-polysaccharide conjugates were isolated from the plant materials in the Department of Organic and Pharmaceutical Technology (Faculty of Chemistry, Wrocław University of Science and Technology) by the procedure previously described [6–9], to receive the *S. officinalis* preparation (**So**), the *E. canadensis* preparation (**Ec**), the *F. vesca* preparation (**Fv**) and the *R. plicatus* preparation (**Rp**), respectively. To obtain the aglycone parts of the plant products each of them was hydrolyzed with 2 M TFA for 5 h, at 120 °C. Each time after hydrolysis the resulting dark brown precipitate was separated from the solution by centrifugation (2000 × *g*; 15 min), washed 5-fold with distilled water, and finally well dried to receive the *S. officinalis* aglycone (**SoA**), the *E. canadensis* aglycone (**EcA**), the *F. vesca* aglycone (**FvA**) and the *R. plicatus* aglycone (**RpA**), respectively.

2.3. Lymphocyte Isolation, Plasma and Cell Treatment

Units of fresh frozen plasma (FFP) and whole blood (WB) were purchased from the Regional Center for Transfusion Medicine in Lodz (Poland). Blood from single healthy adult donors (aged 18–60 years of both sexes), who passed a routine selection criteria and before donation were not exposed to oxidative stress due to cigarette smoking, diet or drug therapy, was collected in citrate-phosphate-dextrose (CPD) anticoagulant preservative solution. This study was approved by the Local Ethics Committee (no KBBN-UŁ/II/4/2013).

Lymphocytes were isolated from WB by centrifugation in a density gradient of Histopaque-1077. Briefly, blood diluted with 10 mM phosphate buffered saline, pH 7.4 (PBS) (1:1) was layered on Histopaque with ratio blood: PBS at 4:3 and centrifuged (540 × *g*, 30 min, 25 °C). The peripheral blood mononuclear cell (lymphocyte)-rich layer was collected; cells were washed twice with PBS and re-suspended in RPMI 1640 medium to give 1×10^6 cells/mL. The viability of lymphocytes, measured by trypan blue exclusion staining, was approximately 99 ± 5%.

Stock solutions (5 mg/mL) of the plant glycoconjugates, their aglycones and the reference polyphenols (**Q**, **R**) were prepared in 50% DMSO and added to plasma or lymphocytes 1 h prior irradiation (DMSO final concentration ≤ 0.25%). Samples (5 mL) of undiluted plasma (for lipid peroxidation measurements) or lymphocyte suspensions (for the comet assay), with (or without) the plant compounds, were irradiated at 20 °C ± 1 °C, to a total dose of 100 and 200 Gy or 15 Gy, respectively. Irradiation was performed at the Institute of Applied Radiation Chemistry (Lodz University of Technology, Lodz, Poland) using a ^{60}Co source (dose rate 0.392 ± 0.027 Gy/min for first category Irradiator BK-1000 (ZZUJ Polon, Poland) and 430 Gy/h for Panoramic Irradiator OB-Servo-D (IZOTOP, Hungary)). Activities of the Cobalt irradiators were 40.4 Ci and 39.95 kCi, respectively. The alanine dosimeters ES200-2106 (Bruker, Poznan, Poland) and radiochromic film dosimeters B3000 (Gex Corporation, CO, USA) for dosimetry were used. Non-irradiated appropriate plasma or lymphocyte samples, served as controls. Plasma samples were stored at − 20 °C until analyzed.