ORIGINAL ARTICLE

ANTIPROLIFERATIVE AND ANTIMICROBIAL PROPERTIES OF PURE AND ENCAPSULATED RUTIN

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Abstract

Rutin, a polyphenolic natural compound is described in the literature for a wide range of biological activities. The study aims to test the antiproliferative potential and the antibacterial effect for pure rutin and its β cyclodextrin complexes. On one hand, the antiproliferative assays against A2780 (human ovarian cells), MDA-MB-231 (human breast cancer cells) and SiHa (cervical cancer cells) did not show any substantial inhibition of cell growth. On the other hand, the antimicrobial screening revealed that rutin presents positive effects against: *Streptococcus pyogenes*, *Enterococcus faecali*s, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. A decreased, but detectable, activity was noticed for *Staphylococcus aureus* and *Streptococcus pneumoniae*. Incorporation in the ramified cyclodextrins had a mixed effect on the antibacterial activity, with increased, constant or decreased effect, depending on the strain. The study concludes that rutin can be re-considerate as a natural active antibacterial agent, while modulation of its solubility by incorporation in β-cyclodextrins is debatable depending on the tested strain.

Rezumat

Rutozida este un compus natural cu structură de tip polifenol, descris în literatură pentru o gamă largă de activități biologice. Studiul present și-a propus să testeze potențialul antiproliferativ și efectul antibacterian pentru rutozida pură, respectiv după încorporare în trei tipuri de β ciclodextrine. Pe de o parte testele antiproliferative pe liniile celulare A2780 (celule de cancer ovarin), MDA-MB-231 (celule de cancer de sân) și SiHa (celule de cancer de de col uterin) nu au evidențiat nici o inhibare substanțială a creșterii celulare.Pe de altă parte evaluarea antimicrobiană arată că rutozida a prezentat un efect pozitiv împotriva următoarelor tulpini: *Streptococcus pyogenes*, *Enterococcus faecali*s, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* și *Escherichia coli*. O activitate scăzută, dar detectabilă, a fost observată pentru *Staphylococcus aureus* și *Streptococcus pneumoniae*. Incorporarea în ciclodextrine ramificate a avut un efect mixt asupra activității antibacteriene, cu efect crescut, constant sau scăzut, în funcție de tulpină. Studiul concluzionează că rutozida poate fi reconsiderată ca agent antibacterian natural. Modularea solubilității prin încorporarea în ciclodextrinele β ramificate este discutabilă în funcție de tulpina testate.

Keywords: rutin, cyclodextrin, antiproliferative, antimicrobial

Introduction

Rutin is a glycosylated polyphenolic phytocompound which is widespread in coloured fruits and vegetables. Medicinal plants with high rutin content include: buckwheat (*Fagopyrum esculentum* Moench) [17], elderberry (*Sambucus nigra* L.) [13], japanese pagoda tree (*Sophora japonica* L) [19] and herb-of-grace (*Ruta graveolens* L.) [22].

The phytocompound was assigned with pleiotropic pharmacological effects (e.g.): capability to increase capillary resistance and decrease capillary permeability [15]; antiplatelet aggregatory effects [9, 10]; important antiinflammatory effects by inhibiting NO, TNF-α

and MPO activity in activated human neutrophils [23] as well the synthesis of pro-inflammatory cytokines, TNF-α and NF-kB in inflammatory bowel disease [35]. Rutin exhibited powerful antioxidant activity by counteracting the reactive oxygen species (ROS) both *in vitro* [43] and *in vivo* [11] and was demonstrated to have an antiproliferative and proapoptotic activity against different cancer cell lines including: GL-15 human glioblastoma, A549 human lung, MCF-7 breast, HepG2 liver and HT-29 colon cancer cells [33, 42, 44]. Finally, rutin has shown antibacterial and antifungal activity as well as synergistic effects against methicillin resistant *S. aureus* (MRSA), when co-administered with classic antibiotics [1]. Moreover, the phyto-compound was able to reduce the toxicity and improve antifungal activity of Amphotericin B when tested against *Cryptococcus sp.* [25]. Despite the multiple pharmacological effects and its therapeutic potential, rutin exhibits a poor water solubility, thus affecting it's *in vivo* bioavailability. For this purpose, many formulation strategies were conducted in order to improve this downside. One of these strategies is represented by encapsulating the flavonoid in cyclodextrins (CDs). CDs are cyclic oligosaccharides made up of α-D-glucopyranoside units, exhibiting a hydrophobic profile on the inside and a hydrophilic one on the outside of the glycosidic ring. Thereby, these features make CDs potential drug delivery systems for hydrophobic molecules in order to increase their bioavailability. Various studies were conducted in order to assess the advantages of CD encapsulation for different types of molecules [12, 36]. In a previous study, several flavonols (fisetin, quercetin and kaempferol) encapsulated in β-CDs showed promising anti-proliferative activity *vs.* native compounds when tested on A2780 human ovarian carcinoma, MDA-MB-231 human breast cancer and SiHa human cervix cancer cells [6]. Other types of molecules, such as nerolidol, a component of essential oils, was investigated after encapsulation in α-CD, β-CD, γ-CD, HP-β-CD, RAMEB (randomly methylated-beta-cyclodextrins), CRYSMEB (methylated beta-cyclo-dextrin) and SBE-β-CD (sulfobutylether-β-cyclodextrin) highlighting their effectiveness as encapsulating agents and solubility enhancers [2]. Also, pentacyclic tri-terpenes such as betulin esters, ursolic and oleanolic acids complexes with CDs showed increased *in vitro* antiproliferative activity when tested on human melanoma cell lines [26, 30, 31]. All these promising results make this formulation strategy a powerful tool in improving the bioavailability and increasing the effectiveness of hydrophobic phytocompounds with important therapeutic potential.

In the present study, our aim was to investigate native rutin compared to its inclusion complex in CDs regarding: i) the antiproliferative activity on three tumour cell lines (A2780 human ovarian cancer, MDA-MB-231 human breast cancer and SiHa cervical cancer) and ii) the antibacterial activity against different bacterial strains (*S. pyogenes*, *E. faecalis*, *B. cereus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*).

Materials and Methods

Reagents

Reagents were purchased as follows: rutin from Sigma Aldrich, Germany, β-cyclodextrin (BCD), hydroxyl-propyl-β-cyclodextrin (HPBCD) and randomly methylated β-cyclodextrin (RAMEB) from Cyclolab, Hungary.

Complexes preparation

For the development of the inclusion complexes the kneading method was used as described in the papers of Danciu *et al*, [6, 7]. All the complexes were prepared using a molar ration 1:2 active substance: CD (Mw_{Rutin} = 610.52 g/mol, Mw_{BCD} = 1134.98 g/mol, Mw_{HPBCD} = 1396 g/mol, Mw_{RAMEB} = 1312 g/mol).

Cell culture

All three gynaecological cancer cell lines, namely: A2780 (human ovarian), MDA-MB-231 (human breast) and SiHa (cervical) (ECACC-European Collection of Cell Cultures, Salisbury, UK) were cultured in minimal essential medium (MEM), supplemented with 10% foetal calf serum (FCS), 1% antibiotic mixture (Pen/Strep), and 1% nonessential aminoacids (NEAA). All media and supplements were obtained from Lonza Ltd. (Basel, Switzerland). The cells were cultured and expanded in standard conditions, at 37°C and 5% CO_2 atmosphere.

MTT assay

Cells where seeded onto a 96-well culture plate at a cellular density of 5,000 cells/well and attached to the bottom of the well during an overnight. After 24 h, 100 µL of new medium containing the tested substances (dissolved in dimethyl sulfoxide - DMSO; purchased from Sigma-Aldrich Ltd., Budapest, Hungary) were added and incubated for 72 h. The cells were then assayed by the addition of 20 µL of 5 mg/mL MTT solution (purchased from Sigma-Aldrich Ltd., Budapest, Hungary). The intact mitochondrial reductase converted MTT into blue formazan crystals during a 4 h contact period and the precipitated crystals were dissolved in 100 µL DMSO. Finally, the reduced MTT was spectro-photometrically analysed at 545 nm, using a Stat Fax microplate reader (Awareness Technology, Palm City, FL, USA); wells with untreated cells were considered as reference for viability. All *in vitro* experiments were carried out on two microplates in quadruplicates for each substance tested and controls. The DMSO concentration (0.3%) of the medium did not have any significant effect on cell proliferation.

In vitro antibacterial activity

Rutin, its BCD complexes and the native CDs were tested for the antimicrobial activity using 11 strains: *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Streptococcus pneumoniae* (ATCC 49619), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Yersinia enterocolitica* (ATCC 23715), *Klebsiella pneumoniae* (ATCC 700603), *Proteus mirabilis* (ATCC 25933), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albican*s (ATCC 10231) by the diffusion method as previously described [27, 29]. The antibacterial and antimycotic activity were determinate according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997). A microbial suspension with a concentration of 10^8 CFU/mL was employed. The compounds were diluted in DMSO at a concentration of 10 mM. The inoculum was dispersed on the Mueller-Hinton agar plates and filter paper discs 6 mm in diameter impregnated with the solution of the tested compounds were distributed on the surface. Paper disks impregnated with BCD, HPBCD and RAMEB were also used. The native CDs did not have any effect on the selected strains. The plates were incubated at 37°C for 24 h. The inhibition zone diameters were determined with a ruler. Triplicate tests were performed and medium values are reported. For positive controls, discs with gentamicin or fluconazole were used, with inhibition zones between 16 - 19 mm.

Results and Discussion

Antiproliferative activity for the selected cell lines A2780, MDA-MB-231 and SiHa showed no substantial grow inhibition when treated with pure rutin and its CDs complexes at 30 μg/mL. In some cases a modest increase in cell viability was evidenced but no practical relevance can be attributed to this phenomenon (Figure 1).

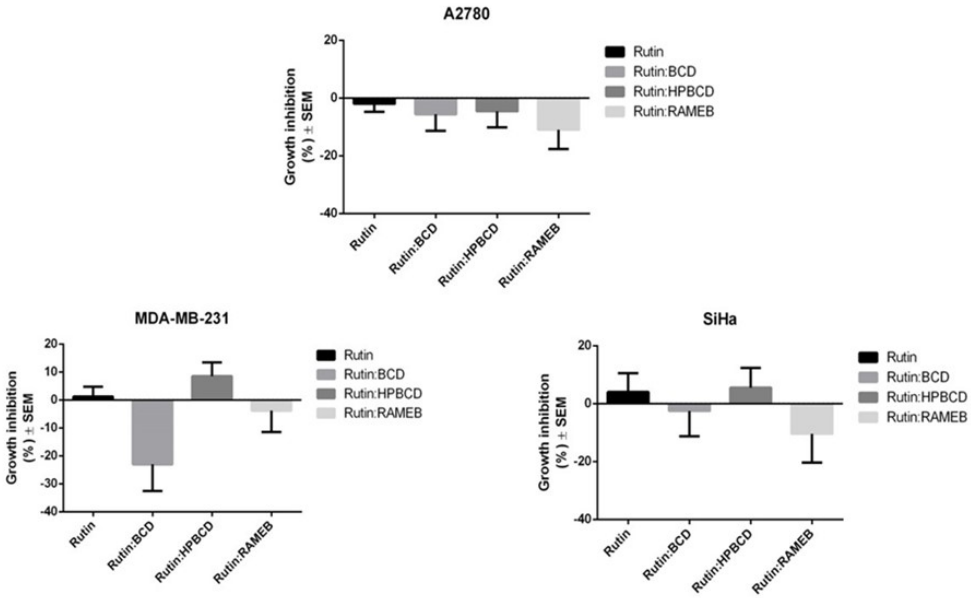

Figure 1.
Antiproliferative activity of rutin and its cyclodextrin inclusion complexes against three tumor cell lines at 30 μg/mL

The antimicrobial screening showed that rutin elicits antibacterial properties on some of the screened strains, namely *S. pyogenes* (19 mm), *E. faecalis* (19 mm), *B. cereus* (16 mm), *P. aeruginosa* (16 mm), *K. pneumoniae* (16 mm) and *E. coli* (16 mm). These strains present comparable inhibition zones with gentamicin, the positive control. A decreased but detectable activity was noticed for *S. aureus* (12 mm) and *S. pneumonia*e (11 mm). Incorporation in the ramified CDs had a mixt effect on the antibacterial activity. For *S. pyogenes* and *E. faecalis,* incorporation in each of the three CDs conduced to a decreased inhibition zone. For *S. pneumoniae*, *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* the activity was constant whereas for *S. aureus* the diameter of the inhibition zone increased from 12 mm for pure rutin to 17 mm for rutin:BCD and rutin:HPBCD, respectively 16 mm for rutin: RAMEB (Table I).

Table I

The antimicrobial activity of rutin and its CDs inclusion complexes (the values are presented as average of three determinations)

Strains	Rutin	Rutin BCD	Rutin HPBCD	Rutin RAMEB
E. coli	16 mm	16 mm	16 mm	16 mm
Klebsiella pneumoniae	16 mm	16 mm	16 mm	16 mm
Proteus mirabilis	6 mm	6 mm	6 mm	6 mm
Yersinia enterocolitica	6 mm	6 mm	6 mm	6 mm
Pseudomonas aeruginosa	16 mm	16 mm	16 mm	16 mm
Bacillus cereus	16 mm	16 mm	16 mm	16 mm
Streptococcus pneumoniae	11 mm	11 mm	11 mm	11 mm
Streptococcus pyogenes	19 mm	13 mm	16 mm	15 mm
Staphylococcus aureus	12 mm	17 mm	17 mm	16 mm
Enterococcus faecalis	19 mm	15 mm	11 mm	12 mm

In terms of natural compounds, flavonoids are one of the most studied class of phytochemicals for cancer chemoprevention [7]. Nano-encapsulation represents a method applied in order to enhance bioactivity and/or bioavailability of biologically active molecules. It was demonstrated that internal water molecules of CDs, are displaced by an increased number of hydrophobic compounds [30]. Ghasemzadeh *et al* evaluating the main bioactive compounds from curry leaf extracts (*Murraya koenigii* L) containing rutin in a concentration of 0.082 mg/g DW as *per* HPLC determination have shown that the extract presents antiproliferative properties against MDA-MB-231 breast cancer cells. The biological effect was assigned to the phytocomplex [10]. It is very well known that rutin is the glycoside of quercetin, and although to the best of our knowledge rutin was not reported in the literature as an active agent on breast, ovarian and cervical cancer cells, quercetin was [32, 38, 40]. Correlated to our results, namely stimulation of proliferation for A2780 cell line, Luo *et al* have described that rutin hydrate stimulates the proliferation of OVCAR-3 ovarian cancer cells [20]. Nevertheless previous studies regarding the *in vitro* and *in vivo* anticancer properties of rutin depict that also this glycoside, not only the aglycone quercetin, acts as an active agent on other cancer cell lines hence our idea to screen its effect on A2780, MDA-MB-231 and SiHa cancer cell lines. Antiproliferative effects were also observed in case of HTC hepatic cells, WEHI-3 murine leukaemia cells, SW480 human colon adenocarcinoma cells and recent papers published informations about its protective effect against induced oxidative stress and apoptosis in human lens epithelial cells as well as against the pro-carcinogenic agent benzo(a)pyrene [5, 18, 46]. Regarding the mechanism of action, rutin acts to various modulators of Wnt signalling. For example, the DNA doxorubicin induced damage was highly diminished when rutin was incubated with HepG2 human liver carcinoma cell line but some studies showed that rutin caused DNA damage on BRCA mutant breast cancer cells [21]. Chemo-preventive effects were detailed in case of animal model of colon cancer and other *in vivo* studies have shown the important effects of rutin in diminishing tumour growth in mice implanted with SW480 human colon adenocarcinoma, metastatic A549 human lung adenocarcinoma, LLC Lewis lung carcinoma and HL-60 human promyelocytic leukaemia [16, 43]. Although active on above mentioned cell lines, rutin showed an overall poor antiproliferative activity against cell lines tested in the present study A2780, MDA-MB-231 and SiHa. Increased dissolution and absorption of rutin may be achieved by complexation with CDs. Paczkowska *et al* have demonstrated that inclusion of rutin in CDs leads to increased chemical solubility, stability, dissolvability and antibacterial activity for some of the selected strains [28]. The antiproliferative effects of rutin proved to be unsubstantial on the selected cell lines which were not changed when it was applied as CD complexes (Figure 1). Some studies have described that the inclusion complex of rutin with HPBCD have been identified to have the highest stabilizing effect in aqueous solutions [24, 37]. The antimicrobial activity of rutin obtained as a methanolic extract from *Pteris vittata* L. was screened against eight intestinal microorganisms by the group of Singh *et al*. Disk diffusion and micro-dilution methods showed efficiency for *P. aeruginosa* and *K. pneumoniae* [34]. Also, aqueous extracts containing rutin from *Castanea sativa* Mill. leaves where analysed in terms of anti-microbial activity against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *Proteus vulgaris*, *E. coli*, *Enterobacter cloacae*, *Salmonella typhi* and *Enterobacter aerogens*. Minimum inhibitory concentrations where detected between 32 and 128 µg/mL [3]. In a similar study, Dubey *et al*, assessed the antimicrobial activity of rutin using concentrations ranging from 10 µg/mL to 0.05 µg/mL and reported positive results for *S. aureus*, *E. coli, Klebsiella oxytoca*, *P. aeruginosa* and *Candida albicans* [8]. As it can be seen from Table I the results of our screening corroborate with the ones presented by other studies in the field. The novelty that comes with this study is the analyse of

the antimicrobial activity after incorporation in CDs. Wang *et al* have showed an increased antibacterial potency for miconazole after incorporation into a cellulosic fabric grafted with BCD [41]. Sun *et al* have shown that chitosan films containing BCD and essential oils have increased the anti-infective properties of the chitosan films, while Iacovino *et al* have concluded that the antibacterial activity of pipemidic acid is increased for some of the tested strains after incorporation in BCD [14, 39]. In a recent study, Corciova *et al*. proven that complexation of hesperidin with BCD led to higher antibacterial and antioxidant effect [4]. On the other hand, Zhao *et al,* analysing BCD inclusion complex of chlorogenic acid, concluded that in terms of antioxidant and antibacterial capacity no significant difference was observed between the pure substance and the inclusion complex [45]. As depicted in the results section, in this study the majority of the tested strains acted in the same manner after incorporation took place. Only in case of *S. aureus* the potency was increased.

Conclusions

Rutin can be re-considerate as a natural active antibacterial agent against *S. pyogenes*, *E. faecalis*, *B. cereus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*. Modulation of the solubility by incorporation in CDs is debatable depending on the tested strain. On the other hand, both the glycoside and its CDs inclusion complexes elicited no substantial antiproliferative effect against A2780 human ovarian, MDA-MB-231 human breast and SiHa cervical cancer cell lines at a relatively high final concentration.

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