Is the antioxidant capacity of flavonoids responsible for their anticancer effects? The case of quercetin in leukemia

Gian Luigi Russo

Institute of Food Sciences – CNR - Avellino, Italy

1 – 15 December 2020
Conflict of Interest Disclosure Statement

I declare the absence of any potential or direct conflict of interest, including honoraria or other funding, related to my participation in the CAHD 2020 Virtual Conference.
### Acknowledgments: the lab...

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Maria Grazia Volpe</td>
<td>Institute of Food Sciences CNR Avellino</td>
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<tr>
<td>Virginia Carbone</td>
<td></td>
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<tr>
<td>EcoNutraPrevention’s group</td>
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<tr>
<td>Silvestro Volpe</td>
<td>IEOS CNR Napoli</td>
</tr>
<tr>
<td>Paola Ungaro</td>
<td>S.Giuseppe Moscati Hospital Avellino</td>
</tr>
<tr>
<td>Marc Diederich</td>
<td>LBMCC (Laboratoire de Biologie Moléculaire et Cellulaire du Cancer) in Luxembourg</td>
</tr>
<tr>
<td>Claudia Cerella</td>
<td>Linköping University Sweden</td>
</tr>
<tr>
<td>Anders Rosén</td>
<td>Stony Brook University L.I., New York, USA</td>
</tr>
<tr>
<td>Nicholas Carpino</td>
<td></td>
</tr>
<tr>
<td>Maria Russo</td>
<td>Institute of Food Sciences CNR Avellino</td>
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<tr>
<td>Carmela Spagnuolo</td>
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<td>Idolo Tedesco</td>
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<td>Stefania Moccia</td>
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<td>Carmen Cervellera</td>
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Overview

- Polyphenols in cancer: a field in *identity crisis*

- Molecular mechanism(s) of quercetin in Chronic Lymphocytic Leukemia: a question of *place* and *time*
Polyphenols (flavonoids) are pleiotropic compounds

Polyphenols are organic compounds present in plants where they play different biological functions. These include the regulation of enzymes involved in the metabolism and the mechanisms of defense against chemical and physical agents (UV rays, oxidative stress) or microbial (viruses, bacteria, fungi).
Chemopreventive mechanisms of polyphenols

Antioxidant activity
scavenging of free radicals and reducing oxidative stress

Phase II enzyme induction
enhancing detoxification

Phase I enzyme inhibition
blocking activation of carcinogens

Inhibition of cell proliferation

Induction of cell differentiation

Inhibition of oncogene expression

Induction of TSG

Induction of cell cycle arrest

Induction of apoptosis

Antiangiogenetic activity

Inhibition of cell adhesion and invasion

Others……

Adapted from
The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM). According to DeFelice, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease…"

Recent Advances in Chemoprevention of Cancer

Waun Ki Hong, Michael B. Sporn
See all authors and affiliations

Chemoprevention is the use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells (1997)

“Our endogenous antioxidant defenses are inadequate to prevent oxidative damage completely. Hence, sources of dietary antioxidants may be especially important to us” (Barry Halliwell, 1994)
The Antioxidant Hypothesis ("free radical theory")

“Epidemiological studies have shown that high consumption of fruit and vegetables reduces risk of developing cancer and cardiovascular disease…

A popular explanation refers to the presence of antioxidant nutrients, including vitamin C, vitamin E, carotenoids, selenium and flavonoids, which prevent carcinogenesis and atherogenesis by interfering passively with oxidative damage to DNA, lipids and proteins.…. [Public Health Nutrition, 2004]
Disease prevention by fruits and vegetables

Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies

Dagfinn Aune¹,²,³*, Edward Giovannucci⁴,⁵,⁶, Paolo Boffetta⁷, Lars T. Fadnes⁸, NaNa Keum⁵,⁶, Teresa Norat², Darren C. Greenwood⁹, Elio Riboli², Lars J. Vatten¹ and Serena Tonstad¹⁰
Cancer prevention by fruits and vegetables

![Graph showing the nonlinear dose-response relationship between fruits and vegetables consumption and total cancer risk.](image)

- **Study**:
  - Choi, 2015: Relative Risk (95% CI) = 0.98 (0.94, 1.03)
  - Hjartåker, 2015: 0.86 (0.72, 1.03)
  - Oyebode, 2014: 0.91 (0.86, 0.96)
  - Wie, 2014: 1.00 (0.92, 1.10)
  - Lof, 2011: 0.99 (0.93, 1.05)
  - Boffetta, 2010: 0.97 (0.96, 0.99)
  - Nechuta, 2010: 1.01 (0.95, 1.08)
  - Takachi, 2008: 0.98 (0.94, 1.02)
  - Genkinger, 2004: 0.81 (0.66, 1.00)
  - Hung, 2004: 1.00 (0.97, 1.02)
  - Jansen, 2004: 0.69 (0.48, 1.00)
  - Shibata, 1992: 0.95 (0.89, 1.01)
  - Overall: 0.97 (0.95, 0.99)
“..The controversial and contradictory issues related to the pros and cons on the use of polyphenols against cancer reflect the confounding assumption that cancer treatment and cancer prevention may overlap…”
### Preclinical vs Clinical Studies

**Table 1**
Observational and interventional studies published in the last 10 years where polyphenols, as single compounds or in mixtures, have been tested against different types of cancers.

- Uncertain – null - negative

**Table 2**
Ongoing clinical studies retrieved (with results) from the ClinicalTrials.gov database on polyphenols and cancer.

- No significant differences

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*Russo GL et al. Seminars Cancer Biol, 2017*
**Preclinical vs Clinical Studies**

Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC)$^{1-4}$

*Kathryn E Bradbury, Paul N Appleby, and Timothy J Key*

There was a **borderline inverse association** of fiber intake with breast cancer risk. For the other 9 cancer sites studied (stomach, biliary tract, pancreas, cervix, endometrium, prostate, kidney, bladder, and lymphoma) there were no reported significant associations of risk with intakes of total fruit, vegetables, or fiber. *Am J Clin Nutr* 2014;100(suppl):394S–8S.

A comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: level of evidence and limitations

**Molecular Nutrition & Food Research, 2017**

Giuseppe Grosso, Justyna Godos, Rosa Lamuela-Raventos, Sumantra Ray, Agnieszka Micek, Andrzej Pajak, Salvatore Sciacca, Nicolantonio D’Orazio, Daniele Del Rio, Fabio Galvano
Preclinical vs Clinical Studies

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Russo GL et al. Seminars Cancer Biol, 2017

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Preclinical vs Clinical Studies

Oxidants, antioxidants and the current incurability of metastatic cancers

Jim Watson

*Open Biol.* 2013 *3*, 120144, published online 8 January 2013

“free radical-destroying antioxidative nutritional supplements may have caused more cancers than they have prevented....”
Antioxidants vs cancer cells

Sayin et al. Science Translational Medicine, 2014
GUIDELINES:
(i) a careful selection of the cellular models based on a robust hypothesis that the given phenolic compound can interfere with cell growth and division;
(ii) quantification of the real uptake of the tested compound (or its metabolites);
(iii) identification of the very early target(s) of the selected compounds, possibly within minutes from the uptake, and before measuring changes in regulatory pathways (e.g., apoptosis).
Quercetin

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>1-2 g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>1 g/day</td>
</tr>
<tr>
<td>Flavonol glycoside</td>
<td>100 mg/day</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5-40 mg/day</td>
</tr>
</tbody>
</table>

Food | Quercetin |
---|-----------|
Lettuce | 0.7-0.3 mg/100g |
Onion | 28.4-48.6 mg/100g |
Apple | 2.1-7.2 mg/100g |
Strawberry | 0.8-1.0 mg/100g |
Black tea | 1.7-2.5 mg/100ml |
Red wine | 0.4-1.6 mg/100ml |
Apple juice | 0.3 mg/100ml |

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Traditional cancer therapy can activate apoptosis, but can encounter tumour resistance. Promising results to bypass resistance are expected from protocols of “combination therapy” approach, where canonical drugs and/or naturally occurring chemotherapeutic agents can be associated with natural occurring agents.

### Quercetin: not apoptotic but pro-apoptotic

<table>
<thead>
<tr>
<th>Molecule</th>
<th>↓ROS(%)</th>
<th>↑Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin 50 μM</td>
<td>60</td>
<td>&gt;50</td>
</tr>
<tr>
<td>(+) Catechin 100 μM</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Myricetin 50 μM</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Quercetetrin 50 μM</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Resveratrol 30 μM</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Gallic Acid 12 μM</td>
<td>0</td>
<td>10</td>
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Quercetin: not apoptotic but pro-apoptotic

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Quercetin enhances apoptosis in cell lines

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Chronic Lymphocytic Leukemia (CLL)

- CLL is one of the most common leukemia in adult population (22-30% of all leukemia cases). In 2016, 18,960 new cases in USA with 4660 deaths.

- CLL is defined a malignant lymphoproliferative disorder of mature clonal B lymphocytes that accumulate in the blood and other lymphoid tissues.

- The diagnosis of CLL occurs when B-cells count is >5,000/μL

- Poor diagnosis and shorter time to treatment and survival is more pronounced for CLL expressing Zap-70 and those with high level of CD38 (both markers are highly expressed in IGHV-UM CLL)

- CLL is an highly heterogeneous and still an incurable disease. Most treated patients became resistant to common chemotherapeutic drugs (combination immunochemotherapy: fludarabine, cyclophosphamide and rituximab, anti-CD20 MAb).

- B-cells are resistant to DR-induced apoptosis
Can Quercetin induce Apoptosis in B-CLL?
BH-3 “mimetic” ABT-737

Adapted from S.-L. Khan et al., Pathology, 2011
Quercetin and ABT-737 in B-CLL

Russo M, Spagnuolo Biochem. Pharmacol., 2013

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Quercetin and ABT-737 in PBMC

Russo M, Spagnuolo Biochem. Pharmacol., 2013
Quercetin and ABT-737 can bypass resistance in B-cells

Quercetin

\[ \text{PI}_3 \text{K} \]

\[ \text{pAkt}^{\text{Ser473}} \]

\[ \text{Mcl-1} \]

ABT-737

\[ \text{Bcl-2}; \text{Bcl-xL} \]

APOPTOSIS

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HG3-CLL Cell Line (the “ideal” cellular model)

- HG3 established by \textit{in vitro} EBV-infection;
- Derives from an IGHV1–2 unmutated CLL patient clone;
- Biallelic 13q14 deletions with genomic loss of DLEU7, miR15a/miR16–1, the two micro-RNAs that are deleted in 50% of CLL cases;
- Expression of CD5/CD20/CD27/CD43
- G-banding are showing identical biallelic deletions of chromosome 13 in HG3 cell line and in HG patient
Quercetin Enhances Apoptosis in HG3-CLL

Russo M. et al. Unpublished

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Quercetin Down-regulates PI₃K-Akt Pathway in HG3-CLL

Densitometric analysis

Russo M. et al. Unpublished

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Quercetin as a direct inhibitor of PI₃K

Table 2  Cellular kinases directly targeted by quercetin

<table>
<thead>
<tr>
<th>Targets</th>
<th>Binding site</th>
<th>Concentration</th>
<th>Cellular effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEK-1</td>
<td>Activation loop</td>
<td>1–2 µM</td>
<td>Apoptosis</td>
<td>[49, 56, 58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cell cycle</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Growth arrest</td>
<td></td>
</tr>
<tr>
<td>PI₃Kγ</td>
<td>ATP-binding site</td>
<td>3.8 µM</td>
<td>Apoptosis</td>
<td>[105]</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Cell cycle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Growth arrest</td>
<td></td>
</tr>
<tr>
<td>IKK α/β</td>
<td>ATP- and IκBz-binding</td>
<td>IC₅₀ 11 µM (α)</td>
<td>Apoptosis</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>sites</td>
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Molecular Cell, Vol. 6, 909-919, October, 2000, Copyright ©2000 by Cell Press

Structural Determinants of Phosphoinositide 3-Kinase Inhibition by Wortmannin, LY294002, Quercetin, Myricetin, and Staurosporine

Edward H. Walker,* Michael E. Pacold,* Olga Perisic,*
Quercetin inhibits PI$_3$K activity in HG3-CLL

Russo M. et al., Oncotarget, 2017
DPBA Staining of Quercetin in HG3-CLL

Russo M. et al., Oncotarget, 2017
**Time-dependent down-regulation of Akt/Mcl-1 (the time..)**

![Graph showing time-dependent down-regulation of Akt/Mcl-1](image)

Russo M. et al., Oncotarget, 2017
CK2 (Casein Kinase 2) in CLL

Adapted from Piazza et al., Leukemia, 2012

Adapted from Prins et al., Leukemia, 2013
Quercetin Inhibits CK2 activity in HG3-CLL

![Graph showing inhibition of CK2 activity with Quercetin concentration and Time (min) vs. Inhibition of CK2 Activity (% of control).]
**Quercetin mechanism of action**

- **Quercetin**
  - PI3K
  - PTEN
  - AKT
  - CK2
- **Mcl-1**
- **Bcl-2 and Bcl-xL**
  - **ABT-737**
  - **ABT-263**
  - **Whei-532**
- **Apoptosis**

Russo M. et al., Oncotarget, 2017

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Conclusions

- Quercetin sensitizes synergistically human malignant cell lines AND B-cells isolated from CLL patients to conventional (fludarabine) and novel (BH3-mimetics, ABT-737) treatments;

- In CLL-derived cells, quercetin targets primarily protein kinases (CK2 and PI₃K) which positively regulate the PI₃K-AKT-Mcl1 pathway;

- Quercetin cytotoxicity on normal peripheral blood leukocytes is acceptable;

**CONSIDERING THAT**

- Phase I clinical trial in humans established a recommended dose of 1400 mg/m² (2.5 g/70 Kg) intravenous weekly interval.

- Overall, these data indicate the potential therapeutic use of quercetin against CLL
Acknowledgments

Regional Products with Healthy Properties to Develop New Functional Foods (RiSaNA)

Well-being from Biotechnology: Innovative Processes and Products to Ameliorate Nutraceuticals, Cosmeceuticals and Human Nutrition (BenTeN)

PO FESR 2014-2020 –CAMPANIA TERRA DEL BUONO”
RIS3 Oncologia
Progetto EcoNutraPrevention