Biological significance of the gut microbial ellagic acid-derived metabolites urolithins

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CONTENT

ELLAGITANNINS, ELLAGIC ACID AND UROLITHINS
- Metabolism
- Urolithins in Nature

BIOLOGICAL ACTIVITY
- Inflammatory bowel disease (IBD)
- Mechanisms of action
- Role of urolithins as anti-inflammatory compounds

TARGET ORGANS
- Human prostate as target organ for urolithins

CONCLUSIONS
Ellagitannin-containing foodstuffs (animal models and humans)

- Cancer
- Diabetes
- Cardiovascular
- Alzheimer’s

Multitarget action (antioxidant, anti-inflammatory, anticarcinogen...),

BUT...

Are ellagitannins or ellagic acid the real active molecules in vivo?
Ellagitannins are metabolized to urolithins

Accumulation of ellagitannins and ellagic acid can be toxic in animals.
The Urolithins

Doyle B, Griffiths LA (1980). The metabolism of ellagic acid in the rat. Xenobiotica, 10, 247-256


Complex-toothed flying squirrel (Trogopterus xanthipes)
Urolithins in the phylogenetic scale

Urolithins are produced by mammals. Not found in birds and insects

**RAT**

**PIG**

**HUMANS**

**BEAVER, MICE, SHEEP, COW...**
METABOLISM OF ELLAGITANNINS (What do we know?)

ABSORPTION AND METABOLISM OF ELLAGITANNINS (Key points)

- Ellagitannins are not absorbed but hydrolyzed to yield ellagic acid.
- Ellagic acid is very poorly absorbed and mainly metabolized by gut microbiota to yield hydroxy-dibenzo-pyran-6-one derivatives (urolithins).
- Human subjects can be divided into high and low-urolithin producers (due to their microbiota).
- Urolithins can reach high micromolar concentrations in the colon (aglycones) and in the bloodstream (glucuronides).
BIOLOGICAL ACTIVITY OF UROLITHINS

TRADITIONAL CHINESE MEDICINE

-Hyaluronidase inhibitor (metastasis, bacterial invasion…)
-Abdominal pain
-Hematological disorders and ischemia…

(Trogopterus xanthipes)
Structure-activity relationship studies suggest that urolithins might exhibit weak estrogenic and/or antiestrogenic activity.


Urolithins bind ER\(\alpha\) and ER\(\beta\)

Estrogenic/Antiestrogenic (MCF-7 assay)
POMEGRANATE EXTRACTS IN INFLAMMATORY BOWEL DISEASE (IBD): The role of urolithins

Fisher 344 rats

Chronic inflammation increases CRC risk in IBD patients

Histological analyses of colon samples

- Crypts damaging (a)
- Epithelium loss (b)
- Infiltration of inflammatory cells (c)

PE and Uro-A protected colon from tissue damage

Inflammatory markers in colon mucosa

Prostaglandins, Nitric Oxide (NO)

ΔPE and Uro-A decreased NO and prostaglandins by downregulating the enzymes involved in their synthesis

Both PE and Uro-A modulate gut microbiota by increasing bifidobacteria, lactobacilli and clostridia and decreasing enterobacteria growth.

The first in vivo evidence of gut microbiota modulation by pomegranate. Uro-A critically contributes to this effect.

Gut microbiota could be involved in the anti-inflammatory effects observed.

Effect of DSS on pomegranate polyphenols metabolism


Colon

Imbalance in gut microbiota prevents normal urolithin formation

Urolithin A: a promising targeting active molecule to the colon
Gene expression in colon mucosa (transcriptomic)

Affymetrix: Approx. 22,000 human genes

Differential expression at least 2-fold, P<0.001 (colon mucosa)

<table>
<thead>
<tr>
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<th>DSS-PE vs DSS</th>
<th>DSS-UroA vs DSS</th>
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</thead>
<tbody>
<tr>
<td>Down-regulated probes</td>
<td>329</td>
<td>3,008</td>
</tr>
<tr>
<td>Up-regulated probes</td>
<td>1,728</td>
<td>3,987</td>
</tr>
</tbody>
</table>

2,057 genes          6,995 genes

667 common genes

✓ Both PE and Uro-A modulate gene profile of colon mucosa

Common: PE-UroA (667 genes)

Gastrointestinal disease

Cellular growth and proliferation

Cancer

Organismal survival

Cell cycle
MOLECULAR MECHANISMS OF CANCER

Pomegranate extract

Up-regulated

Down-regulated

AKT

p53

BclXL

Urolithin A

MOLECULAR MECHANISMS OF CANCER
Inflammation: Mechanistic studies. Confirming the responsible

Inflammation of human colon normal cells with IL-1β

- EA, Uro-A or Uro-B (10 and 1μM) and 1ng/mL IL-1β

- PGE₂
- COX-2 and mPGES-1 (RT-PCR and WB)
- NF-κB activation
- MAPKs pathways activation
- Cell metabolism

Prostaglandins
Effects on COX-2 and mPGES-1

Gene expression

Proteins

NF-κB and MAPKs pathways

Urolithins metabolism in colon cells

Ellagic acid (the precursor of urolithins) does not exert anti-inflammatory activity

- Very low metabolism (characteristic in ‘normal’ cells)

In vitro assays confirm in vivo effects: Uro-A is the main anti-inflammatory compound

- Trace amount inside the cells (pM)

The anti-inflammatory activity is NFκB-dependent

Cell receptor-mediated mechanism

Uro-A exerts the activity at lower concentrations than those found in the colon lumen

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CONCLUSIONS
Pomegranate juice and prostate cancer: Could urolithins be behind these effects? The human prostate as target organ

Analyses of human prostates

- High interindividual variability
- Metabolites in 8 prostate samples (high urolithin producers): 24% of patients
  - Uro-A glc: 6 samples (0.5-2 ng/g tissue) → UV, MS, MS/MS
  - Uro-B glc: 2 samples → MS and MS/MS
- Dimethyl ellagic acid (DMEA): 4 samples → MS and MS/MS

No correlation was observed between type of tissue (prostate cancer or benign hyperplasia) and metabolites detection

Metabolites were detected at very low concentration: Fasting period before the surgery?

Analyses of rat prostates: Influence of the fasting period

In both groups, urolithin A glucuronide was only detected in rats with free access to feed, with no fasting period

Urolithins (mainly Uro-A glucuronide) can reach the human prostate upon ingestion of ellagitannins-rich foodstuffs

These metabolites could be involved in the protective effects of pomegranate juice intake against prostate cancer

(Without fasting) The presence of higher urolithins levels cannot be discarded in the human prostate

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CONCLUSIONS
UROLITHINS: MULTITARGET MOLECULES PRODUCED BY THE GUT MICROBIOTA (anti-inflammatory, cancer cell regulation....)

- Urolithin A
- Urolithin B

**CELL CYCLE ARREST**
- (IN VITRO AND IN VIVO)

**REGULATION OF MAPKS SIGNALLING**
- (IN VITRO AND IN VIVO)

**INHIBITION OF NF-KB ACTIVATION**
- (IN VITRO AND IN VIVO)

**INHIBITION OF PROSTAGLANDIN SYNTHESIS**
- (IN VITRO AND IN VIVO)

**REGULATION OF GENE EXPRESSION:**
- tumor suppressor genes,
- transcription factors,
- COX-2, mPGES-1, iNOS……
- (IN VITRO AND IN VIVO)

**HIGH CONCENTRATION IN THE GUT**
- HIGH BIOAVAILABILITY
- THE HUMAN PROSTATE AS TARGET ORGAN (IN VIVO)
- REGULATION OF GUT MICROBIOTA (IN VIVO)

**ESTROGENIC/ANTIESTROGENIC ACTIVITY** (IN VITRO)
Urolithins as an iceberg: A long way to go for this emerging topic...

Identification of the microbiota involved in urolithins production

To study in depth the role of urolithins in colon inflammation and cancer: Many important markers

‘Systemic’ effect of urolithin conjugates: cardiovascular, other cancers....

Metabolism of ellagitannins in very low urolithin producers: What happens? Toxicity? Other effects?

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UROLITHINS: Multitarget molecules produced by the gut microbiota

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THANK YOU FOR YOUR ATTENTION!