Cytotoxic effect of hydroxytyrosol and its semisynthetic derivatives against prostate cancer cells

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Intake of olive oil as the main source of fat in Mediterranean diet is related to positive effects on human health. The olive biophenol hydroxytyrosol (HT) is considered a promising cancer chemopreventive compound against different types of cancer. The aim of the present study was to compare the cytotoxic activity against prostate cancer (PCa) cell lines of HT, obtained from olive mill wastewaters, and five semisynthetic alkyl ether, ester, and nitro-derivatives. HT, hydroxytyrosyl acetate (HT-Ac) and ethyl hydroxytyrosyl ether (HT-Et) exerted higher cytotoxic effect against 22Rv1 and PC-3 PCa cell lines than in non-malignant RWPE-1 cells. These compounds also significantly decreased the migration rate of RWPE-1 and PC-3 cells and the colony and prostatosphere formation of 22Rv1 cells. However, HT-Ac and HT-Et, but not HT, were able to decrease p-AKT levels and colony and prostatosphere formation in PC-3. In sum, our results together with previous studies showing the antioxidant capacity of HT and its lipophilic derivatives suggest that they could be considered as potential therapeutic tools in PCa.

Keywords: Anticancer; extra virgin olive oil; hydroxytyrosol; hydroxytyrosyl acetate; prostate cancer; semisynthetic derivatives.
Prostate Cancer (PCa): Epidemiology and diet chemoprevention

PCa is one of the most prevalent cancers among men worldwide.

A high adherence to the Mediterranean diet with a lower risk, aggressiveness and mortality of PCa.

Extra virgin olive oil phytochemicals exert health promoting effects and a PCa cytotoxic effect.

GLOBOCAN 2018.
Objective

Chemical modifications improved the absorption and pharmacological activities of hydroxytyrosol

The aim of the present study was to compare the cytotoxic activity of hydroxytyrosol and five semisynthetic derivatives, against prostate cancer cell lines
Methodology

Human prostate cell lines

<table>
<thead>
<tr>
<th>Non-tumor</th>
<th>Tumor</th>
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<tbody>
<tr>
<td>RWPE-1</td>
<td>22Rv1</td>
</tr>
<tr>
<td>PC-3</td>
<td></td>
</tr>
</tbody>
</table>

Experiments

- Cell viability
- Cell migration
  - Wound healing
- Colony formation
- Prostaspheres
  - Clonogenic assay
  - Tumorsphere formation
- Protein expression
  - Western blotting
Results. Cell proliferation

HT and five semisynthetic derivatives exert a concentration-dependent effect in proliferation of prostate cells. HT, AT-Ac and HT-Et were more cytotoxic in cancer cells.
**Results. Cell migration**

HT, HT-Ac, and HT-Et treatment decrease migration capacity of prostate cells in a concentration-dependent manner.

**RWPE-1**

Migration (% of control)

0h

- **Ctrl**
- **10 µM**
- **30 µM**
- **100 µM**

16h

- **Ctrl**
- **10 µM**
- **30 µM**
- **100 µM**

**PC-3**

Migration (% of control)

0h

- **Ctrl**
- **10 µM**
- **30 µM**
- **100 µM**

24h

- **Ctrl**
- **10 µM**
- **30 µM**
- **100 µM**

Cell migration rate of RWPE-1 (16h), and PC-3 (24h) treated with 0-100 µM of HT, HT-Ac, and HT-Et.
**Results. Cell stemness**

HT, HT-Ac, and HT-Et differently affect cancer stemness of prostate cancer cells.

**Tumorspheres**

- 22Rv1
- PC-3

Effect of HT, HT-Ac, and HT-Et treatment (20 µM, 14 days) in the size of 22Rv1 and PC-3 prostatospheres.

**Clonogenic assay**

- 22Rv1
- PC-3

Effect of HT, HT-Ac, and HT-Et treatment (20 µM, 10 days) in the clonogenic assay in 22Rv1 and PC-3 cells.
Results. Protein phosphorylation

HT-Ac and HT-Et, but not HT, reduce the activation of AKT in PC-3 cells.

Effect of HT-Ac, HT, and HT-Et treatment (20 µM, 24h) in the phosphorylation of AKT and ERK in 22Rv1 and PC-3 cells.
Conclusions

Altogether, our data demonstrate that the lipophilic derivatives HT-Ac and HT-Et, not only maintained the anticancer effect of the parent compound HT against PC-3 PCa cells, but also improved its anticancer effect at selected concentrations. These results, together with earlier studies showing increase in the antioxidant and antiangiogenic capacity of HT-Ac and HT-Et, suggest that they could be considered as potential therapeutic tools in PCa.
Acknowledgments

OncObesity and Metabolism group

Raúl M. Luque Huertas
Manuel D. Gahete Ortiz
Antonio J. Martínez Fuentes
Antonio J. León González
André M. Sarmento B. Cabral
Juan Manuel Jiménez Vacas
Emilia Alors Pérez
Vicente Herrero Aguayo
Antonio C. Fuentes Fayos
Prudencio Sáez Martínez
Juan Luis López Cánovas
Antonio J. Montero Hidalgo
Jesús Miguel Pérez Gómez
Fernando López López
Auxiliadora Pérez Repiso