Effects of extra virgin olive oil phenols on HL60 cell lines sensitive and resistant to anthracyclines

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Abstract

The aim of our study was to evaluate the capability of a crude extract of phenols from extra virgin olive oil of Moraioiolo cultivar to induce apoptosis and/or differentiation in sensitive and resistant HL60 cell lines to anticancer drugs (Typical Multidrug Resistance). Our data highlight that the crude extract is able to induce apoptosis on both sensitive and resistant cells, whereas the exposure to a number of anticancer drugs does not induce apoptosis in resistant cells. In differentiation experiments we investigated the capability of crude extract of phenols to induce the expression of CD11 granulocytic or CD14 mononcytic cell surface antigen in sensitive and resistant HL60 cell lines. At IC50 dose level (17 μg/ml and 32 μg/ml respectively for sensitive and resistant cell lines), the crude extract induced differentiation associated with the expression of CD14 mononcytic cell surface antigen either in sensitive or resistant cell lines but not that of CD11 granulocytic cell surface antigen. Further investigations are in progress to better clarify the mechanism by which olive oil phenols induce differentiation on this cell line.

Introduction

A number of in vitro studies highlight that the polyphenolic fraction of extrarvigir olive oil contains several molecules which may induce direct cytotoxic effects on human tumour lines. Although the mechanisms by which these molecules may affect tumor cell growth are not fully understood, a number of experimental evidence suggests that these substance may exert their antiproliferative effects by activating specific apoptosis pathways with a mechanisms which resemble those of antitumor agents (Della Ragione et al., 2000; Fabiani et al., 2006; Fabiani et al., 2002.). Interestingly, the apoptotic pathways activated by molecules present in the polyphenolic fraction of extrarvigir olive oil are those which result to be disregulated in cancer cells resistant to antineoplastic agents (Longley and Johnston, 2005). Several experimental indicated that caspases, enzymes belonging to the cysteine-proteinase family, play an important role in apoptosis activation (Oliver and Valletta, 2005). The intracellular activity of these proteases is regulated by IAPs proteins (apoptosis inhibiting proteins) that play a central role in the regulation of caspase-dependent apoptosis and in cell differentiation (Oliver and Valletta, 2005; Vucic and Fairbrother, 2007; Tamm et al., 2004). The pharmacological modulation of the expression of apoptosis regulating proteins may be of clinical relevance in order to find out new and more effective and selective therapeutic strategies. On the basis of these considerations we have undertaken some investigations to evaluate the ability of a crude extract of phenols (CE) from extra virgin olive oil of Moraioiolo Cultivar to induce apoptosis and/or differentiation in sensitive and resistant HL60 cell lines.

Materials and Methods

Cell culture and chemicals: Mouse anti-human CD14 and mouse anti-human CD11c were purchased from Becton Dickinson, Mountain View C.A. Human promyelocytic leukemia HL60 cells as parental (HL60/S) or resistant (HL60/R) variants were grown in RPMI and 10% FBS (GIBCO, BRL Gran Island, New York, U.S.A.) with 2mM glutamine (GIBCO), 100 U/ml penicillin and 100 μg/ml streptomycin).

The phenols crude extract was obtained from extra virgin olive oil of Moraioiolo Cultivar as previously described (Gill at
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Phenolic Compounds | mg/100mg Crude Extract
--- | ---
3,4-Dihydroxynaphthyl Ethanol (3,4 DHPEA) | 0.67
p-Hydroxyphenyl Ethanol (p-HPEA) | 6.79
Dialdehydic form of elenolic ac. linked to 3,4 DHPEA | 19.12
Dialdehydic form of elenolic ac. Linked to p-HPEA | 22.21
(+)-1-acetoxy-pinoresinol and (+)-pinoresinol | 3.32
Oleuropein aglycon (3,4-HPEA-EA) | 15.76

Table 1 - Content of Phenolic Compounds in crude extract of Moraiolo extra-virgin olive oil

Results

Antiproliferative effects of Crude Extract.
The IC50 value of CE and DXR in the HL60 cell lines sensitive and resistant is reported in table 2.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>IC50* HL60S</th>
<th>IC50* HL60R</th>
<th>RI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXR</td>
<td>0.022 μM</td>
<td>0.60 μM</td>
<td>27</td>
</tr>
<tr>
<td>CE</td>
<td>17 μg/ml</td>
<td>32 μg/ml</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 2 - Drug sensitivity profiles of HL60S and HL60/R Human leukaemia cell lines

Morphological evaluation of apoptosis.
The effects of crude extract on the induction of apoptosis is reported in table 3. The concentration level able to induce 50% apoptosis in cells (AC50) was 20 μg/ml for sensitive cells and 45 μg/ml for resistant cells. The CE was able to induce apoptosis in both sensitive and resistant cells, whereas the exposure to a number of anticancer drugs did not induce apoptosis at all in resistant cells lines (Fig. 1).

![Fig. 1 - Proapoptotic effect of cytosine arabinoside (ArA-C), metotrexate (MTX), 5-fluorouracil, (5FU), cis-platin (cisPt) and daunorubicin (DNR) on HL60/S and HL60/R Human leukaemia cell lines](image)

Table 3 - Effect of EG on induction of apoptosis in HL60/S and HL60/R Human leukaemia cell lines

Data are the mean of at least four separate experiments. The standard deviation are less than 10%.

Evaluation of expression of Caspase 3

The results reported in Fig. 2 showed that CE was able to induce activation and increased expression of caspase 3 in sensitive HL60 variant. No activation was observed in resistant cell line.

Induction of differentiation.

To evaluate the capability of CE to induce differentiation as shown by morphological examination (Fig. 1) and by the increase of expression CD14 antigen (Fig. 2), HL60/S and HL60/R were exposed to the IC50 concentrations of CE, namely, 17 μg/ml and 32 μg/ml respectively up to four days.
No expression of CD11c granulocytic cell surface antigen was observed in sensitive and resistant cell lines.

Discussion

The good sensibility to the cytotoxic effect of CE observed in HL60/S and HL60/R led us to investigate the mechanisms involved in the growth inhibitory effects and to assess whether the lower sensibility of resistant variant may be due to an escape from apoptosis. In line with the data reported by other studies (Fabiani et al., 2008), our results suggest that CE is able to induce apoptosis on both sensitive and resistant cell line and that caspase 3 seems to be involved at least in the induction of apoptosis only in HL60/S but not in HL60/R. In the last cell lines CE could activate other caspase independent apoptotic pathways (Della Ragione et al., 2000). In order to study the possible relationship between apoptosis and cell differentiation we assessed the capability of CE to induce differentiation in both cell lines. Our results showed that CE was able to induce differentiation as assessed by the expression of monocytic (CD14) cell surface antigen either in sensitive or resistant cell lines. As it has been reported that XIAP proteins are involved in the expression of monocytic CD14 cell antigen surface (Tamm et al., 2004), further studies are in progress to assess the role of XIAP proteins in the modulation of CD14 surface antigen expression during the monocytic differentiation. In conclusion the good activity of CE on induction of apoptosis and differentiation of resistant variant could bring out new strategy in the treatment of resistant tumors. Further studies in aimed at better assessing the therapeutic possibilities of CE against sensitive and resistant tumors are warranted by these observations.

References


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