POLYMERIZATION AND GALLYLATION: TWO IMPORTANT ASPECTS FOR ANTIPROLIFERATIVE PROPERTIES OF PROCYANIDIN-RICH NATURAL EXTRACTS.

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ABSTRACT

Natural polyphenolic compounds from plants and fruits have been reported to be potent antioxidants and to present antitumoral properties. The difficulty to clearly identify structure/antitumoral activity of this family of compounds makes difficult to optimize the formulation of combinations of polyphenolic extracts from different plants or fruits to achieve the best antitumoral potency. Recent advances have been achieved in this field from the exhaustive characterization of polyphenolic extracts with different degree of polymerization and percentage of galloylation. Here we review the results obtained in this field from our team (Lizarraga et al., 2007; Lozano et al., 2006; Lozano et al., 2005; Matito et al., 2003; Tourino et al., 2008; Tourino et al., 2005) that relate degree of polymerization and galloylation to radical scavenging capacity, tumor cell proliferation inhibition and induction of apoptosis. From this review we concluded that natural polyphenolic extract with high degree of polymerization and galloylation will present the higher antiproliferative and induction of apoptosis capacities.

KEYWORDS

Polymerization, galloylation, antiproliferative properties, procyanidin-rich.
INTRODUCTION
Cancer is one of the most important disorders that cause alarming mortality in humans. Results of research efforts over the past 30 suggest that dietary habits and lifestyle may reduce the risk. Many bioactive food components thought to be involved with cancer prevention have been described (Auger et al., 2004). Polyphenols are the most abundant antioxidant compounds in our diet and are widespread constituents of fruits, vegetables and beverages, such as tea, coffee and wine. Epidemiology studies have suggested that dietary phytochemicals such as polyphenols could be related to protection in prostate, breast and colon cancer (Bemis et al., 2006; Kumar et al., 2007; Sun et al., 2006).

Even benefits of plants and fruits rich in polyphenols on human health have been described from centuries, has been only during the last two decades that the potential health attributes of dietary polyphenols have been intensively researched. The main reason to the delayed research on polyphenols is the variety and the complexity of their chemical structures. The role of polyphenols in the prevention and protection against several pathologies characterized by ROS overproduction and deregulated apoptosis and cell cycle such as cancer has been largely studied (Di Chen, 2007; Lizarraga et al., 2007; Lozano et al., 2006; Matito et al., 2003). Specifically, the imbalance between high level oxidant exposure and antioxidant capacity in the colon has been linked to increased cancer risk and is strongly influenced by dietary antioxidants (Bruce et al., 2000; Hietanen et al., 1994; Theodoratou et al., 2007).

The importance of the molecular structure of polyphenolic compounds on their scavenger capacity has been largely studied (Lizarraga et al., 2007; Lozano et al., 2006; Tourino et al., 2008). Large efforts have been also devoted to establishing structure/antitumoral relationships of polyphenolic compounds. Research with different cell lines has shown that the most widely studied natural polyphenol, resveratrol from grape, is a potent antioxidant and chemopreventive agent (Alkhalaif et al., 2008; Baxter, 2008; Park et al., 2007). Additionally, some derivatives of gallic acid, such as epigallocatechin gallate, have been shown the same benefits (Siddiqui et al., 2006; Zhang et al., 2006). These and other results suggest that those benefits can be related to galloylation of catechins and the presence of gallocatechin moieties in natural extracts. Particularly, it has been proposed that gallate esters are more stable than simple catechins, upon less metabolized and more bioavailable in the colon (Meselhy et al., 1997). Moreover, in colon cancer the gallic acids derivatives could be considered a potent chemopreventive compounds, and it has been reported that oligomeric procyanidins are not significantly absorbed in the intestinal tract and they can reach the colon mainly intact (Kuhnle et al., 2000). From these studies it has been concluded that galloylation and polymerization are important structural factors that influence its scavenger capacity and antitumoral properties even not permit to conclude a direct correlation structure-function.

To establish relationships between polyphenolic compounds structural characteristics (degree of polymerization and percentage of galloylation) and its antitumoral properties we have used a complete collection of polyphenolic fractions of Pinus pinaster and Vitis vinifera differing in size and pyrogallol content generated over the last years (Lizarraga et al., 2007; Matito et al., 2003; Torres et al., 2002; Tourino et al., 2005). Vitis vinifera and Pinus pinaster both are polyphenolic natural sources with similar composition with respect to the degree of polymerization of the polyphenolic compounds which permit to prepare equivalent collections of fractions from extracts when compared respect to the degree of polymerization. However, they differ in the fact that whereas in Vitis vinifera galloylated
polyphenols exist in monomeric and polymeric forms, in *Pinus pinaster* galloylated polyphenols are absent. These homologies have permitted us to study the influence of polymerization and galloylation of polyphenolic compounds on its antitumoral properties (Lizarraga et al., 2007).

Results obtained on the antitumoral effects of the fractions from *Vitis vinifera* and *Pinus pinaster*, with different degree of polymerization and galloylation and different hydroxyl/superoxide radical scavenging capacity, are presented in table 1. In particular we used HT29 colon adenocarcinoma cells and we analyzed the effects on cell proliferation, and capacity to induce apoptosis.

### Table 1. Comparative chemical characteristics and HT29 cell growth inhibition of grape and pine polyphenolic fractions (Data from Lizarraga et al., 2007).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>mDP</th>
<th>%G</th>
<th>mMW (µg/ml)</th>
<th>IC50 (%) of apoptosis induced at IC50 (µg/ml)</th>
<th>ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grape</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIIIG</td>
<td>3.4</td>
<td>34</td>
<td>1160</td>
<td>64±4, EA(18%<em>), LA(7%</em>), N(5%*)</td>
<td>70%<strong>/42%</strong></td>
</tr>
<tr>
<td>IVG</td>
<td>2.7</td>
<td>25</td>
<td>880</td>
<td>59±3, EA(11%*), LA(5%), N(3%)</td>
<td>60%<strong>/39%</strong></td>
</tr>
<tr>
<td>VIG</td>
<td>2.4</td>
<td>16</td>
<td>751</td>
<td>42±6, EA(4%), LA(3%), N(3%)</td>
<td>ND</td>
</tr>
<tr>
<td>OWG</td>
<td>1.7</td>
<td>15</td>
<td>552</td>
<td>55±10, EA(5%), LA(2%), N(2%)</td>
<td>20%<strong>/15%</strong></td>
</tr>
<tr>
<td>VG</td>
<td>1.0</td>
<td>0</td>
<td>290</td>
<td>119±6, EA(%), LA(2%), N(3%)</td>
<td>20%**/0%</td>
</tr>
<tr>
<td><strong>Pine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XIP</td>
<td>3.4</td>
<td>0</td>
<td>999</td>
<td>107±4, ND</td>
<td>ND</td>
</tr>
<tr>
<td>VIIIP</td>
<td>3.0</td>
<td>0</td>
<td>876</td>
<td>108±5, EA(10%), LA(7%), N(2%)</td>
<td>50%<strong>/18%</strong></td>
</tr>
<tr>
<td>IVP</td>
<td>2.9</td>
<td>0</td>
<td>833</td>
<td>106±5, EA(10%), LA(4%), N(3%)</td>
<td>45%<strong>/20%</strong></td>
</tr>
<tr>
<td>VIP</td>
<td>2.7</td>
<td>0</td>
<td>777</td>
<td>111±5, ND</td>
<td>ND</td>
</tr>
<tr>
<td>OWP</td>
<td>2.1</td>
<td>0</td>
<td>601</td>
<td>114±3, ND</td>
<td>20%<strong>/10%</strong></td>
</tr>
<tr>
<td>VP</td>
<td>1.0</td>
<td>0</td>
<td>290</td>
<td>422±4, ND</td>
<td>10%*/0%</td>
</tr>
</tbody>
</table>

Percentage of galloylation (%G), mean degree of polymerization (mDP) and mean molecular weight (mMW) from Torres 2002, Touriño 2005. Non determined (ND). *Inhibition of Hydroxyl/superoxide radical system by polyphenolic fractions at 50 µM. The capacity to inhibit cell proliferation has been expressed as the amount of compound necessary to inhibit the cell growth in a 50% (IC50). Apoptosis stages (EA, early apoptosis; LA, late apoptosis; N, necrosis) induced in HT29 at IC50. Percentages were significant respect to their control (p<0.05 *; p<0.001 **).*  

As it is observed in table 1, pine bark extracts containing oligomers (XIP, VIIIP, IVP, VIP and OWP) reduce proliferation of the carcinoma cell line HT29 with IC50 values between 100-115 µg/ml, whereas IC50 of the fraction VP containing monomers was almost one order of magnitude higher. Additionally, grape polyphenolic fractions (with an equivalent degree of polymerization but also with a percentage of galloylation ≥ 15%) (VIIIG, IVG, VIG and OWG), showed an IC50 approximately the half with respect to the homologous pine fractions. From these results it has been concluded that both polymerization and galloylation enhance the antiproliferative capacity of polyphenolic fractions being the most suitable polyphenolic extracts as antiproliferative agents those that contain polymers and high degree of galloylation (Lizarraga et al., 2007).
Regarding apoptosis, it has been observed that at IC50 concentration, the grape polyphenolic fractions VIIIG and IVG induced significant percentages of apoptosis (approximately 25% and 17% respectively) as measured by FACS analysis. A significant percentage of apoptosis was also induced by the equivalent non galloylated pine fractions VIIIP and IVP at their respective IC50 but the percentages were lower than those obtained for the grape fractions. Moreover, it should be noted that IC50 for grape were lower than those for pine, and thus (for an identical concentration) it should be expected a lower apoptosis for pine fractions. These results show that both galloylation and polymerization contribute to the apoptosis induction. However, the fact that grape fraction IVG (with a similar percentage of polymerization than VIG but with much higher degree of galloylation), induces a percentage of apoptosis three times higher than fraction VIG, suggest that galloylation plays a more important role than polymerization in apoptosis induction.

Moreover the results on scavenger capacity obtained by Lizarraga et al (2007) and presented in the last column of table 1, also showed that the oligomeric fractions (VIIIG and IVG) were the most efficient as hydroxyl radical and superoxide scavengers followed by the oligomeric fractions of pine (VIIIP and IVP) when compared at 50 µM.

CONCLUDING REMARKS

Results show that high degree of polymerization is a suitable characteristic to enhance polyphenolic fractions capacity to inhibit cell proliferation and induce apoptosis. Interestingly, the efficiencies observed for grape oligomeric fractions, which proved to be better apoptotic inducers and better ROS scavengers than pine oligomeric fractions, are apparently related to the degree of galloylation.

The studies performed with grape and pine fractions (Lizarraga et al., 2007) provide useful insights on the polyphenolic structure/antitumoral relationships which should help to rationally design formulations for potent chemopreventive or antiproliferative natural vegetable based on apoptosis-inducing activity. According to our results it can be predicted that natural plants rich in high polymerized and galloylated polyphenolic compounds will be suitable sources for natural antioxidant extracts with high antitumoral properties.

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