Abstract:
The investigation of Complimentary and Alternative Medicine (CAM) modalities has garnered much interest in the scientific community. Hydroxycinnamaldehyde (HCA) is a purified extract isolated from cinnamon bark which has been purported to have effects on cell division. In the current study, we examine HCA for potential antineoplastic efficacy or modulatory activity with traditional antineoplastic agents in 32Dc13 mouse promyelocytic cells. Co-treatment of cells with HCA and paclitaxel has a greater effect at decreasing cell survival than treatment with paclitaxel alone. Interestingly, HCA does not appear to alter the efficacy of methotrexate, doxorubicin, busulfan, or etoposide. In addition to the effect on cell survival, we have seen that HCA induces both a G1 and G2/M phase cell cycle arrest in myeloid cells. Further investigation of the mechanism of action of HCA and its effect alone and in combination with other antineoplastic agents may identify new chemotherapeutic strategies for the treatment of cancer.

Cell death can be measured by an increase in M1 percentage or an increase in M1/M2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Doxorubicin (0.1μM)</th>
<th>Paclitaxel (0.1μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2.9%</td>
<td>13.3%</td>
<td>18.6%</td>
</tr>
<tr>
<td>M2</td>
<td>97.1%</td>
<td>86.7%</td>
<td>81.4%</td>
</tr>
<tr>
<td>M1/M2</td>
<td>0.029</td>
<td>0.153</td>
<td>0.228</td>
</tr>
</tbody>
</table>

Figure 1. Factor-dependent mouse myeloid cells, 32D, were treated with doxorubicin or paclitaxel as compared to control for 24 hours. Cells were then washed in PBS and stained with propidium iodide prior to analyzing by flow cytometry. Populations of cells in the two marker regions (M1 or M2) were counted and percentages were determined using WinMDI software.

Caspase-3 activation in 32D cells treated with paclitaxel in the presence or absence of HCA

A. Paclitaxel + HCA (15μM)

Figure 2. 32D cells were treated with increasing concentrations of 2′hydroxycinnamaldehyde (HCA) for 24 hours. Cells were then washed in PBS and stained with propidium iodide prior to analyzing by flow cytometry. The percentage of cells in M1 were determined using WinMDI software.

Dose response curves for chemotherapeutic agents +/- HCA (15μM)

Figure 3. 32D cells were treated with increasing concentrations of the chemotherapeutic agents indicated +/- HCA for 24 hours. Cells were then washed in PBS and stained with propidium iodide prior to analyzing by flow cytometry. The ratios of M1/M2 were determined using WinMDI software.

Conclusions:
1. The herbal extract HCA decreases cell survival in a dose dependent manner as indicated by flow cytometry.
2. HCA appears to enhance the effectiveness of paclitaxel, but not other chemotherapeutic agents, at decreasing cell survival when measured by flow cytometry.
3. However, HCA does not appear to be more effective at increasing a paclitaxel-induced caspase-3 activation.
4. Although HCA enhances the efficacy of paclitaxel, it is unclear as to whether this is mediated through a apoptotic or necrotic mechanism. Further studies must be performed to elucidate a synergistic relationship between HCA and other chemotherapeutic agents.

We would like to thank Dr. B.M. Kwon of the Korean Research Institute of Bioscience & Biotechnology, Taejon, South Korea for his generosity in providing purified 2′hydroxycinnamaldehyde.