

INTRODUCTION

The stilbene resveratrol has attracted a good deal of attention as a possible cancer chemopreventative agent (Gusman et al., 2001). Resveratrol (trans-3,4',5-trihydroxystilbene) is found in a variety of plant species including Polygonum cuspidatum (Japanese knotweed), several grass species and pine bark (Mannila and Talvitie, 1992; Powell et al., 1994; Vastano et al., 2000). The main dietary sources of resveratrol are grapes (Vitis vinifera) and wine, peanuts (Arachis hypogea L.) and peanut products (Sobolev and Cole, 1999). Resveratrol is produced as a phytoalexin by peanuts and grapes in response to fungal infection and high levels are also induced following exposure to UV light (Burns et al., 2002).

In 1997, resveratrol was shown to inhibit carcinogenesis in vitro and tumourigenesis in vivo in a mouse skin cancer model (Jang et al., 1997). Subsequent studies demonstrated that resveratrol inhibits the growth of several types of tumours in animal models (Bhat et al., 2001; Sale et al., 2004). In vitro, resveratrol has antioxidant activity, modulates carcinogen metabolism, has antiinflammatory activity, and modulates the cell cycle, cell proliferation and apoptosis (Gusman et al., 2001). Resveratrol may also have cardio-protective activity by inhibiting platelet aggregation and coagulation and reducing LDL oxidation (Pace-Asciak et al., 1995).

Several groups have shown that resveratrol inhibits the expression of cyclo-oxygenase-2 (COX-2), an inducible enzyme that metabolizes arachidonic acid to prostaglandins (Gusman et al., 2001). COX-2 is produced in response to inflammatory stimuli and in cancerous tissue (Subbaramaiah and Dannenberg, 2003). Since over-production of pro-inflammatory prostaglandins by COX-2 increases cell proliferation and tumour angiogenesis, it is likely that at least part of the antiinflammatory and anticancer activities of resveratrol can be attributed to inhibition of COX-2 expression. Resveratrol has little or no direct effect on the activity of COX-2, although it does inhibit the activity of the constitutive form COX-1 (Gusman et al., 2001).

The key role of COX-2 in mediating inflammatory conditions has prompted a search for naturally occurring resveratrol analogues as cyclo-oxygenase inhibitors. Alpha-viniferin, a trimer of resveratrol, was isolated from the root of Carex humilis Leyss and was found to inhibit COX-2 induction in mouse macrophages and carrageenin-induced paw oedema (Lee et al., 1998; Chung et al., 2003). Su and colleagues isolated several stilbenoids related to resveratrol from the evergreen tree Atrocarpus dadah that inhibited COX-1 and COX-2 enzyme activity and showed moderate inhibition of 7,12-dimethylben[z]anthracene-induced preneoplastic lesions with mouse mammary organ culture (Su et al., 2002). Two stilbene dimer glucosides of resveratrol from Vitis vinifera cell cultures inhibited both COX-1 and COX-2 activities (Waffo-Teguo et al., 2001).

Peanuts produce several stilbene phytoalexins structurally related to resveratrol (Aguamah et al., 1981). These compounds were isolated and evaluated as inhibitors of COX-2 expression induced in mouse macrophages by lipopolysaccharide.

MATERIALS AND METHODS

Isolation of resveratrol derivatives. The isolation and characterization of the resveratrol derivatives was based on Aguamah et al. (1981). Peanuts (500 g) were shelled and the seeds were incubated overnight on

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Resveratrol derivatives are of interest as inhibitors of cyclo-oxygenase-2 and as antiinflammatory agents. The prenylated resveratrol derivative 4-(3-methyl-but-1-enyl)-3,5,3',4'-tetrahydroxystilbene was purified from fungally infected peanuts by thin layer chromatography and its structure was confirmed by mass spectrometry. 4-(3-Methyl-but-1-enyl)-3,5,3',4'-tetrahydroxystilbene inhibited lipopolysaccharide-induced expression of cyclo-oxygenase-2 protein and cyclo-oxygenase-2 mRNA in mouse macrophages at concentrations that were non-cytotoxic. 4-(3-Methyl-but-1-enyl)-3,5,3',4'-tetrahydroxystilbene warrants further evaluation as an antiinflammatory agent. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: peanuts; resveratrol; cyclo-oxygenase-2; J774; macrophage.
filter paper moistened with sterile distilled water. The seeds were then cut into slices approx. 2 mm thick and incubated at 25 °C for 48 h during which time the sliced seeds became brownish-yellow and mouldy indicating the establishment of microflora. The sliced seeds were homogenized in 95% ethanol at room temperature. Extracts were concentrated under reduced pressure at 40 °C using a Rotaevaporator-R (Buchi) and fractionated by thin layer chromatography using hexane/ethyl acetate/methanol solvent mixture (60:40:1) and re-chromatographed using chloroform/acetonitrile/acetone/180 µL Tris-HCl pH 6.8, 180 µg/mL) for 24 h. Test substance and protease cocktail inhibitors) for 5 min while keeping the plate on ice. Cells lysates were centrifuged (13000 rpm for 5 min), 0.2 m dithiothreitol and 0.02% w/v bromphenol blue were added and the samples were boiled for 5 min. Equivalent amounts of protein (20 µg) were electrophorezed by 12.5% SDS-PAGE. Proteins were transferred onto nitrocellulose membrane, the membranes blocked (5% milk in PBS-Tween) and were probed with polyclonal anti-COX-2 (1 in 1000) (Santa-Cruz Biotechnology, USA) followed by anti-goat IgG-HRP (1 in 5000) and enhanced chemiluminescence detection (Amersham, UK). COX-2 protein was estimated by densitometry.

COX-2 mRNA. COX-2 mRNA levels were quantitated using a Quantikine mRNA base kit (R&D Systems, UK) and Quantikine mRNA Probes and Calibrator kit Human COX-2 (R&D Systems, UK). mRNA was extracted according to the manufacturer's protocol. Each experiment was performed in duplicate.

Protein determination. The protein concentration was determined by the Bradford reagent (Bradford, 1976).

Statistics. Comparisons between groups were made using Student’s t-test.

RESULTS AND DISCUSSION

Three compounds were induced in fungally infected peanut seeds compared with non-infected peanuts as observed by thin layer chromatography. The major compound was isolated and analysed by mass spectrometry. A comparison of the mass spectrometry data with that given by Aguamah et al. (1981) indicated that the structure was 4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxystilbene (Fig. 1). The yield was 35 mg from 500 g of peanuts. The other two compounds were identified as 4-(3-methyl-but-2-enyl)-3,5,4′-trihydroxystilbene (yield 4 mg) and 4-(3-methyl-but-1-enyl)-3,5,4′-trihydroxystilbene (yield 19.1 mg). Our initial studies indicated that 4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxystilbene, hereafter referred to as BS2, was more active than the two tri-hydroxy compounds and so further studies were carried out with BS2 only.

The cytotoxicity of BS2 was compared with that of resveratrol. There was a dose-dependent increase in cytotoxicity when mouse macrophages were treated with resveratrol for 3 days (Fig. 2). BS2 was less cytotoxic than resveratrol (Fig. 2). There was less
cytotoxicity after treating the macrophages with either resveratrol or BS2 for only 1 day (Fig. 2). Similar results were obtained after 1 day or 3 days treatment of LPS-stimulated macrophages (data not shown). The low cytotoxicity of BS2 is of interest since a potential problem for the clinical use of resveratrol is that it decreases the growth of cycling normal human peripheral blood lymphocytes at concentrations comparable to those required to inhibit most leukaemia cells (Ferry-Dumazet et al., 2002).

Resveratrol inhibits cyclo-oxygenase-2 (COX-2) expression in several cell lines (Surh et al., 2001). For example, in phorbol ester-treated mammary epithelial cells, resveratrol caused a noticeable reduction in COX-2 expression at concentrations as low as 5 μM (Subbaramaiah et al., 1998). The effects of resveratrol and BS2 on COX-2 expression were examined in J774 mouse macrophages, a cell line that expresses COX-2 in response to LPS (D’Aquisto et al., 1997). COX-2 expression was suppressed by doses of BS2 down to 10 μM (Fig. 3). From three independent experiments, it was found that BS2 reduced COX-2 expression to 71.3% of control values by 10 μM BS2 and to 38.6% by 30 μM BS2 (Table 1). By contrast, resveratrol was a weaker inhibitor of LPS-induced COX-2 protein expression in J774 cells and the inhibition was not statistically significant (Table 1). The weaker effect of resveratrol on COX-2 induction in J774 cells compared with the effect on mammary epithelial cells suggests that the ability of resveratrol to inhibit COX-2 induction is cell-type dependent. However, resveratrol at 30 μM has been reported to inhibit COX-2 expression in freshly isolated mouse macrophages (Martinez and Moreno, 2000).

Next the effects of resveratrol and BS2 on COX-2 mRNA expression induced in J774 cells by LPS were examined. Resveratrol suppressed COX-2 mRNA levels by 27% and 35% at 10 μM and 30 μM, respectively (Table 1), and BS2 suppressed COX-2 mRNA levels by 23% and 53% at 10 μM and 30 μM, respectively (Table 1). Several groups have shown that inhibition of COX-2 expression by resveratrol is due to inhibition of the activation of the transcription factor NFκB (Kundu and Surh, 2004). Hence, it is of interest to determine if this pathway is also a target for the suppression of COX-2 expression by BS2.

In conclusion, it was shown that BS2 is a good inhibitor of COX-2 expression in mouse macrophages. Coupled with its low cytotoxicity, this indicates that BS2 warrants further evaluation as a non-toxic anti-inflammatory agent and inhibitor of tumourigenesis.

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Table 1. Inhibition of COX-2 protein and COX-2 mRNA by resveratrol and BS2 induced in J774 cells by lipopolysaccharide

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μM)</th>
<th>COX-2 protein (% of control)</th>
<th>COX-2 mRNA (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resveratrol</td>
<td>10</td>
<td>82.1 ± 15.6*</td>
<td>73.0 ± 2.0*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>93.9 ± 14.7*</td>
<td>65.4 ± 17.9*</td>
</tr>
<tr>
<td>BS2</td>
<td>10</td>
<td>71.3 ± 9.9*</td>
<td>77.6 ± 27.8*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>58.5 ± 3.1d</td>
<td>47.1 ± 5.1*</td>
</tr>
</tbody>
</table>

* Not statistically significant.
* Statistical significance from control at p ≤ 0.05.
* Statistical significance from control at p ≤ 0.001.
* Statistical significance from control at p ≤ 0.001.

REFERENCES


