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Short communication

Synergistic effects of triterpenic compounds with prostaglandin A1 on vaccinia virus infected L929 cells

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Abstract

Triterpenic compounds, such as glycyrrhizic acid (GRa) and carbenoxolone (CBX), have a synergistic effect with prostaglandin A1 on the inhibition of vaccinia virus (VV) replication in L929 cells. The fractional inhibitory concentration (FIC) values for GRa and CBX were 0.5 and 0.25, respectively. In the supernatant of triterpene treated cells, increased production of some prostaglandins was shown, whilst cell-associated prostaglandins and prostaglandins of the A series were only slightly influenced by the presence of triterpenes. From these findings there is no evidence that prostaglandin production and metabolism could be involved in the antiviral activity of triterpenes.

Keywords: Antiviral compounds; Prostaglandins; Triterpenes; Glycyrrhizic acid; Carbenoxolone

Triterpenic compounds of the saponine group, such as glycyrrhizic acid (GRa), glycyrrhetinic acid (GNa), carbenoxolone (CBX), cicloxolone (CCX) and some of their derivatives, have demonstrated activity against both RNA and DNA viruses (Baran et al., 1973; Pompei et al., 1979, 1983; Dargan and Subak-Sharpe, 1985). Viral inhibition has been found in vitro and has also been confirmed in vivo in some herpetic human infections (Poswillo and Roberts, 1981).

Studies on the mechanism of action of triterpenes have not yet allowed any specific targets in viral synthesis to be identified, but rather these compounds have been found to affect one or more steps in the cellular processes that control viral morphogenesis (Dargan and Subak-Sharpe, 1985, 1986). Furthermore, viral glycoprotein syn-

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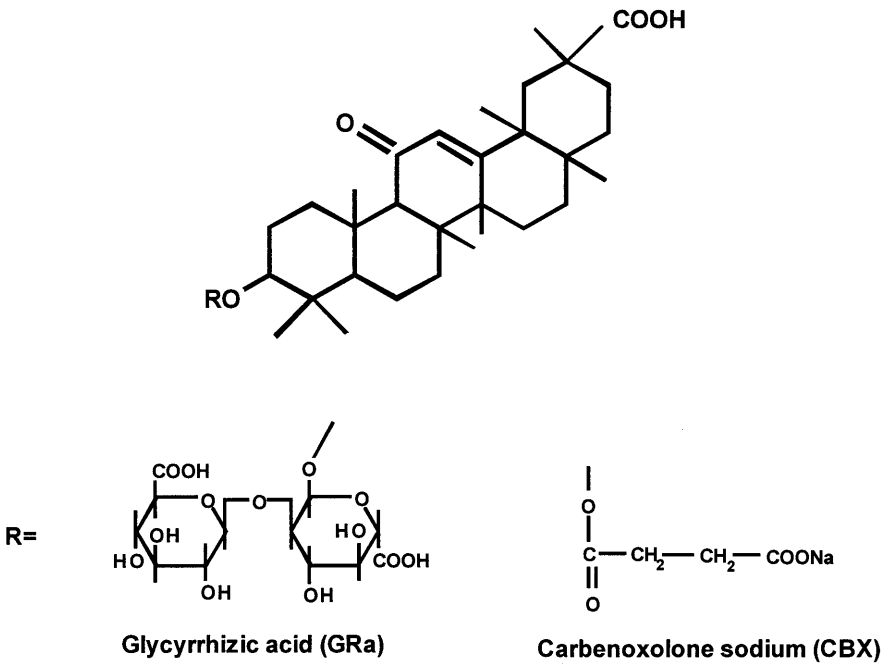

Fig. 1. Chemical formulae of carbenoxolone and glycyrrhizic acid.

thesis is irreversibly impaired by GRa at non-toxic doses in herpes simplex virus-infected cells, (Pompei and Marcialis, 1985), but higher doses of the drug also inhibit cellular glycoprotein synthesis.

Dargan et al. (1992a,b) subsequently claimed that CCX and CBX act, partly by inhibiting virus particle production, but predominantly through a dramatic impairment of the quality of the progeny virions that are produced. They concluded that CBX and CCX exhibit little specificity in their antiviral activity. Moreover, triterpenes did not directly affect HSV DNA synthesis, whilst virus glycoproteins are markedly reduced by triterpenoid treatment. Also protein sulfation is reduced, but phosphorylation is only slightly affected.

GRa and CBX are known to possess anti-inflammatory and anti-ulcer properties (Van Huis and Kramer, 1981; Capasso et al., 1983; Wan and Gottfried, 1985; Robert, 1986). The possible role of prostaglandins in these effects has been proposed, because CBX was shown to induce prostaglandin synthetase and to inhibit prostaglandin-metabolizing enzymes (Peskar et al., 1976; Vapaatalo et al., 1978). In addition, Santoro et al. (1982, 1983, 1987) have found that prostaglandins (PGs) of the A and J series exert a highly selective inhibition of the replication of several animal viruses. These findings were confirmed by other authors with different viruses and cells (Ankel et al., 1985; Zavagno et al., 1987; Parker et al., 1995). Furthermore, Amici et al. (1994) recently reported that the induction of a heat-shock protein was responsible for a specific viral protein inhibition in prostaglandin A1-treated virus-infected cells.

The possible interactions between the triterpenes GRa and CBX (Fig. 1) and PGs in their antiviral activity against vaccinia virus (VV) replication in L929 cells were analyzed in this paper.

1. Synthesis of prostaglandins in the presence of triterpenes

In order to define whether the prostaglandin metabolism could be involved in the antiviral

activity of triterpenes, the synthesis of PGs in the presence of either GRa (Sigma) or CBX (ISF, Milan, Italy) was studied in L929 fibroblasts (ICN-Flow, Irvine, UK). L929 monolayers in 35 mm petri dishes (2×10^6 cells per plate) were grown in T199 medium supplemented with 8% fetal calf serum (FCS, ICN-Flow). Then, the medium was substituted by T199 with 2% FCS containing 0.5 μCi/ml of [^{14}C] arachidonic acid (AA, Amersham, UK, $\approx$600 000 CPM/plate). To some of the plates CBX and GRa were added at concentrations of 20 μg/ml and 2 mg/ml, respectively. The cells were incubated for a further 6 h. The supernatant was then collected and the PGs were concentrated in a SEPAK C18 column (Millipore Italia, Milan, Italy). Columns were first washed with H_2O, next, with H_2O-ethanol 85/15 v/v and then with petrol ether. Finally the PGs were eluted with 3 ml of acetonitrile. This sample was dried under a flux of nitrogen and dissolved again in 0.2 ml of acetonitrile, which was kept at -30°C until it was used for thin layer chromatography (TLC).

To investigate cell associated PGs, cell monolayers were washed three times with fresh Hanks' solution (HBSS). PGs were extracted with 10 ml acetonitrile–acetic acid 99/1 v/v. The solution was dried under nitrogen flux. The extract, resuspended in 0.2 ml of acetonitrile, was separated on 0.25 mm TLC plates. The elution solution was chloroform (10 ml), methanol (7 ml), formic acid (1 ml). Each sample was spotted onto the 0.25 mm TLC plates (Carlo Erba, Milan, Italy) in an amount containing exactly 15 000 CPM. After drying, the TLC plates underwent autoradiography on Kodak × AR films for 5 days at -70°C. A standard mixture of commercial PGs (Sigma, St. Louis) was run in the TLC with the radioactive extracts and was used as control for recognizing the corresponding PG spots on the autoradiogram. PG spots, corresponding to the PG standards, were scraped off from the TLC plates, put in vials with 4.5 ml of scintillation liquid and counted in a LKB 1211 Rackbeta β-counter. The amount of PGA1 recovered from the cells was detected by the use of an HPLC apparatus (Waters 440) according to the method described by Watkins and Peterson (1982).

Preliminary results of the TLC analysis of cell associated and medium released prostaglandins in lipid extracts from cultures grown in the presence of [^{14}C] labeled arachidonic acid showed no major differences of radioactive metabolites (data not shown).

2. Synergistic effect with triterpenes and prostaglandin A1

A virus yield reduction assay was used to study the effect of the association of PGA1 with triterpenes on VV. L929 cell monolayers were used in all the experiments of virus inhibition and the viral titer was determined in VERO cells. All the assays were done in triplicate. The fractional inhibitory concentrations (FICs) of the drugs were determined as described by Ishii et al. (1993). A FIC equal or lower than 0.5 indicate a significant synergistic effect between the combined drugs. Cytotoxicity was detected as the percent of inhibition of cell multiplication.

The antiviral activity of GRa and CBX in association with PGA1 is reported in Table 1. Both GRa and CBX, at subinhibitory doses, significantly potentiate the suppressive effect of various concentrations of PGA1 on VV growth in L929 mouse fibroblasts. The IC_{50}'s of PGA1, GRa and CBX were 2, 1000 and 20 μg/ml, respectively. Synergistic effects were evident when 10 μg and 5 μg/ml of CBX were added to 0.5 and 1 μg/ml of PGA1, respectively. Similarly, a potentiating effect was observed when 500 μg/ml of GRa was associated with 1 μg/ml of PGA1. The FIC values determined for CBX in combination with PGA1 were from 0.5 to 0.25—for GRa it was 0.5. No significant increase of cytotoxicity was observed with the various drug combinations.

Experiments designed to define whether triterpenes could elicit their activity by interacting with prostaglandin metabolism, led to the conclusion that PGA, which is known to possess a potent antiviral activity, is slightly influenced by

Table 1
Antiviral activity and cytotoxicity of glycyrrhizic acid and carbenoxolone alone and in combination with various concentrations of prostaglandin A1

Prostaglandin A1 μg/ml	Antiviral activity			Cytotoxicity % of control
	Compounds	50% inhibitory concentration μg/ml	(FIC)[a]	
0	Glycyrrhizic acid	1000	—	10
0.5		1000	—	12
1		500	(0.5)	10
2		125	—	15
0	Carbenoxolone	20	—	5
0.5		10	(0.5)	8
1		5	(0.25)	10
2		2.5	—	14

[a] The fractional inhibitory concentrations (FICs) are reported for those combinations that showed a clear synergistic effect.

cell treatment with triterpenes and its increase cannot explain the inhibitory effect of triterpenes on viruses or the enhancement of anti-VV action resulting from the association of triterpenes with PGA1. Although it is clear that triterpenes could influence prostaglandin metabolism by increasing the arachidonic acid mobilization from the intracellular pool (Pompei et al., 1989), there is no direct evidence that triterpenes exert their antiviral activity through an increase in cellular antiviral prostaglandins.

Thus, although triterpenes and prostaglandins present some analogies in their action on viral replication, it is likely that their effects are independent from each other. In addition, the enhancement of viral inhibition observed by the combined use of these two types of molecules, suggests that they act on different steps of viral replication, resulting in a more potent viral suppression. Santoro et al. (1982) suggested that the antiviral action of PGA on VV-infected cells could be due to a specific block of protein synthesis and that this effect could be mediated at the level of transcription and/or translation of viral mRNA. Since triterpenes such as CBX and GRa were claimed to cause a strong suppression of HSV-1 glycoprotein production in various types of cells (Dargan and Subak-Sharpe, 1986), different steps in both protein and glycoprotein synthesis may be the main targets for the combined action of triterpenes and prostaglandin A1.

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