The Pathogenesis of Cianidanol-Induced Fever

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Summary. Recently, there has been a variety of reports of adverse drug reactions during therapy with the flavonoid Cianidanol (Ci), a cytoprotective radical scavenger, especially involving haemolytic anaemia and drug fever.

To elucidate whether the fever was due to a direct, antigen-independent interaction of Ci with immune competent cells, its effect on macrophage (MΦ) function and early biochemical events during lymphocyte activation has been examined.

A direct interaction of Ci with MΦ was demonstrated, resulting in increased secretion of interleukin-1 (IL-1). The influence of Ci on lymphocyte activation was assessed by measuring levels of cyclic AMP and GMP. At high concentrations of Ci, cAMP levels were increased, and at low Ci concentrations cGMP levels were elevated. Both findings are correlated with lymphocyte proliferation and function, which is increased at low and decreased at high concentrations of Ci. The synthesis of prostaglandin E2 by MΦ, an important factor in MΦ-mediated suppression, was reduced by increasing doses of Ci, which inhibited MΦ-cyclooxygenase. Ci did not affect phospholipase A2 activity.

These findings indicate that flavonoid-induced fever may be due to allergic as well as pseudo-allergic mechanisms, the latter probably caused by increased antigen-independent release of IL-1, the endogenous mediator of fever.

Key words: cianidanol, drug fever; (+)-cyanidanol-3, flavonoid, macrophage function, interleukin-1, adverse drug effect, immunotoxicity, cyclooxygenase

There are several reports of a favourable effect of the flavonoid Cianidanol (Ci; = (+)-Catechin = (+)-Cyanidanol-3) on acute and chronic liver disorders (Blum et al. 1977; Demeulenaere et al. 1981). Ci and other flavonoids have been shown to decrease the hepatotoxicity of various agents by acting as free radical scavengers and by protecting membranes by preventing lipid peroxidation (Kappus et al. 1977; Ring et al. 1976; Somani et al. 1980).

Recently, haemolytic anaemias and drug fever have been reported as adverse drug reactions during therapy with Ci (Neftel et al. 1980; Wälti et al. 1986; Brattig et al. 1981). Using the Radioallergosorbert Test (RAST), Wälti et al. (1986) found Ci-specific IgG antibodies in sera from patients with haemolytic anaemia. In contrast, the same test for antibodies was negative in patients with drug fever. There are also reports of positive in-vitro lymphocyte transformation testing both in haemolytic anaemia and drug fever in the presence of Ci, indicating either an allergic process (Brattig et al. 1981; Berg et al. 1987, Wälti et al. 1986) or antigen-independent activation of the immune system.

A similar immunoenhancing effect on various functions of peripheral blood mononuclear cells (PBMC) from young healthy donors who had not previously ingested the drug (Brattig et al. 1984; Daniel et al. 1986) has also been demonstrated, indicating that Ci may activate immune cells through antigen-independent mechanisms. A significant stimulant effect at Ci concentrations up to 64 µg/ml has been shown for spontaneous blastogenesis, mixed lymphocyte reaction, primed lymphocyte typing and other activities. In contrast, at Ci concentrations up to 256 µg/ml humoral and cellular parameters were significantly reduced.
In order to elucidate these immunomodulating properties and the mechanism of drug induced fever, the interactions of Ci with human MΦ have been investigated, as well as early events in activation of lymphocytes by various stimuli, viz. calcium influx, phospholipase A2 activation and synthesis of the cyclic nucleotides cAMP and cGMP.

Materials and Methods

Preparation of Cells

Isolation of PBMC. Peripheral blood to which was added 1% heparin 5000 IU/ml (Medac, Hamburg) was obtained from healthy volunteers. The blood was diluted 1:2 with 0.9% saline, and PBMC were isolated by discontinuous density gradient centrifugation on Ficoll Hypaque (Seromed, Berlin). PBMC were washed twice with Hanks’ BSS (Gibco) by centrifugation at 150 x g.

Preparation of MΦ for MΦ Cyclooxygenase Assays. PBMC were isolated at 4 °C and adjusted to a concentration of 2 x 10^6/ml in RPMI 1640 (Gibco), supplemented with 2 mM glutamine (Gibco), 100 μg/ml gentamicin and 5% heat-inactivated (56 °C, 30 min) human AB serum. MΦ were allowed to adhere to plastic surfaces in 250-ml culture flasks (Greiner, Nürtingen) for 1 h at 37 °C. Non-adherent cells were obtained by further incubation (1 h at 4°C) with PBS without Ca^2+ or Mg^2+, (Seromed) containing 2 mM EDTA. Purification of MΦ was assessed by alpha-naphthyl acetate esterase staining.

Preparation of MΦ for IL-1 Assays. PBMC were isolated at 4 °C and adjusted to 5 x 10^6/ml in RPMI 1640 (Gibco), supplemented with 2 mM glutamine (Gibco), 100 μg/ml gentamicin and 5% heat-inactivated (56 °C, 30 min) human AB serum. MΦ were allowed to adhere to plastic surfaces in 250-ml culture flasks (Greiner, Nürtingen) for 1 h at 37 °C. Non-adherent cells were obtained by further incubation (1 h at 4°C) with PBS without Ca^2+ or Mg^2+, (Seromed) containing 2 mM EDTA. Purification of MΦ was assessed by alpha-naphthyl acetate esterase staining.

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Assays

Phospholipase A2 Assay. Buffer substrate solution 2 ml was mixed with 1 ml Ci-containing 0.9% saline (molecular weight of Cianidanol = 290). Buffer substrate solution: mix 0.8 ml lecithin solution (dissolve 1 g lecithin (Merck) in 10 ml methanol and 3 ml Tween 20, make up to 20 ml with redist. water and filter) 1.4 ml indicator solution (dissolve 6 mg cresol red (Merck) in 1 ml methanol, add 0.1 ml 1 M MgCl2, make up to 200 ml with redist. water), 0.6 ml 0.1 M glycylglycine, 0.3 ml CaCl2 solution (10 mg anhydrous CaCl2/ml); adjust to pH 9.5 with 0.1 M NaOH and make up to 6 ml with redist. water. The reaction was started with 10 μl of a 1:5 diluted, ice-cold, commercially available phospholipase A2 preparation from porcine pancreas (Merck). Increasing hydrolysis of fatty acids from lecithin results in decreased light absorption by cresol red. Enzyme kinetics were measured at 578 nm for 20 min at 25 °C, in a Beckman DU-50 spectrophotometer. Data are given as the slope of the linear section of the graph (= d(extinction)/d(t) = reaction speed), which is at proportional to enzyme activity at substrate saturation.

Radioimmunoassay (RIA) of Prostaglandin (PG) E2. (Spontaneous Synthesis of PGE2 by MΦ). MΦ were isolated as described above, adjusted to a final concentration of 1 x 10^5/ml in serum free RPMI 1640 (supplemented with 2 mM glutamine and 100 μg/ml gentamicin, containing Ci at various concentrations) and incubated in culture tubes (Greiner) for 20 h at 37 °C. The supernatant was centrifuged at 2000 x g and frozen at −70 °C until PGE2 was determined by 125I-PGE2 RIA (NEA).

Radioimmunoassay (RIA) of Prostaglandin (PG) E2. (Fatty Acid Cyclooxygenase Assay), 1 x 10^5 MΦ were incubated for 20 h as described above and then homogenised by freezing rapidly (−70 °C) and heating gently in a water bath (37 °C) three times, followed by 3 sonication cycles of 10 s at lowest power, using the microtip of a Branson sonifier. Homogenates were incubated with shaking for 30 min, at 37 °C with 1 μg arachidonic acid (AA; Sigma). PGE2 was determined by RIA after centrifugation and filtration (Millipore 0.22 μm) of the homogenate. Cyclooxygenase activity was calculated as: PGE2=(PGE2 synthesis after addition of AA) - (PGE2 synthesis by MΦ during incubation). To allow for AA crossreactivity in the PGE2 RIA, the effect of 1 μg AA on RIA blank probes was determined.

IL-1 Bioassay. MΦ were plated as described above and incubated with or without phytohemagglutinin (PHA (Wellcome) 0.5 μg/ml final dilution) at sub-optimal concentrations. Following incubation for 20 h at 37 °C, the supernatants were removed, centrifuged at 2000 x g for 20 min and dialyzed in autoclaved standard dialysis bags for 24 h against