A POSSIBLE ACETALDEHYDE-MEDIATED CARDIOPROTECTIVE MECHANISM

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Excess alcohol consumption may affect the cardiovascular system in various ways. Adverse effects include hypertension (McMahon, 1987), arrhythmias (Ettinger et al., 1978), cardiomyopathy (Urbano-Marquez et al., 1989) and haemorrhagic stroke (Camargo, 1989). One beneficial effect of moderate alcohol consumption appears to be some protection against morbidity and mortality from coronary heart disease. This effect has been attributed to a number of mechanisms. These include an increase in the reverse transport of cholesterol as a consequence of increased levels of high density lipoprotein (Gaziano et al., 1993), decreased ADP-, collagen- and adrenaline-induced aggregability of platelets (Renaud et al., 1981; Pikaar et al., 1987; Veenstra et al., 1990; Thaulow et al., 1991), effects on the fibrinolytic system (Veenstra, 1990) and the potentially anti-atherogenic action of antioxidants in red wine (Witztum, 1994; Maxwell et al., 1994). Another possible mechanism stems from a report that acetaldehyde (10-100 μM) stimulates rat aortic rings to produce prostacyclin (PGI₂) which is both a potent vasodilator and a potent inhibitor of platelet aggregation (Guivernau et al., 1987). However, this study did not determine whether prostacyclin release was from endothelial cells, smooth muscle cells or both. We have therefore investigated whether ethanol, acetaldehyde or acetaldehyde-albumin complexes found in serum after alcohol consumption influence prostacyclin release from cultured human umbilical vein-derived endothelial cells.

After not drinking any alcohol for a minimum of 3 days, seven healthy adult males drank 750 ml of white wine (94 g of ethanol) in 45 min. Serum was separated from venous blood taken before alcohol consumption (pre-alcohol serum) and 8–9 h after consumption began (post-alcohol serum), and was stored at −70°C. Albumin was separated from 2 ml of each of the pre- and post-alcohol sera by affinity chromatography on Blue Sepharose CL-6B as described previously (Wickramasinghe et al., 1987). The fractions containing the albumin peak were pooled and dialysed against Medium 199 at 4°C for 24 h. The protein concentration in the dialysed material (pre- and post-alcohol albumin) was adjusted to 6.9 mg/ml and the material stored at −70°C until use.

Endothelial cells were isolated from human umbilical cords and cultured as described by Frearson et al. (1995). At the third passage, cells were detached by treatment with 0.05% (w/v) trypsin and 0.02% (w/v) EDTA and suspended in endothelial cell growth medium supplemented with endothelial growth factor. One-millilitre portions of the cell suspension were placed within each of the wells of 24-well tissue culture plates (0.6–0.7 x 10⁵ cells per well) and incubated for 2–3 days until confluence. The medium was then removed and the monolayers washed once with Hanks’ solution. Fresh growth medium (plus endothelial growth factor) with or without various concentrations of ethanol or acetaldehyde was added to the wells (0.5 ml per well; three wells per experimental condition) and the plates incubated for 2 h. The final concentration of ethanol was 0, 10, 25 or 50 mM and that of acetaldehyde was added to the wells (0.5 ml per well; three wells per experimental condition) and the plates incubated for 2 h. The final concentration of ethanol was 0, 10, 25 or 50 mM and that of acetaldehyde 0, 10, 30, 50 or 100 μM. In some experiments, 0.5 ml portions of pre- or post-alcohol albumin were placed over the washed cells in each of three wells and the plates incubated for 2 h: seven pairs of pre- and post-alcohol albumin from seven volun-
acetaldehyde, the concentration of 6-keto-PGF₁₂ was increased by an average of 13.3, 57.3, 327.5 and 504.8 pg/100 μl, respectively (SD 6.3, 28.0, 65.7 and 76.0 pg/100 μl, respectively; \( P < 0.01, <0.02, <0.001 \) and \( <0.001, \) respectively).

Supernatants from microcultures of cells with albumin prepared from post-alcohol sera always contained higher concentrations of 6-keto-PGF₁₂ than those from microcultures with albumin prepared from the corresponding pre-alcohol sera (Fig. 1). The increases were statistically significant (\( P < 0.001 \), paired \( t \)-test after logarithmic transformation of the data) and are attributable to the presence of unstable acetaldehyde–albumin complexes in post-alcohol sera. It has been shown that acetaldehyde can be transferred from such complexes to macromolecules in or on target cells (Wickramasinghe et al., 1987, 1994).

These in-vitro data imply that acetaldehyde and unstable acetaldehyde–albumin complexes may cause an enhanced release of prostacyclin from human endothelial cells \( \text{in vivo} \). Thus, an increased release of prostacyclin after alcohol consumption may be one of the mechanisms involved in alcohol-related vasodilatation and the protective effect of moderate alcohol consumption against coronary heart disease. Possibly, prostacyclin mediates the cardioprotective effect by its well-known action as a powerful inhibitor of platelet aggregation.

REFERENCES


