Calcium: the best gift for your baby?

The effects of calcium on endothelial cell activation: relevance to preeclampsia

Mancy Tong, Qi Chen, Man Wu, Peter Stone, Saul Snowise, Larry Chamley

Department of Obstetrics and Gynaecology, The University of Auckland.

Background & Research Aims

Preeclampsia is one of the most common diseases of pregnancy, affecting 2-7% of otherwise healthy pregnant women (1). If uncontrolled, preeclampsia can become life-threatening for both the mother and baby (2, 3). Despite these dangerous outcomes, because the precise causes of preeclampsia are still unclear, the only treatment available is delivery (3).

Preeclampsia is characterised by endothelial cell activation which occurs prior to the onset of clinical symptoms (4). Endothelial cells are cells that line blood vessel walls and they can become activated by many different factors, including inflammatory cytokines (5).

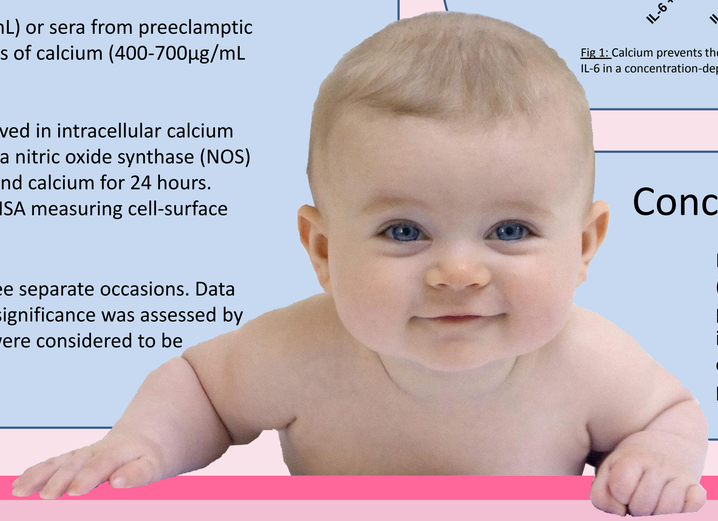
Many epidemiological and clinical studies have shown that calcium supplementation during pregnancy can greatly reduce the risk of developing preeclampsia (6, 7). This inverse relationship was first described in the 1980s but the mechanism of how calcium works is still unclear (2). Therefore, this study aimed to explore the effects of calcium on endothelial cell activation. The potential intracellular mechanisms by which calcium functions to protect the endothelium were also investigated.

Methods

Endothelial cells (HMEC-1) were cultured with IL-6 (5ng/mL) or sera from preeclamptic women (20%) in the presence of increasing concentrations of calcium (400-700µg/mL $CaCl_2$) for 24 hours.

To investigate whether nitric oxide synthase (NOS) is involved in intracellular calcium signalling, some HMEC-1 were also treated with L-NAME, a nitric oxide synthase (NOS) inhibitor (1mM), for 24 hours before the addition of IL-6 and calcium for 24 hours. Endothelial cell activation was quantified by cell-based ELISA measuring cell-surface ICAM-1 expression.

All experiments were conducted in quadruplicates on three separate occasions. Data are presented as a median ± 5/95th percentile. Statistical significance was assessed by the Mann-Whitney U-test or Kruskal-Wallis test. Results were considered to be statistically significant if $p < 0.05$.



Results

Treatment of endothelial cells with IL-6, an inflammatory cytokine, resulted in increased ICAM-1 expression which was reversed by calcium in a concentration-dependent fashion ($p = 0.001$).

In order to investigate whether calcium supplementation can prevent endothelial cell activation in a more physiological model, endothelial cells were treated with sera from women with preeclampsia. In concordance with previous published results (7), sera from women with preeclampsia activated endothelial cells compared with control sera from gestation-matched healthy pregnant women (n = 5). The addition of calcium into the cell culture prevented the activation of endothelial cells induced by preeclamptic sera ($p = 0.001$).

The treatment of endothelial cells with L-NAME, a NOS inhibitor, significantly blocked the ability of calcium to prevent the increase in endothelial ICAM-1 expression induced by IL-6 ($p < 0.03$).

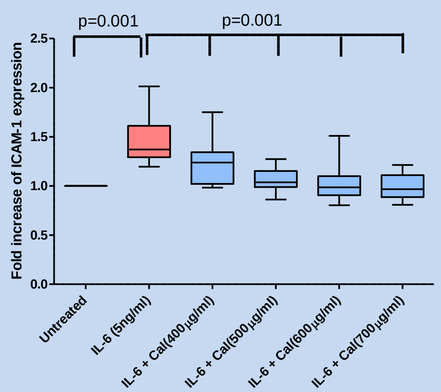


Fig 1: Calcium prevents the increase in ICAM-1 expression induced by IL-6 in a concentration-dependent manner.

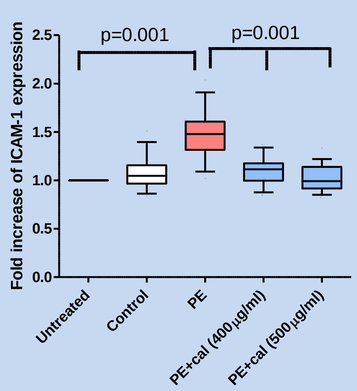


Fig 2: Calcium prevents the increase in ICAM-1 expression induced by sera from women with preeclampsia.

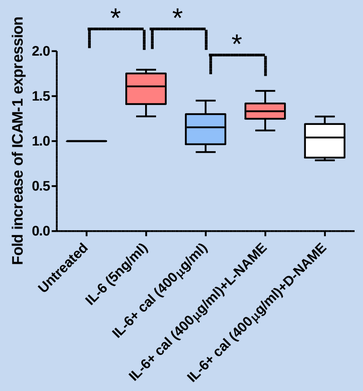


Fig 3: L-NAME prevents calcium's ability to protect the endothelium from activation induced by IL-6 (*$p < 0.03$).

Conclusion and Discussion:

Preeclampsia is a leading cause of maternal and fetal mortality and it affects over 8 million pregnant women annually (8). Recently, it has been shown that calcium supplementation during pregnancy can decrease the risk for developing preeclampsia (7). Our data suggests that calcium may increase the activity of NOS to prevent endothelial cell activation induced by inflammatory IL-6 and sera from women with preeclampsia. These findings contribute to the growing body of evidence that calcium supplementation can decrease the risk of developing preeclampsia and has suggested a possible mechanism of action of calcium.

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