Low Molecular Weight Heparin (LMWH) modulation of placental HMGB1/RAGE axis: novel understanding of Preeclampsia management.

Zenerino, C[1]; Nuzzo, AM[1]; Giuffrida, D[1]; Barrile, R[1]; Zicari, A[2]; Todros, T[1]; Rolfo, A[1].

[1]Department of Surgical Sciences, University of Turin, Turin, Italy; [2] Department of Experimental Medicine, University La Sapienza, Rome, Italy

Introduzione/Obiettivo

Preeclampsia (PE) is a severe pregnancy-related syndrome characterized by exacerbated placental inflammation. LMWH has been used during PE since several trials described its anti-inflammatory effect. Nevertheless, LMWH mechanisms of action on the placental tissue are still unclear. HMGB1 is a transcription factor with an extracellular cytokine-like function that plays a pivotal role in the inflammatory process. HMGB1 bound to its receptor RAGE (Receptor for Advanced Glycation End products) is able to induce the transcription of the pro-inflammatory cytokine TNF-α and IL-6. LMWH binds to HMGB1 in-vitro, thus changing its structural conformation and inhibiting its activity. Since HMGB1 has been previously found up-regulated in PE, in the present study we investigated LMWH's modulation of HMGB1/RAGE axis and its targets TNF-α and IL-6 in the placental tissues

Materiali e metodi

Human placental villous explants (n=32) were excised from physiological placentae (n=4), cultured in HAM F12 medium and treated for 24h by LMWH 0.5 units (U;

Parnaparin, Alfa Wassermann, Italy) or plain culture medium (controls). HMGB1, TNF-α and IL-6 mRNA expression was assessed by Real Time PCR while HMGB1 and RAGE protein expression was determined by Western Blot assay. HMGB1/RAGE binding affinity was assessed by RAGE immunoprecipitation (IP) followed by HMGB1 immunoblot performed on the resulting pellets and RAGE-deprived placental lysates.

Risultati

We reported significantly decreased gene expression levels of HMGB1 and TNF-α (p<0.05, 2.45 and 1.85 Fold Decrease) and a markedly decreased IL-6 (1.27 Fold Decrease) in placental villous explants treated by LMWH 0.5U vsuntreated controls. In contrast, HMGB1 protein levels were increased (p=0.044, 2.13 Fold Increase), while no differences in RAGE protein levels were found in placental villous explants treated by 0.5U LMWH compared to untreated controls (p>0.05). Finally, HMGB1 significantly decreased in the IP pellet (p=0.022, 1.60 Fold Decrease) and it increased in the RAGE-deprived lysates (p=0.003, 4.45 Fold Increase) of LMWH-treated villous explants compared to controls.

Conclusioni

Herein, we described a direct effect of LMWH on placental HMGB1/RAGE axis modulation. Indeed, HMGB1 protein increase accompanied by decreased HMGB1, TNF-α and IL-6 gene expression level in LMWH-treated villous explants confirm the anti-inflammatory effect of heparin and it suggest an impairment of HMGB1 extracellular functions. Indeed, HMGB1 in the LMWH-treated placental lysates after RAGE IP strongly suggest an heparin-mediated HMGB1 conformation change able to diminish its affinity for RAGE, as previously demonstrated in other systems. Our study provides new important insights on the LMWH's anti-inflammatory effect observed in PE women.