Department of Clinical Science, Intervention and Technology (CLINTEC),
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Critical evaluation of nitric oxide as an immuno-modulator in humans

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ABSTRACT

Introduction: Nitric oxide (NO) has been shown to possess anti-inflammatoryproperties. In a porcine endotoxin model the combination of intravenously (iv) administrated glucocorticoid (gc) and inhaled NO (iNO) compared to gc or iNO given separately, blunted the inflammatory response in vital organs. The ischemia and reperfusion (I/R) syndrome has an essential role regarding the pathogenesis in a number of clinical conditions. Animal and human studies have shown favourable effects with exogenously administrated NO in different I/R models.

Aim: The overall aim of this thesis was to study the potential of inhaled NO as amodulator of the inflammatory response in humans. In study I-III two different concentration of iNO in combination with gc was studied and evaluated using clinical parameters and cytokines in plasma (study I-II) as well as microparticles in plasma (study III). In study IV we evaluated whether knee surgery in spinal anesthesia during tourniquet could be used as a model to study I/R, and if so, if this could be attenuated by iNO.

Methods: In study I-III an endotoxin model was employed in 30 healthy human volunteers participating in 60 experiments. Study III is based on samples taken separately in study II. The studies were double-blind, cross-over and randomised with regard to iNO and placebo (nitrogen, N2), i.e. every volunteer had iv endotoxin (2 ng/kg) and iv gc (2 mg/kg) combined with iNO in one experiment (iNO/gc) and withplacebo (placebo/gc) in the other. In study I endotoxin was given before gc and iNO(30 ppm lasting for 5 hours), while in study II iNO (80 ppm, lasting for 7.5 hours) wasinitiated first, followed by endotoxin and gc. Clinical symptoms were recorded and blood samples collected. In study IV patients consecutively submitted to kneearthroplasty in spinal anaesthesia were included. As a standard procedure a tourniquet was used to create a bloodless surgical field. The patients were randomised into three groups (n=15). Groups 1 and 3 were either receiving iNO 80 ppm or placebo throughout the entire operation, whereas group 2 received iNO 80 ppm just in the beginning and in the end of the operation, hence, no iNO during the period with activated tourniquet. Blood samples and muscle biopsies were collected during theoperation. Adhesion molecules before and after the ischemic period were analysed.

Results: In study I and II endotoxin elicited typical flulike symptoms e.g. headache and fever as well as activation of cytokine levels. In study III there was an increase in platelet and monocyte microparticles (MP) while endothelial derived MP were unaltered. Also, platelet derived MP positive to CD40L and monocyte MP positive to HMGB1 showed an early increase. There was no difference between the two treatments (iNO/gc and placebo/gc) in study I-III. In study IV no signs of endothelial cell activation or inflammatory response neither systemically nor locally in adjacent muscle were seen.

Conclusions: The present human endotoxaemic model exhibited reproducible results, thereby providing a stable and safe model for randomized studies. The combination of intravenously administered gc and iNO, elicited no anti-inflammatory effect. The endotoxin infusion in healthy volunteers resulted in an increase in plasma cytokines as well as in microparticles released from platelets and from monocytes but not from theendothelium. In patients undergoing knee arthroplasty in spinal anesthesia, the ischemia/reperfusion created by a tourniquet did not cause any signs of endothelial cell activation or inflammatory response.