Citrate reduces complement and leukocyte activation *in vitro* in human blood – already at low concentrations

Viktoria Hancock[1], Shan Huang[2], Anders Nilsson[1], Gunilla Grundström[1] and Kristina Nilsson Ekdahl[2]
[1]Gambro Lundia AB, Lund, Sweden, [2]Linnaeus Center of Biomaterials Chemistry, Linnaeus University, Kalmar, Sweden

INTRODUCTION AND AIMS

Acetate as acidifier in haemodialysis fluid is known to induce negative effects, such as nausea and increased inflammatory response. Haemodialysis fluid where acetate is substituted by citrate was developed recently; the SelectBag® Citrate concentrate gives a fluid with 1 mM citrate resulting in ~0.66 mM citrate in the blood returning to the patient. Citrate-containing dialysis fluids have shown promising results in terms of improving clearance and treatment tolerance. In the present study, the biocompatibility of citrate was examined by investigating the effects of citrate on complement and leukocyte activation in human whole blood.

METHODS

Human blood from five healthy donors was collected in the presence of 50 µg/ml of the thrombin inhibitor hirudin and mixed with small aliquots of citrate to final concentrations ranging from 0 to 6 mM citrate. After 1 hour of incubation at 37°C, EDTA was added to terminate the activation. Subsequently, complement activation was measured as generation of C3a, C5a and TCC, and leukocyte activation was measured as up-regulation of CD11b expression on granulocytes [1].

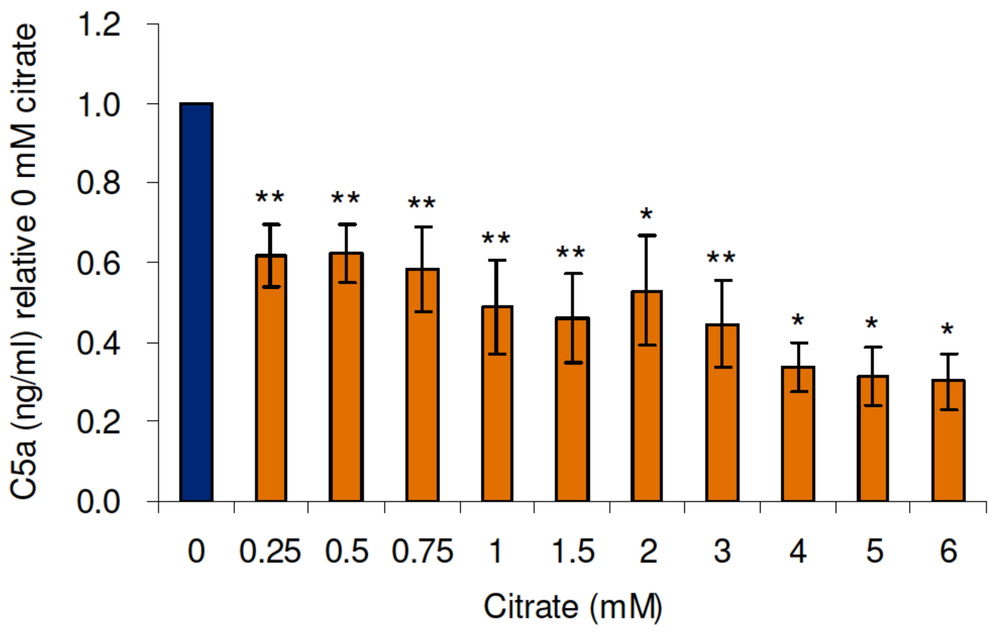

Figure 1. Complement activation as indicated by the generation of C5a in human blood. Error bars indicate SEM. **P* <0.05, ***P* <0.01.

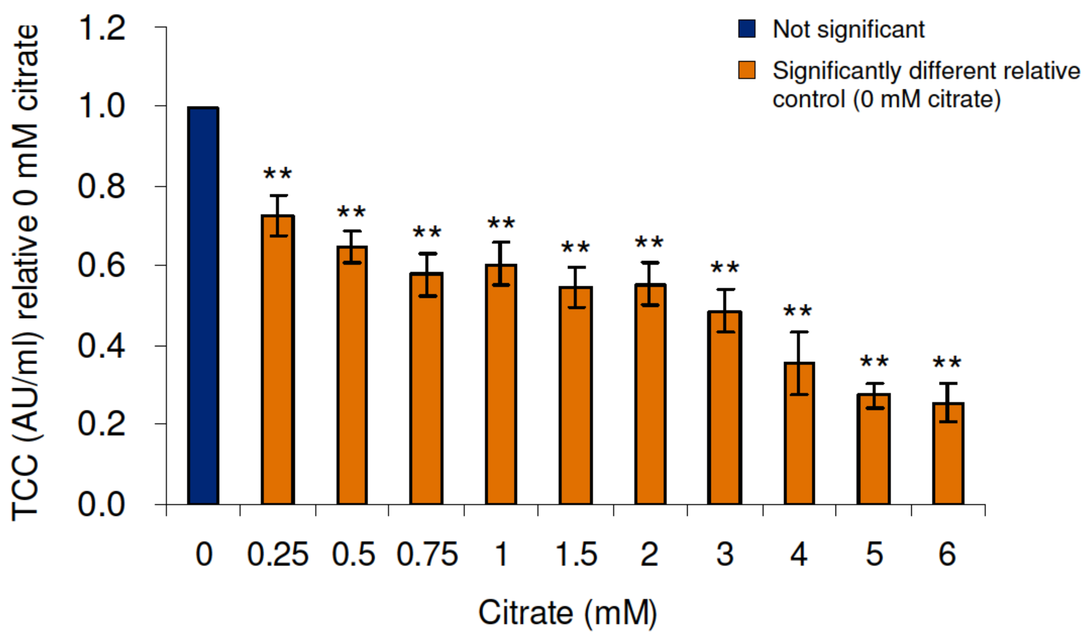

Figure 2. Complement activation as indicated by the generation of terminal complement complex C5b-9 (TCC) in human blood. Error bars indicate SEM. ***P* <0.01.

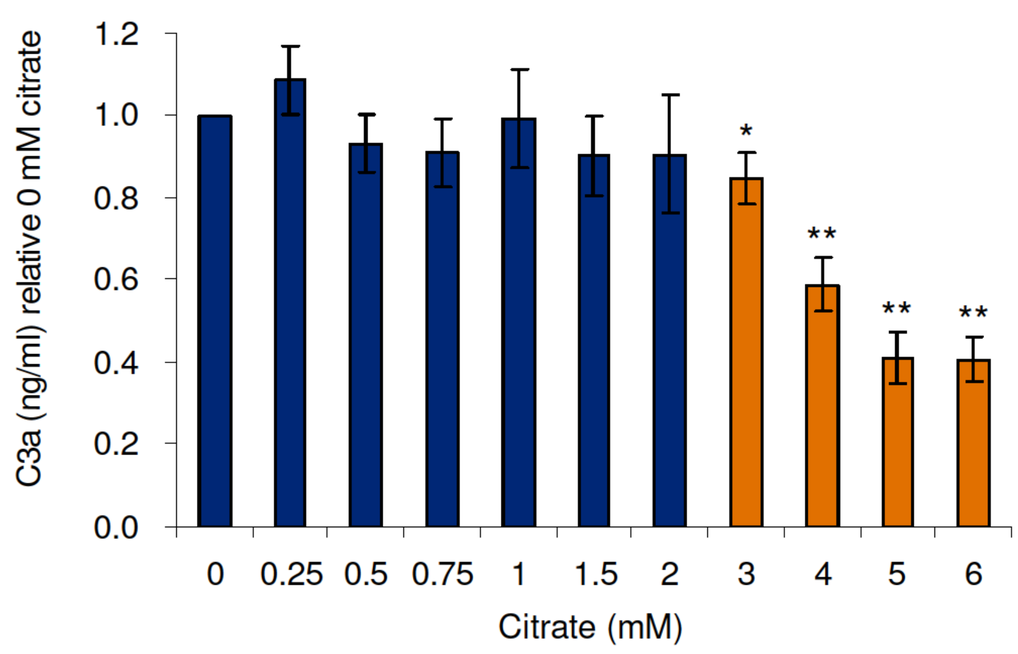

Figure 3. Complement activation as indicated by the generation of C3a in human blood. Error bars indicate SEM. **P* <0.05, ***P* <0.01.

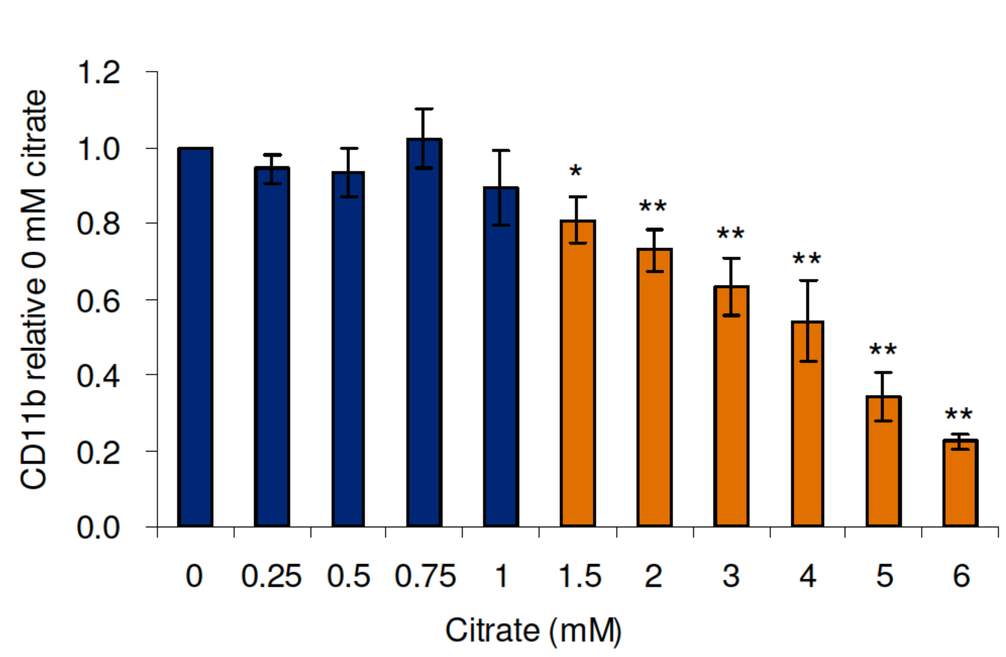

Figure 4. Granulocyte activation measured as expression of CD11b, immunoassay technique described in [1]. Error bars indicate SEM. **P* <0.05, ***P* <0.01.

RESULTS

Complement activation was significantly reduced in the presence of citrate. C5a and TCC showed a reduction of 38% and 27%, respectively, already in the presence of 0.25 mM citrate compared with control without citrate, and the reduction was further enhanced at higher citrate concentrations up to 74% and 70%, respectively, in the presence of 6 mM citrate (Figs 1 and 2). C3a showed significant reduction only at the higher concentrations of citrate – by 41-59% in the presence of 4-6 mM citrate (Fig. 3). **Leukocyte activation** was significantly reduced in the presence of 1.5 mM citrate and above, measured as a reduction in expression of CD11b by 19% with 1.5 mM citrate – up to a 78% reduction at the highest citrate concentration applied, 6 mM (Fig. 4).

DISCUSSION / CONCLUSIONS

- Citrate is a potent reducer of leukocyte and complement activation in human whole blood *in vitro*. Complement and leukocyte activation were significantly reduced at relatively low citrate concentrations (0.25 mM and 1.5 mM, respectively), similar to those currently employed in citrate haemodialysis.
- Mathematical modelling indicates that during a typical dialysis treatment with a fluid containing 1 mM citrate the blood returning to the patient contains about 0.66 mM citrate. The systemic concentration is highly dependent on the patient's citrate metabolism; mathematical modelling indicates a systemic concentration of 0.1-0.4 mM citrate after 4 hours of dialysis – which is in agreement with our measured systemic concentrations of around 0.3 mM citrate post dialysis.
- Our results reveal that substituting acetate for citrate in dialysis fluids might contribute to a more biocompatible dialysis by reducing activation of the innate immune response.

REFERENCES

1. Nilsson *et al*. 1998. Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation. Blood 92:1661-7.

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Viktoria Hancock
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