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The Effect of Citrate on Human Cells

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Background

Citrate is widely used in haemodialysis (HD), either as a buffer in dialysis fluids for chronic HD or as a regional anticoagulant in continuous renal replacement therapy (CRRT). In both cases, the concentration of citrate in the blood of the patient will increase above physiological levels.

Citrate is metabolised in the citric acid (TCA) cycle, a mitochondrial metabolic pathway providing energy for the cell. In previous studies, citrate in dialysis fluid has been associated with advantages such as reduced treatment-induced inflammation[1] and reduced oxidative stress[2]. However, the effect of elevated levels of citrate on human cells has not been studied in great detail.

The objective of this study was to investigate to what extent citrate is transported into the cells and elucidate the effects of citrate on central metabolic pathways.

Methods

Human lung fibroblast cells, (MRC-5, ATCC® CCL-171™) were seeded and treated with citrate (1, 2, 4 and 8 mM). Whole genome expression analysis (Affymetrix) was performed in triplicates in the presence of 0, 1 and 8 mM citrate after 1 hour exposure. The metabolites citrate, pyruvate, succinate, NADH and lactate were measured using colourimetric enzymatic assay kits (Sigma-Aldrich). Intracellular ATP generation was measured using ATPlite® kit (Perkin Elmer).

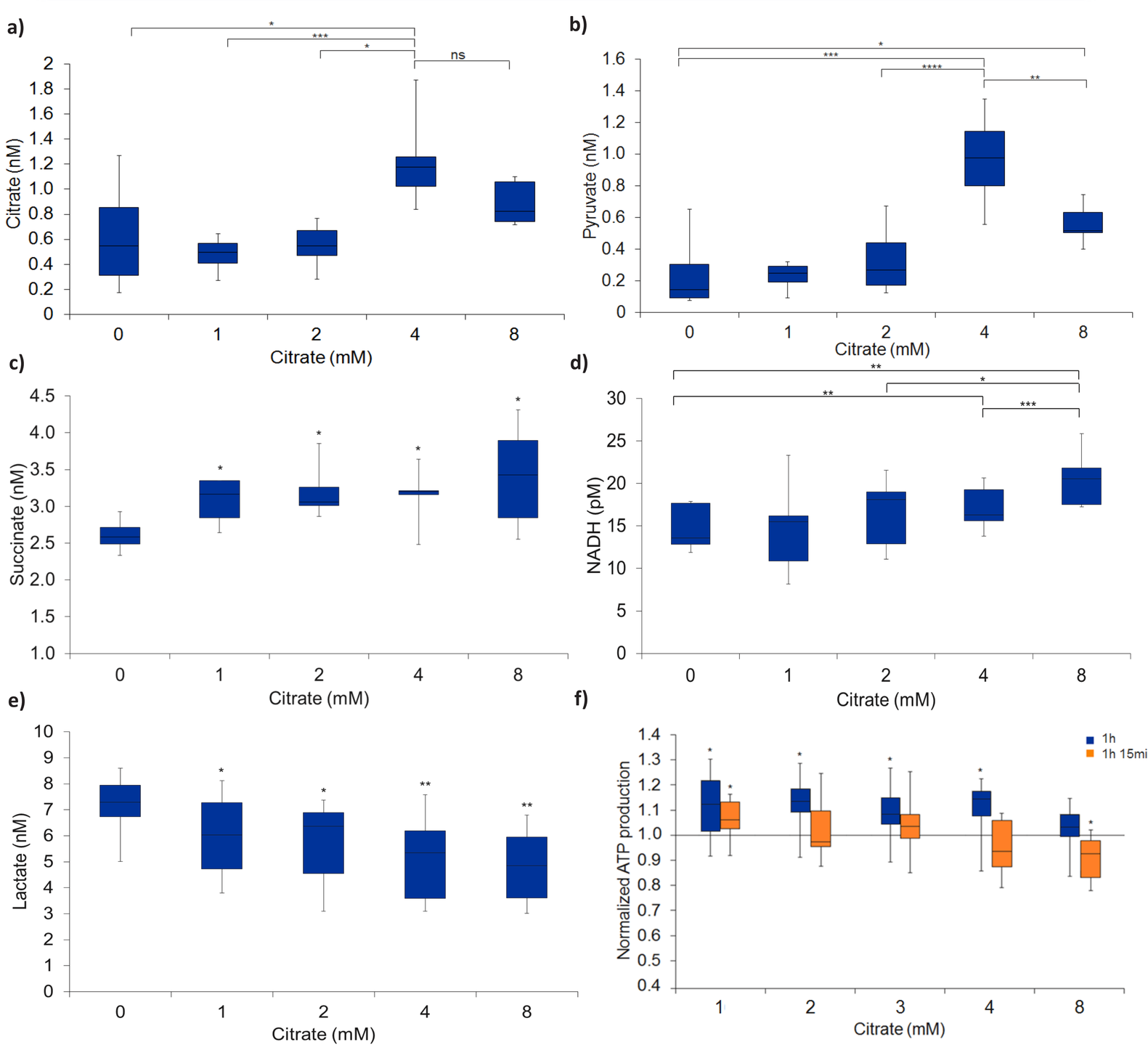

Figure 1. Intracellular levels of a) citrate, b) pyruvate, c) succinate, and d) NADH after 1-h treatment with different concentrations of citrate (0-8 mM). e) Lactate level after 3-h treatment with citrate. f) Change in ATP production after treatment with citrate for 1 h and 1 h 15 min compared with control without citrate. Asterisks indicate significant differences compared with control (without citrate), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Results

- No genes encoding proteins in the citric acid cycle showed any change in gene expression after citrate treatment (Table 1).
- Genes encoding proteins in the electron transport chain, such as complex 1, were upregulated after 1 h both in samples treated with 1 and 8 mM citrate (Table 1).
- Gene data showed a down-regulation of genes encoding subunits in the ATP synthase in samples treated with 1 and 8 mM citrate for 1 h (Table 1).
- The intracellular citrate level was significantly increased 1 h after addition of 4 mM citrate compared with control (Figure 1a). No significant differences in the level of citrate after treatment with 1, 2 and 8 mM citrate were detected.
- Exposure to high citrate levels (i.e. 4 and 8 mM) resulted in increased intracellular levels of pyruvate, succinate and NADH after 1 h (Figure 1b-d).
- Exposure to lower citrate levels (i.e. 1 and 2 mM) revealed a significant increase in intracellular succinate level – but did not show any differences in the concentration of citrate, pyruvate or NADH compared with control (Figure 1b-d).
- After 3 h of treatment with citrate the intracellular lactate level was decreased in all samples compared with control (Figure 1e).
- A significantly higher level of ATP was observed in the presence of citrate compared with control without citrate – with exception for cells treated with the highest citrate concentration, i.e. 8 mM citrate (Figure 1f).

Conclusions

- The increased levels of intermediators involved in the metabolic pathway of citrate indicate that citrate is transported into the cells and metabolised in the TCA cycle. However, these increased levels are not reflected in the results of the gene transcription analysis.
- The presence of 1-4 mM citrate resulted in an increased ATP production in human fibroblasts. Conversely, the presence of a high citrate concentration (i.e. 8 mM) resulted in a reduced ATP production as well as a down-regulation of ATP synthase genes.
- It has been shown that a high activity of complex 1 can protect against oxidative stress[3]. Our observed up-regulation of complex 1 encoding genes in the presence of citrate, infers that complex 1 activity might be involved in the mechanism of citrate's anti-oxidative properties.
- Our results indicate that citrate is metabolised to pyruvate – an α-ketoacid with anti-oxidative properties[4]; another possible mechanism by which citrate exerts its anti-oxidative properties.

Table 1: Up- or down-regulated genes in the presence of 1 and 8 mM citrate compared with 0 mM citrate after 1 h. The genes are grouped in mitochondrial pathways according to the Reactome pathway database.

Pathways	1 h	
	1 mM	8 mM
Citric acid cycle (19 genes)	0	0
Respiratory electron transport (107 genes)	↑ 2	↑ 2
Formation of ATP by chemiosmotic coupling (18 genes)	↓ 3	↓ 4

References

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