Ali Tunç Tuncel
Dr. med.

Maleic Acid-Induced Nephrotoxicity in Proximal Tubule Epithelial Cells of the Human Kidney

Fach: Kinderheilkunde
Doktorvater: Prof. Dr. med. Stefan Kölker

The human kidney is one of the most essential organs of the human body with the important task of filtrating blood. In order to fulfill this task the cells of the human kidneys rely on continuous and efficient production of molecular energy. Even the slightest disruption of the energy metabolism can lead to fatal results within the renal cells. The De Toni-Debré-Fanconi syndrome is a disease of the proximal tubule epithelial cells of the kidney, which leads to clinical consequences such as hypophosphatemic rickets, growth failure and osteomalacia, due to insufficient reabsorption of numerous metabolites. Application of maleic acid is one of the most established methods in examining these effects *in vitro*.

This study was conducted to examine the nephrotoxic effects of maleic acid on human proximal tubule epithelial cells *in vitro* to induce renal Fanconi syndrome and to answer the following questions:

1. Are maleic acid and structurally similar methylmalonic acid toxic to cultivated human proximal tubule epithelial cells?
2. Does maleic acid impair the energy metabolism?
3. Is calcium involved in maleic acid-induced cytotoxicity?
4. Can maleic acid-induced cytotoxicity be prevented by therapeutic interventions?

In order to answer these questions human proximal tubule epithelial cells have been treated primarily with maleic acid. Lactate dehydrogenase concentrations in treatment buffers have been used as a marker for cell death as well as stainings with ethidium homodimer and calcein-AM. Activities of glycolysis enzymes, citric acid cycle enzymes and respiratory chain complexes were analyzed with a spectrophotometer before and after maleic acid treatment. Moreover possible rescue mechanisms have been examined using different amino acids and inhibitory substances (e.g. Ca^{2+} channel blockers). All statistical analyses were performed by SPSS for Windows 16.0 software.

The present study shows that maleic acid exerts toxic effects on human renal proximal tubule epithelial cells. On the other hand, methylmalonic acid, a structurally similar organic acid, does not have nephrotoxic effects. *Figure 32* is a synopsis of maleic acid-induced effects on the human proximal tubule epithelial cell summarizing major results of this study. The

intake of maleic acid probably occurs through the organic anion transporter OAT4, whose inhibition reduces maleic acid cytotoxicity. Once in the cell, maleic acid induces Ca^{2+} overload by opening nifedipine-sensitive L-type Ca^{2+} channels and activating calcium-dependent signaling pathways, e.g. inositol-1,4,5-triphosphate receptor, which also acts as a Ca^{2+} channel found on the membrane of the endoplasmic reticulum. The excess calcium in the intracellular space leads to cell death through:

1. Opening of chloride channels, resulting in cell swelling;
2. Activation of the calpain pathway, which could be inhibited by PD150606;
3. Mitochondrial dysfunction, followed by a decline in energy production.

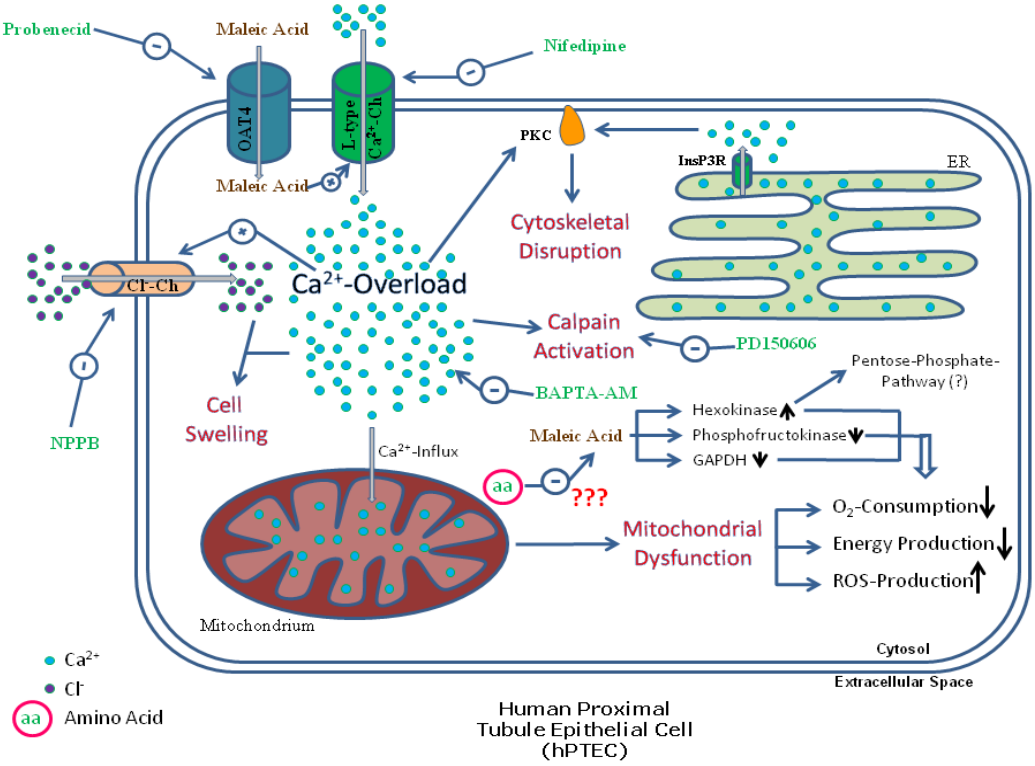

Figure 32 An overview of maleic acid cytotoxicity on a human proximal tubule epithelial cell.

In addition, maleic acid affects the activities of the glycolytic enzymes reinforcing the disruption of the ATP-producing pathways. Although the cytotoxicity of maleic acid could be attenuated with some amino acids, such as L-glutamate, glycine and L-alanine, the exact mechanism of this effect and its therapeutic impact remains yet to be elucidated.

In conclusion, this study provides experimental evidence that maleic acid-induced toxicity is mediated by disturbed calcium homeostasis, in particular calcium overload of the endoplasmic reticulum and subsequently activation of calpain proteases. It is accompanied by disturbed energy homeostasis, by directly affecting enzyme activities of glycolysis, tricarboxylic acid cycle and respiratory chain. This disruption in energy metabolism constitutes the basis of transport disorders, especially of the Na^{+}-K^{+}-ATPase (Castano et al.,

1997), resulting in a resorption deficit of the proximal tubule epithelial cells and finally, renal Fanconi syndrome (Maesaka and McCaffery, 1980).