INTRACELLULAR CALCIUM AND TRANSMEMBRANE CALCIUM FLUXES IN CHRONIC RENAL FAILURE PATIENTS

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Dedicated to my parents

ABSTRACT

Candidate: **AM Koorts**
Title: **Intracellular calcium and transmembrane calcium fluxes in chronic renal failure patients**
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Intracellular calcium is a major determinant of a wide variety of cell functions and thus of organ function. In order to get a clear picture of the intracellular calcium status it is preferable to assess the content of the various intracellular calcium pools as well as the characteristics of the transmembrane calcium movements, i.e., the magnitude of the transmembrane Ca^{2+} flux upon stimulation and the rate of the subsequent return to baseline levels. The first aim of this study was to establish and evaluate the methods in the laboratory. The methods investigated include atomic absorption spectrometry, graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry for the determination of the total cell calcium content, fluorescence spectrophotometry for the determinations of intracellular free Ca^{2+} and transmembrane Ca^{2+} movements and transmission electron microscopy for the localisation of intracellular calcium. The methods eventually identified as feasible included fluorescence spectrophotometry for the determination of intracellular free Ca^{2+} and transmembrane Ca^{2+} movements and transmission electron microscopy for the localisation of intracellular calcium. The newly developed fluorescent calcium indicator, fura-PE3, was presently shown to be the most reliable fluorescent indicator for the intracellular free Ca^{2+} determinations. The best method for the calcium localisation by transmission electron microscopy was an adaptation of the antimonate precipitation technique. The following objectives were set in order to contribute to the knowledge in chronic renal failure; examination of the intracellular free Ca^{2+} content in the neutrophils of end stage renal failure patients on maintenance haemodialysis treatment, as the result of renal failure, dialysis treatment and medication combined; examination of the characteristics of the transmembrane Ca^{2+} movements; investigation of the intracellular calcium distribution in the neutrophils; exploration of a possible link between the alterations in intracellular calcium status and factors known to influence the calcium status, including the lipid composition of the membrane, the oxidative status as reflected by anti-oxidant vitamin levels, as well as the levels of parathyroid hormone, and ionised serum calcium.
This study involved 14 chronic renal failure patients on maintenance haemodialysis. An increase in intracellular free Ca^{2+}, the magnitude of the transmembrane Ca^{2+} flux upon fMLP stimulation and an increase in the rate of the subsequent decrease in intracellular free calcium were found. In separating the patients into those receiving rHuEPO and those not receiving rHuEPO, it was seen that the significance in the increase in intracellular free Ca^{2+} could be ascribed to the values obtained in those patients receiving rHuEPO – despite the fact that they were the only patients receiving calcium channel blockers. No overt indications of oxidative stress could be detected by anti-oxidant vitamin levels. Nevertheless, a decrease in the content of specific membrane fatty acids occurred, supporting the previous suggestions of the presence of a mild chronic inflammatory condition in the chronic renal failure patient on maintenance haemodialysis treatment. These results suggest that factors other than those associated with uraemia, such as rHuEPO administration, might result in an increase in intracellular free Ca^{2+} in cells of CRF/MHT patients. The magnitude of the rHuEPO–induced increase in intracellular free Ca^{2+} and the effects of the various calcium channel blockers need urgent further investigation as ineffective counteraction of the rHuEPO effect, as indicated by the relative ineffectivity of Norvasc, may have serious side-effects.

Keywords: Intracellular calcium, fluorescent calcium indicator, transmission electron microscopy, haemodialysis patients, recombinant human erythropoietin

ABSTRAK

Kandidaat: **AM Koorts**
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Intrasellulêre kalsium speel 'n hoofrol in die regulering van verskeie selfunksies en dus van orgaanfunksie. Om 'n duidelike beeld te bekom van die intrasellulêre kalsium status is dit verkieslik nodig om die verskeie intrasellulêre kalsiumstore te ondersoek asook die eienskappe van die beweging van kalsium oor die membraan, insluitend die grootte van die kalsium fluks met stimulering van die sel en die tempo van die daaropvolgende verlaging na basaal vlakke. Die eerste doel van hierdie studie was die daarstelling en evaluering van die metodes in die laboratorium. Die volgende metodes was ondersoek, atoom absorpsie spektrometrie, grafiet-oond atoom absorbsie spektrometrie, induktief-gekoppelde plasma massa spektrometrie vir die bepaling van die totale kalsium inhoud in die sel, fluoressente spektrofotometrie vir die bepaling van intrasellulêre vry kalsium en kalsium flukse en transmissie elektron mikroskopie vir die lokalisering van intrasellulêre kalsium. Die metodes wat as voldoende geïdentifiseer was het die volgende ingesluit fluoressente spetrofotometrie vir die bepaling van intrasellulêre vry kalsium en kalsium flukse en transmissie elektron mikroskopie vir die lokalisering van intrasellulêre kalsium. Die onlangs ontwikkelde fluoressente kalsium indikator fura-PE3 was as die mees betroubare fluoressente indikator vir kalsium aangewys. Die mees betroubare metode vir kalsium lokalisering was 'n adaptasie van die antimoon-presipiterings tegniek. Die volgende was as mikpunte gestel vir die moontlike bydrae van nuwe inligting t.o.v. chroniese nierversaking; die ondersoek van die intrasellulêre vry Ca^{2+} inhoud in die neutrofiel van eindstadium nierversakings pasiënte wat hemodialise behandeling ontvang, soos bepaal deur die gesamentlike invloed van nierversaking, dialise en medikasie; die ondersoek van die eienskappe van die kalsium flukse; die ondersoek van die intrasellulêre kalsium distribusie in die neutrofiel; ondersoek na die moontlike verband tussen die verandering in die intrasellulêre kalsiumstatus en faktore wat die kalsiumstatus kan beïnvloed insluitend die volgende, die lipied komposisie van die membrane, die oksidatiewe skade soos gereflekteer deur die anti-oksidatiewe vitamien vlakke, asook paratiroïedhormoonvlakke en geïoniseerde serum kalsium.
Veertien chroniese nierversakingspasiënte wat hemodialise behandeling ontvang was ingesluit in die studie. Die volgende veranderings is aangetoon, 'n verhoging in die intrasellulêre vry kalsiumvlakke, 'n verhoging in die grootte van die intrasellulêre kalsium fluks met fMLP stimulering en 'n verhoogde tempo van die daaropvolgende verlaging in intrasellulêre vry kalsium. Met die verdeling van die pasiënte op grond van eritropoïetien behandeling of nie kan die waargenome verhoging in intrasellulêre vry kalsium toegeskryf word aan die pasiënte wat eritropoïetien ontvang, t.s.v. die feit dat hierdie pasiënte kalsium kanaal blokkers ontvang. Die vitamienvlakke dui nie op 'n verhoging in oksidatiewe stres nie, alhoewel 'n verlaging in spesifieke vetsure in die membrane aangetoon word. Hierdie verlaging in spesifieke membraan vetsure ondersteun vorige aanduidings van 'n chroniese lae-graadse inflammatoriese toestand teenwoordig in chroniese nierversakings pasiënte wat hemodialise behandeling ontvang. Die resultate impliseer dat faktore anders as die wat met uremie geassosieër word, soos rekombinante eritropoiëtien toediening, moontlik 'n verhoging in intrasellulêre vry kalsium in selle van chroniese nierversakings pasiënte wat hemodialise behandeling ontvang mag veroorsaak. Die omvang van die rekombinante eritropoiëtien geïnduseerde toename in intrasellulêre vry kalsium en die effekte van verskeie kalsium kanaal blokkers benodig dringende verdere ondersoek omrede die oneffektiewe blokkering van die rekombinante eritropoiëtien effekte, soos tans aangedui vir Norvasc, mag lei tot ernstige newe-effekte.

Sleutelterme: Intrasellulêre kalsium, fluoressente kalsium indikator, transmissie elektron mikroskopie, hemodialise pasiënte, menslike rekombinante eritropoïetien

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