Expanded Haemodialysis Therapy of Chronic Haemodialysis Patients Prevents Calcification and Apoptosis of Vascular Smooth Muscle Cells in vitro

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BACKGROUND

Vascular calcification is common in patients with chronic kidney disease and is associated with cardiovascular mortality. Vascular calcification is an active process promoted by hyperphosphatemia. This process is mediated in part by inflammatory processes in vascular smooth muscle cells (VSMC) and inhibited by anti-calcific proteins such as matrix Gla protein (MGP) and Fetuin-A under normal conditions. The increased risk of mortality is associated with increased levels of the proinflammatory cytokine growth differentiation factor 15 (GDF-15). Insufficient removal of certain cytokines using conventional hemodialysis may contribute to vascular calcification.

High cut-off (**HCO**) membranes showed a reduction in the serum levels of soluble TNF receptors, vascular cell adhesion molecule (VCAM) and other markers of inflammation, but this is accompanied by marked loss of albumin.

High retention onset (HRO) dialysis membranes, or middle cut-off, with a steeper cutoff were developed and have been shown to provide clearance for middle-sized molecules while limiting albumin filtration. The potential impact of expanded hemodialysis therapy with an HRO membrane on vascular calcification has not yet been reported.

OBJECTIVE

The study examined the impact of HRO dialysis membrane on the process of the in vitro calcification of VSMC in a well-established cell culture model.

METHODS

The study used serum samples from the Permeability Enhancement to Reduce Chronic Inflammation (PERCI II) clinical trial,[1] which included 45 patients. Every patient was dialyzed 3x/week using medium cut-off membrane (MCO-Ci 400) and high-flux (HF) membranes (Revaclear 400 dialyzer) for 4 weeks in a randomized order. After the second phase, an extension period of 8 weeks was added to assess the long-term effects. No dialysis performed. In a consecutive manner, half of the patients who were randomized to the HRO dialysis membrane group in the second phase were dialyzed using HRO membrane for 12 weeks.

Serum samples were collected before every first dialysis session of the week, centrifuged, frozen and then thawed for cell culture experiments. No freeze-thaw-refreeze cycles existed before the samples were used in the experiments.

Human VSMC were purchased from LifeLine Technology (Frederick, MD, USA). All cells used in these experiments came from the same donor. Cells were characterized as VSMC with alpha-SMA antibodies and passaged up to a maximum of 6 passages. During incubation, VSMC were stored in a humidified incubator at 37°C and 5% CO_2. Cells were cultured in 25-mL cell culture flasks until they were confluent. Cell number and viability were determined in a Neubauer counting chamber using Trypan blue, and cells were seeded at 100,000 cells/well on 24-well plates.

Induction and Determination of Calcification

An osteogenic medium was used to induce calcification in VSMC, and 5% of the serum samples collected from the 45 patients during the clinical trial was added. Calcification was evaluated using alkaline phosphatase (ALP) and alizarin red (AZR) after 7 and 10 days of incubation, respectively.

The water-soluble terazolium salt (WST-8) assay was used to measure the marker activity of viable cells, which served to normalize the activity of the calcification markers (ALP and AZR) of living cells.

Cell culture supernatants were collected at days 3 and 7 of incubation and later pooled, and the concentrations of calcification-associated proteins were measured.

Apoptosis rates caused by the different sera were measured via fluorescence spectroscopy after 7 days of incubation.

Results are presented as mean (standard error of the mean).

RESULTS

Calcification

ALP activity assay (Figure 1a):

- After 4 weeks of dialysis: HRO dialysis membrane induced 24% less calcification than HF dialysis (HF 2.91 (0.11) vs. HRO 2.21 (0.088), $p < 0.0001$)
- After 12 weeks of dialysis: HRO dialysis membrane induced 38% less calcification than HF dialysis (HF 3.14 (0.1) vs. HRO 1.96 (0.081), $p < 0.0001$)

AZR staining (Figure 1b)

- After 4 weeks of dialysis: HRO dialysis membrane induced 36% less calcification than HF dialysis (HF 0.31 (0.01) vs. HRO 0.19 (0.008), respectively, $p < 0.0001$)
- After 12 weeks of dialysis: HRO dialysis membrane induced 48% less calcification than HF dialysis (HF 0.30 (0.01) vs. HRO 12 weeks 0.16 (0.007), $p < 0.0001$)