Can Vitamin D Reduce Heart Muscle Damage After Bypass Surgery?

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Study protocol:

Trial Design

This randomized, double-blind, placebo-controlled study was conducted for 70 patients undergoing coronary surgery with CPB. The study was approved by the local Ethical Committee. The informed written consent was obtained from the patients prior to enrollment. Enrollment was started in December 2018 and was accomplished in April 2019. The inclusion criteria were as follows: the patients who were referred for elective and isolated CABG using CPB with vitamin D deficiency (defined as 25-hydroxyvitamin D < 20 ng/mL) and normal kidney function (creatinine < 1.5 mg/dL). Vitamin D was measured by an
immunoluminometric assay. The exclusion criteria were as follows: recent myocardial infarction, urgent CABG, nonisolated coronary surgery, redo surgery, malignant disease, the presence of acute or chronic inflammatory diseases, history of vitamin D treatment within previous 6 months, or unwillingness to participate.

Intervention

Following the informed consent, eligible study participants were randomly assigned (using a computer-generated random code) in a 1:1 ratio to receive either placebo or a total of 450 000 international units (IU) vitamin D3 (three 50 000 IU of vitamin D3 tablet daily for 3 days) before operation. The placebo group received 3 inactive medication tablets similar to those for other group, daily at the same time point (Figure 1).

Except the pharmacists, all the investigators, patients, and the medical team were blinded to the group allocation. Coronary artery bypass was performed in the culprit lesions for both groups by one surgical team. The standard protocols for general anesthesia, surgical, and CPB management were performed for all patients and have already been described in detail.

Outcome Measures

The primary outcome was the degree of heart apoptosis by measuring the caspases 2, 3, and 7 activity from right atrial specimen with immunohistochemistry staining, the serum level of anti-inflammatory interleukin-10 (IL-10) and insulin-like growth factor 1 (IGF-1), and N-terminal (NT)-pro-brain natriuretic peptide (pro-BNP). The biopsy from right atrial appendage was taken at the end of surgery after removing the venous cannula in a nontraumatic manner and then was kept in formalin and parafinized in less than 24 hours. Blood samples were collected firstly at the baseline (by passing 24 hours from hospital admission; T1), before anesthesia induction (T2), at the end of surgery after protamine reversal (T3), and also at the first postoperative day (T4) in order to measure the serum level of IGF-1, IL-10, and pro-BNP. The blood samples were centrifuged at 2500 rpm for 15 minutes within 1 hour after blood sampling, and the serum was stored at 20C until assayed.

Enzyme-Linked Immunosorbent Assay

The concentration of IL-10 was measured using a quantitative enzyme-linked immunosorbent assay (ELISA) kit (Ref: BE53101, IBL International GmbH, Hamburg, Germany). The concentration of the IGF-1 was also measured by a quantitative ELISA kit (Ref: MD58011, IBL International GmbH). The proBNP measurement was done using a commercially available 2- site chemiluminescent immunometric assay (IMMULITE 2000
NT-pro-BNP, Siemens Healthcare, Mannheim, Germany). Serum vitamin D was detected using the high-

performance liquid chromatography method (Agilent 1100 series HPLC, Germany).

Immunohistochemistry Studies

Immunohistochemical staining was performed on 5-mm thick sections. The slides were incubated at 37C for 24 hours and then deparaffinized in preheated xylene and rehydrated through descending grades of alcohol, and after that they were washed in distilled water. Heat-induced antigen retrieval was done by microwave oven with citrate buffer (pH 6.0) for anti-caspase 7 and Tris–EDTA (pH ¼ 8) for anticaspases 2 and 3. Endoperoxidase blocking was done by adding hydrogen peroxide onto the sections. Then, the protein block was added for 5 minutes, and slides were washed in Tris-buffered saline (TBS). The primary
antibody as anti-caspase 2 antibody, rabbit monoclonal (clone EPR16790, abcam), anti-caspase 3 antibody, rabbit monoclonal (clone EPR16888, abcam), anti-caspase 7 antibody, and mouse monoclonal (clone 7-1-11, abcam) antibody were added and kept for 30 minutes, then they were washed in TBS. Mouse and Rabbit
Specific HRP/DABIHC DetectionMicro-polymerKit (ab80436) were used and incubated for 15 minutes, and then were washed with TBS. 3,30-Diaminobenzidine (DAB) chromogen was added and kept for 5 minutes. Slides were washed in distilled water, and counterwas stainedwith hematoxylin. Sections containing lymph
node tissue were used as positive control. Negative control included primary antibody replaced with phosphate-buffered