



PROTEIN EXTRACTION FROM HUMAN BLOOD CONDUITS WITH MINILYS

The Cardiovascular and Thoracic research Institute, Hadassah Medical Center, Ein Kerem, Jerusalem, Israel

CONTEXT

Up to 50% of saphenous vein (SV) grafts will be occluded ten years after a coronary bypass operation, compared to only 2-5% of the mammary (IMA) or radial (RA) artery grafts. The superior patency of the arterial graft translates into improved long-term survival and freedom from cardiac-related adverse events. SV graft occlusion after bypass surgery is primarily related to accelerated graft atherosclerosis. The superiority of arterial grafts compared to SVs has been attributed to their striking resistance to atherosclerosis. In this study, we evaluated one of the primary protective cellular mechanisms against oxidative stress and atherosclerosis, Thioredoxin-1 (Trx-1) in arterial and venous conduits.

MATERIALS

- Minilys homogenizer
- Precellys lysing kit : CK28_2mL (KT03961-1-002.2) + Bulk Beads 5.0mm Zirconium Oxide Beads (KT03961-1-106)
- Samples : segments of Human Saphenous Vein (SV), Internal Mammary Artery (IMA) and Radial Artery (RA)
- Buffer : RIPA Buffer (Cell Signaling #9806, Danvers, MA, USA) + Protease inhibitors (I3786, Sigma Aldrich, St Louis, MO, USA)

PROTOCOL

- Segments of the IMA, RA and SV were placed in CK28 tubes supplemented with one 5.0mm ZO bead. 200 μ l RIPA buffer was added per 50mg tissue.
- All above samples were kept in ice.
- Samples were homogenized at RT with the following parameters : 3 runs of 90 sec at 5000rpm. Samples were placed on ice for 1 min after each run.
- Samples were centrifuged at 14000rpm at 4°C for 20 minutes.
- Supernatant was collected and frozen at -80°C before analysis by Gel electrophoresis and Immunoblotting using anti Trx-1 and anti-actin

RESULTS

The expression of Trx-1 in IMA and RA was similar and significantly higher compared to SV. These data suggest that arterial conduits express significantly higher amounts of Trx-1 compared to saphenous veins, possibly rendering them more resilient to oxidative stress and accelerated graft atherosclerosis.

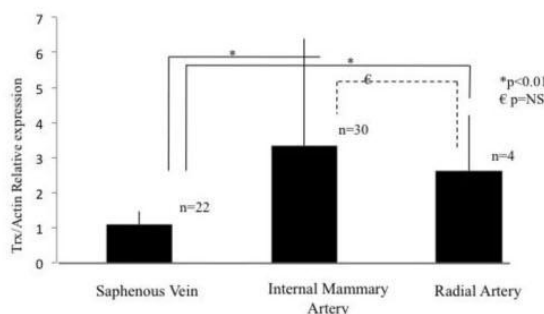


Figure 1. Differential Trx expression between conduits. (A) Protein extracts from patient conduit tissue was prepared and immunoblot analysis was performed using anti-Trx. Trx bands were quantified and normalized to the corresponding beta actin. Average from all the patients is shown. Statistical significance is indicated in the figure. NS= not significant. Number of patients is indicated for each column.

CUSTOMER

Author : Didi Matza, PhD, didi.matza@hadassah.org.il



CONCLUSION

Protein extracts from human blood conduits can be effectively recovered using Minilys. Blood conduits are hard tissues and the current short Minilys protocol replaces previous, much longer and less efficient protocols in our lab. The current Minilys protocol also prevents cross contamination between samples.

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□東京 〒162-0805 東京都新宿区矢来町 113 番地 TEL (03) 3235-0661(代) / FAX (03) 3235-0669
 □大阪 〒532-0005 大阪市淀川区三国本町2丁目12番4号 TEL (06) 6396-0501(代) / FAX (06) 6396-0508
 □福岡 〒812-0054 福岡市東区馬出 1 丁目 2 番 23 号 TEL (092) 631-1012(代) / FAX (092) 641-1285