Protective Role of Dimethyltryptamine in Preserving Donor Heart Function in a Heterotopic Rat Transplantation Model

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Results

Western blot analysis

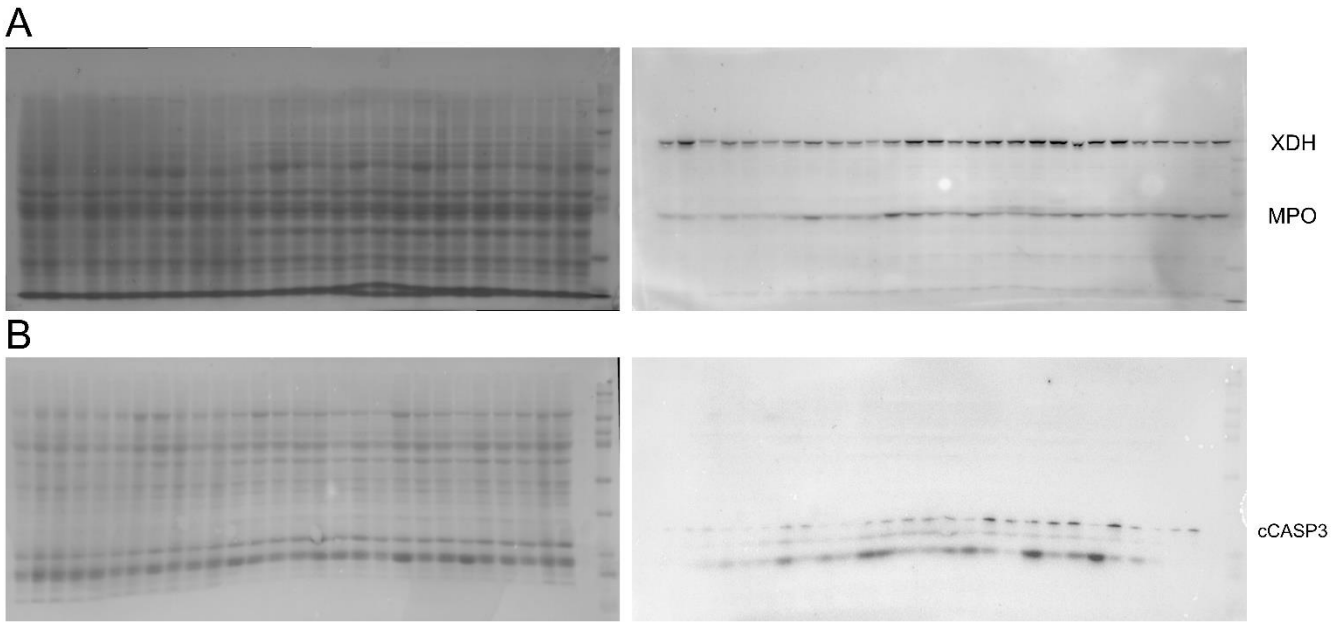

Figure S1 Representative Western blots showing total protein staining and target protein expression. (A) Total protein staining (left) and immunoblot analysis of xanthine dehydrogenase (XDH) and myeloperoxidase (MPO; right). (B) Total protein staining (left) and immunoblot analysis of cleaved caspase-3 (cCASP3; right). Samples from control (C) and DMT-treated (DMT) animals are presented on the blots in the following order (left to right): C1, C2, DMT1, DMT2, DMT3, DMT4, DMT5, DMT6, DMT7, DMT8, DMT9, DMT11, DMT13, C3, C4, C5, C6, C7, C8, C9, DMT14, DMT15, DMT16, DMT17, C10, C11, C12, and DMT18.

Methods

Preparation of DMT solution and DMT enriched Custodiol

The N,N-Dimethyltryptamine (DMT) was initially dissolved in 2.78 µL of 70% ethanol and subsequently diluted with 0.047 mL of saline solution. Donor hearts were preserved in 20 mL of Custodiol solution at 4 °C, supplemented with DMT. The preservation solution was enriched with 10.4 µL of DMT stock solution, corresponding to 0.1875 mg of pure DMT, dissolved in 0.83 µL of 70% ethanol and 9.57 µL of Custodiol.

Experimental protocol

Donor rats were anesthetized with isoflurane (3-5% for induction and 1-2% for maintenance) and heparinized (25000 IU IV), after which the heart was exposed via bilateral thoracotomy. Cardiac arrest was induced by slow infusion of cold (4 °C) Custodiol cardioplegic solution into the aortic root to perfuse the coronary circulation. The great veins and pulmonary veins were ligated en masse, and the heart was excised with the ascending aorta and aortic arch preserved to enable subsequent assessment of coronary blood flow. Following procurement, grafts were stored in cold (4 °C) Custodiol, and cold ischemic time (static storage time plus implantation time) was standardized to 60 minutes.

Recipient rats were anesthetized with isoflurane (3-5% for induction and 1-2% for maintenance), heparinized (400 IU/kg IV), and maintained at 37 °C using a heating pad. After midline laparotomy, approximately 2 cm segments of the infrarenal abdominal aorta and inferior vena cava were isolated and temporarily occluded. The donor aorta and pulmonary artery were anastomosed end-to-side to the recipient abdominal aorta and inferior vena cava, respectively, establishing coronary perfusion

of the graft in an unloaded heterotopic configuration. Upon completion of the anastomoses, heparin was antagonized with protamine (400 IU/kg IV), vascular clamps were released, and in situ blood reperfusion was maintained for 60 minutes before functional measurements.

H-Score Calculation

Immunohistochemical staining intensity was quantified using the H-score method. Following color deconvolution, the DAB optical density (OD) of each evaluated unit (pixel or cell) was classified into four intensity categories using the following thresholds:

Table S1. Predefined intensity categories used for H-Score calculation.

Category	DAB OD range	Weight
Negative	< 0.2	0
Weak positive (1+)	≥ 0.2 and < 0.4	1
Moderate positive (2+)	≥ 0.4 and < 0.6	2
Strong positive (3+)	≥ 0.6	3

3,3'-diaminobenzidine (DAB), optical density (OD)

The DAB OD thresholds (0.2, 0.4, and 0.6) correspond to the default intensity classification values used in the QuPath open-source digital pathology platform and are expressed in log10-based optical density units.

The H-score was computed as: H-score = (1 × n1+ + 2 × n2+ + 3 × n3+) / N × 100; where n1+, n2+, and n3+ denote the number of units in each positive intensity category, and N is the total number of evaluated units. The resulting H-score ranges from 0 (entirely negative) to 300 (all units strongly positive).

List of Abbreviations

BSA	bovine serum albumin
cCASP3	cleaved caspase-3
DAB	3,3’-diaminobenzidine
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HIER	heat-induced epitope retrieval
HRP	horseradish peroxidase
IOD	integrated optical density
MPO	myeloperoxidase
OD	optical density
TBS	tris-buffered saline
XDH	xanthine dehydrogenase