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Data Article

Data on sodium tetraborate as a modulation of hypertrophic intracellular signals

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ABSTRACT

The present work benefits the use of sodium tetraborate to prevent and treat hypertrophic cardiac. The data obtained from the work could serve as a reference point to compare with data obtained in vivo studies with cardiac damage. This research will be an advantage for future researches to stimulate the ones focused on developing food supplements to prevent heart diseases such as cardiac hypertrophic. This article also indicates the data on the optimal concentration of isoproterenol as an inducer of hypertrophy in cardiomyocytes. Also, data of the cytotoxic effect of sodium tetraborate on normal cardiomyocytes is revealed. Finally, data of viability, cell size, proliferation nuclear antigen (PCNA) and apoptosis is shown. The expression of transcription factors linked to hypertrophy such as GATA-4, MEF2c, NFAT, CDk9, and myogenin was also quantified by immunofluorescence. The mRNA expression of adrenergic receptors (alpha and beta), AKT1 and Erk1 / 2 and genes of early response to hypertrophy (*c-myc, c-fos, c-jun*) are also shown as Cts of RT-qPCR. GAPDH and 18 s were used as housekeeping genes.

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Specifications Table

Subject	Cardiology and Cardiovascular Medicine
Specific subject area	Cell Biology and Molecular Biology
Type of data	Photomicrographs and raw data in excel were presented
How data were acquired	The data were acquired using a conventional 40 X microscope; a confocal microscope; and a thermal cycler. As well as the corresponding tools as image software and qPCR software Instruments used: conventional 40 X microscope (Zeiss, Oberkochen, Germany); confocal microscope L100 (Zeiss, Oberkochen, Germany); thermal cycler Mx3005p (Agilent Technologies, USA), Axio-Vision software 4.8sp1 /12/2011 (Zeiss, Oberkochen, Germany); Zen version 2009 software and MxPro qPCR v.3.00 software (Agilent Technologies, USA)
Data format	Raw data in excell tables and micrographs in lsm and TIFF format
Parameters for data collection	Primary culture of mouse heart cardiomyocytes of the Balb / c strain with hypertrophy induced with isoproterenol and treated with sodium tetraborate
Description of data collection	**Sheet 1**. Raw Data Sets of Viability: The cellular viability measured by the trypan blue dye exclusion test was analyzed in the groups: CONTROL, ISOPROTERENOL AND SODIUM TETRABORATE. Two trials (test 1 and test 2) in triplicate of each group at times 0 h, 24 h, 48 h 72 h, 96 h, 120 h, 168 h (column A), were performed. **Sheet 2.** Raw Cell Area Standardization Test: The cell area was analyzed in 100 cells for the groups: ISOPROTERENOL (column B to M) and SODIUM TETRABORATE (Column N to Y). Four concentrations per group were measured at 3 different times (4 columns for each time). **Sheet 3.** Raw Data for GATA-4 vs PCNA: Raw data of nuclei positive to the transcription factor GATA-4 vs proliferation (PCNA) by immunofluorescence. **Sheet 4**. Raw Cell Area for COMPETITIVE Assay: The cell area was analyzed in 100 cardiomyocytes (Column A) in the groups: CONTROL (column B-D), ISOPROTERENOL (column E-G), SODIUM TETRABORATE (Column H-J) and COMPETITIVE Borax and Isoproterenol (Column K-M). Three times per group were evaluated. **Sheet 5**. Raw Cell Area for CARDIOPROTECTIVE Assay: The cell area was analyzed in 100 cardiomyocytes (Column A) in the groups: CONTROL (column B-D), ISOPROTERENOL (column E-G), SODIUM TETRABORATE (Column H-J) and CARDIOPROTECTIVE (Column K-M). Three times per group were evaluated. **Sheet 6.** Raw Data of apoptosis vs proliferation: Raw data of nuclei positive for apoptosis (TUNEL) vs proliferation (PCNA) by immunofluorescence. **Sheet 7.** Raw Data for GATA-4 vs MEF2c: Raw data of nuclei positive to the transcription factor GATA-4 vs MEF2c by immunofluorescence. **Sheet 8.** Raw Data for NKx2.5 vs NFAT: Raw data of nuclei positive to the transcription factor NKx2.5 vs NFAT by immunofluorescence. **Sheet 9.** Raw Data for Myogenine vs CDK9: Raw data of nuclei positive to the transcription factor myogenine vs CDK9 by immunofluorescence. **Sheet 10**. Raw Cts of RT-qPCR: Cts of expression of 1AR, β1AR, Akt1, Ekr1 / 2, *c-myc, c-fos, c-jun* by real time qPCR in different groups of cardiomyocytes treated with sodium tetraborate and isoproterenol. **Folder 1**. Micrographs for cell area analyses: Five micrographs of the standardization groups (isoproterenol and sodium tetraborate) in their different concentrations. **Folder 2.** Immunoflorescence micrographs: Five immunofluorescence micrographs of each study group organized in GATA-4 (green) vs PCNA (red), GATA-4 (green) vs MEF2c (red), apoptosis (green) vs PCNA (red) and NKx2.5 (green) vs NFAT (red).
Data source location	Institution: Hospital Infantil de México Federico Gómez City/Town/Region: Ciudad de Mexico (CDMX) Country: Mexico

(*continued on next page*)

Data accessibility	Repository name: [Mendeley Data] Data identification number: CONTRERAS-RAMOS, ALEJANDRA (2020), "Data of role of Sodium tetraborate in the Modulation of Cardiac Hypertrophic", Mendeley Data, V7, http://dx.doi.org/10.17632/tdngvdyx35.7 Direct URL to data: http://dx.doi.org/10.17632/tdngvdyx35.8
Related research article	S. Hernández-Gutiérrez[1*], J. Roque-Jorge[2], A. López-Torres[2], G. Díaz-Rosas[3], A. J. García-Chequer[3], A. Contreras-Ramos[3**]. Article JTEMB_126,569. In Press. https://doi.org/10.1016/j.jtemb.2020.126569

Value of the Data

- The present work benefits the use of sodium tetraborate to prevent and treat hypertrophic cardiac.
- This protocol can be used as a reference to compare with data obtained from in vivo studies with cardiac damage.
- This study will be an asset for future clinicians and help them focus on the development of food supplements to heart diseases as cardiac hypertrophic.

1. Data Description

Primary cultures of cardiomyocytes derived from mouse heart of the Balb /c strain were performed. To validate the optimal concentration of isoproterenol (ISO) and sodium tetraborate (Bx), cell viability was evaluated at different times (0 h, 24 h, 48 h, 72 h, 96 h, 120 h and 168 h) (Sheet 1), the cell area was also measured of 100 cardiomyocytes (Sheet 2), since the effect of size increase is a typical response of the hypertrophied cardiomyocytes. In these same groups, proliferation and the transcription factor GATA-4 were determined by immunofluorescence (Sheet 3 and raw microscopy images are shown in the Mendeley dataset). Subsequently, with the optimal concentration, the protective and competitive capacity of sodium tetraborate was evaluated in the cardiomyocytes treated with ISO. All cell area data is shown in Sheets 4 and 5. The apoptosis and proliferation data by immunofluorescence were placed on Sheet 6 and raw microscopy images are shown in the Mendeley dataset. While the hypertrophy linked transcription, factors were placed on Sheets 7, 8, 9. and raw microscopy images are shown in the Mendeley dataset. Finally, mRNAs expression of the adrenergic receptors (alpha and beta), AKT1 and ERk 1 / 2, as well as genes of early response to hypertrophy (c-myc, c-fos, c-jun) are shown as Cts of Rt-qPCR. GAPDH and 18S were used as housekeeping genes.

2. Experimental Design, Materials and Methods

Myocyte culture: Neonatal mice aged 2–3 days from Balb / c strain were used under conditions exposed in the NOM-062-ZOO-199 [1], the hearts were dissected, washed and perfused (using an insulin syringe) 3 times with PBS 1% EDTA 0.5% sterile. Myocytes were isolated using the commercial Neonatal Heart dissociation for mouse and rats kit (MACS, Miltenyi Biotec, USA). Once the myocytes were isolated, it was seeded in P35 dishes or 8-well Chamber Slide boxes (depending on the assay) in 2 mL per plate or 0.2 mL per chamber of DMEM culture medium supplemented with 10% SFB + 1X Antibiotic (100 U of Penicillin, 100 µg of Streptomycin, 0.25 µg Amphotericin B) and incubated at 37 °C and 5% CO_2 atmosphere for 24 h according to the manufacturer's instructions [2] until reaching a 75% confluence.

Sodium tetraborate and isoproterenol standardization: The myocyte cultures were treated at concentrations of 0, 13, 26 and 39 µM with sodium tetraborate (Na2B4O7. 10H20 Sigma-Aldrich,

USA) or at 0.5, 10 and 15 µM of Isoproterenol as hypertrophic inductor with exposure times of 24, 48 and 72 h in both cases.

The toxicity of the treatments in cardiomyocytes was evaluated by analyzing cell viability, cell area, quantification of proliferation nuclear antigen (PCNA), and expression of the transcription factor GATA-4 by immunofluorescence (raw data shown in http://dx.doi.org/10.17632/tdngvdyx35.8).

2.1. Study groups

Once the conditions were standardized, the following study groups were carried out: **a) The control group (CTR)**, without treatment, cultivated for 24, 48 and 72 h; **b) the hypertrophic group (ISO)**, cardiomyocytes treated with 10 µM / 24 h of Isoproterenol (Wen et al., 2020); **c) Sodium tetraborate (BX) group**, cardiomyocytes treated with 13 µM / 24 h; **d) The Competitive Test**, the cardiomyocytes will be treated simultaneously with (10 µM) isoproterenol and (13 µM) sodium tetraborate for 24 h; 48 h and 72 h. **e) The Cardioprotective Test**, after inducing hypertrophy in the cultured cardiomyocytes, these were treated with sodium tetraborate for 24 h; 48 h and 72 h.

Cell viability assay: Cell viability was evaluated by the Trypan blue dye exclusion assay, Cells were prepared adding 10 µl of cell suspension to 10 µl of Trypan blue, 1:1 ratio [3].

2.2. Hematoxylin and Eosin staining

The culture medium was removed from plate P35. Washes with 1% PBS were performed. The cells were fixed with 4% PFA and incubated for 24 h. Washes with 1% PBS were performed. Cells were post-fixed with 70% Ethanol and incubated for 24 h. All Ethanol was removed, and the cells were hydrated with distilled water for 5 min. The cytoplasm was stained with Hematoxylin, for a period of 5 min. Washes with 1% PBS were performed. The nuclei were stained with Eosin, for a period of 1 min. Washes with 1% PBS were performed. 25 µL of mounting solution (PBS / Glycerol 1: 1) was added. A coverslip was placed on the surface. We proceeded to visualize under the microscope [4].

Immunofluorescence: Cells were previously fixed with 4% paraformaldehyde, post-fixed with 70% ethanol, permeabilized with 1% PBS-0.05% Triton and blocked with universal blocker (Biogenex, USA). The primary antibodies: Proliferation Nuclear Antigen (anti-PCNA) and those for transcription factors associated with cardiac hypertrophy (anti-GATA-4, anti-Nkx2–5, anti-MEF2c, anti-NFAT, anti-CDK9 and anti-Myogenin) from Santa Cruz Biotechnology, Inc. were incubated 1hr at room temperature (RT), inside a humid chamber, with gentle shaking. Subsequently, the secondary antibody coupled to a fluorophore was incubated for 1 h at RT. The nuclei were stained with Draq7 (BioStatus, United Kingdom) for 20 min and PBS-Glycerol 1: 1 was used as the mounting medium. The coverslips were sealed with varnish. The samples were analyzed using an Axiovision LSM100 confocal microscope (Zeiss, Oberkochen, Germany) and the Zen version 2009 capture program (Zeiss, Oberkochen, Germany). Five micrographs were captured at 40X for each antibody test and the percentage of positive cells was determinate by the average of [number of positive cells / numbers of total cells].

Tunnel assay: The detection of apoptosis was carried out through DNA fragmentation by the analysis of Terminal Deoxynucleotidyl Transferase (Tdt) dUTP Nick-End Labeling (TUNEL) labeled at the free 3′-OH end with modified nucleotides coupled to fluorescein, following the instructions of the Apotag Kit (Millipore, USA). The nuclei were contrasted with Draq7 (BioStatus, United Kingdom), and PBS-Glycerol 1:1 was used as the mounting medium. The samples were analyzed by confocal microscopy. The rate of apoptosis was determined by the average of positive nuclei / total nuclei of the five fields analyzed.

RNA extraction: After the treatments, the culture medium was removed, the Petri dish was washed 3 times with 1% PBS. Cells were disaggregated using 0.05% trypsin by the method

described in Appendix. Then the cells were collected in a 1.5 mL micro tube and stored at −70 °C. RNA extraction was isolated from cells using TRIzol reagent (Life Technologies Corporation, Carlsbad CA, USA) following the manufacturerś instructions. Subsequently RNA was quantified at an absorbance 260 / 280 nm.

cDNA synthesis: The cDNA was synthesized by retro-transcription using the enzyme M-MuLV-RT (New England BioLabs, USA), according to instructions from the data sheet. 1 µg of total RNA, plus 2 µL of hexamers, plus 2 µL of dNTPs, 2 µL of buffer, 0.5 µL of RNAse Inhibitor, 0.5 µL of RT (mmlv) were used, in a final volume of 20 µL. Reactions were performed at 25 °C/15 min, 37 °C/1 h, and 70 °C/15 min. At the end, nuclease-free H2O were added to having a final concentration of 20 ng / µL of cDNA.

Real time- qPCR: The expression of α1AR(3ÁGTTCTGGCTGAGAGGGACA and 5ǴGGGGTGT GGAGGAGATAAT), β1AR (3ĆCTCTTCCTTCCAGTGCTTG and 5ŕTCTGGAGCGTTGGAGAGTT); Akt1 (3ĆACACAGCTGGAGAACCTCA and 5ÁGGGAACACACAGGAAGTGG); Ekr ½ (3ÁGGTAGTGG GAGCCTTGGTT and 5´GGTCAGAGCTGCTCCCATAG) c-myc (3ǴCCCAGTGAGGATATCTGGA and 5ÁTCGCA GATGAAGCTCTGGT); c-fos (3ĆTCCCGTGGTCACCTGTACT and 5´TTGCCTTCTCTG ACTGCTCA); c-jun (3ÁAAACCTTGAAAGCGCAAAA and 5´CGCAACCAGTCA AGT TCT CA) messengers RNA were analyzed by real-time qPCR. GAPDH and 18S were used as housekeeping genes. Gene expression profiles were visualized with SYBR Green as a detection method. The qPCR was performed on an Mx30005Tm system, using the MxPro QPCR version 3.00 software (Agilent Technologies, USA). The reagents used per reaction were as follows: 10µL of SYBR Green (1X), Primer FW (0.4 µM), Primer RV (0.4 µM), H2O ccp 17.5µL, 2.5µL cDNA (50 ng). The primers were designed with the Primer3 bioinformatics program (http://promer3.ut.ee) on a sequence of the mouse genes.

2.3. Solutions

PBS 1X: Sodium chloride (NaCl): 11. 94 g, Sodium phosphate (NaH2PO4): 8.51 g, Potassium phosphate (KH PO4): 3.48 g, make up to 1000 mL with distilled water. Sterilize at 121 °C at 15 lb pressure and store at 4 °C.

Paraformaldehyde 4% in PBS 1X: Paraformaldehyde: 4 g, PBS 1X pH 7.4: 100 mL. Procedure: Heat the PBS to 60 °C, gradually add the PFA, and stir until the solution is clear. If turbidity occurs add 1 mL of 1 N NAOH.I.3 Culture medium (DMEM + FBS 10% + AB 1x).

DMEM culture medium: DMEM: 44.9 mL, 10% Fetal Bovine Serum: 5 mL, Antibiotic 1000 U / mL: 500 µL. Procedure: In a 50 mL falcon tube, add 44.9 mL of DMEM culture medium, then add the sterile Bovine Fetal Serum, and antibiotic, close the falcon tube and mix by gentle inversion until the reagents are integrated. Store at 4 °C until use, heat to 37 °C before use.

Ethics Statement

The research protocols used in this research were approved by the ethics committee HIMFG (HIM/2014/045 SSA 1131) and all procedures performed in animal studies were compatible with ethical standards and standard “NOM-062-ZOO-199”.

CRediT Author Statement

S. Hernández-Gutiérrez and **A. Contreras-Ramos:** Conceptualization, Data curation, Formal analysis and Writing - review & editing; **Roque-Jorge, A. López-Torres, A.J. García-Chequer:** Roles/Writing - original draft; **A. Contreras-Ramos:** Funding acquisition, Project administration; **S. Hernández-Gutiérrez, A. López-Torres:** Investigation; **J. Roque-Jorge, A.J. García-Chequer** and **G. Díaz-Rosas:** Methodology, Resources; **A. Contreras-Ramos, A.J. García-Chequer** and **G. Díaz-Rosas:** Software, Supervision; Validation; Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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