THE IMMUNOMODULATOR DIMETHYL ITACONATE INHIBITS SEVERAL KEY STEPS OF ANGIOGENESIS IN CULTURED ENDOTHELIAL CELLS

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INTRODUCTION

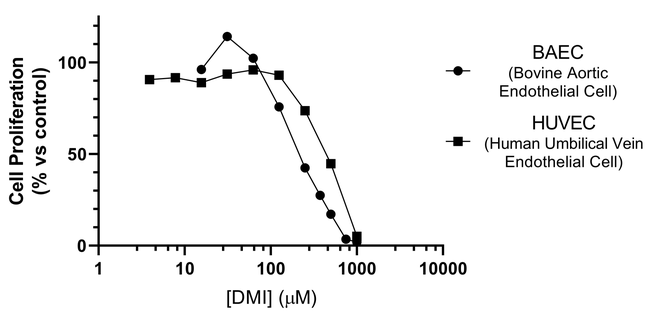
Dimethyl itaconate (DMI) is a cell-permeable derivative of itaconate, a known immunomodulator metabolite synthesized from the Krebs cycle intermediate cis-aconitate by the enzyme aconitate dehydrogenase[1]. Although DMI is not metabolized into itaconate intracellularly[2], immunomodulatory, anti-inflammatory and anti-oxidative effects has been described for this compound[3,4,5]. Some of these processes affected by DMI are directly related to angiogenesis[6]; however, nothing has been published yet in this field. The aim of this work is to evaluate the potential effect of DMI in different key steps of the angiogenic process.

METHODS:

1. Cell survival assay[7]: performed under proliferative conditions; methylthiazolyldiphenyl-tetrazolium bromide (MTT) was used after 72 h of incubation of cells with different concentrations of DMI and IC_{50} values were calculated from the survival curves.
2. Tubular-like structures formation on Matrigel[7]: endothelial cells (BAECs) were seeded in absence of serum onto Matrigel layers in presence of different concentrations of DMI, and after 5 h (when tubular-like structures were formed in negative control) photos were captured. Images were analysed by Fiji.
3. Cell cycle analysis by flow cytometry[8]: BAECs in each cell cycle phase were analysed through propidium iodide staining, after an incubation of 16 h with different concentrations of DMI.
4. Migration assay[7]: wound-healing assay was conducted in monolayers of BAECs in presence of DMI. Photos were captured at time 0, 4 h and 8 h and analysed by Fiji software.
5. Invasion assay[8]: BAECs that invade the Boyden's chamber coated with Matrigel were photographed after 16 h of incubation in presence of DMI.
6. Determination of matrix metalloproteinase-2 (MMP-2) activity[8]: gelatin zymography was performed with conditioned media of BAECs treated 24 h with DMI.

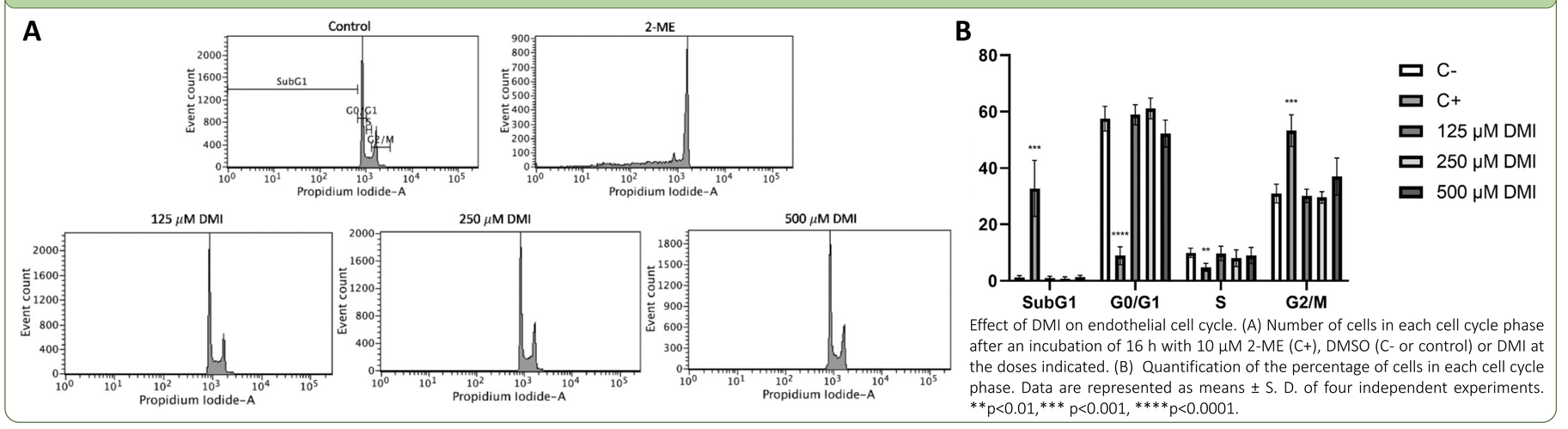
- Replicates from at least three independent experiments were performed in all assays. Statistical analysis of obtained results were performed through t-test with GraphPad Prism software and p values < 0.05 were considered statistically significant.

Endothelial cell growth is affected by DMI concentrations in the range of submillimolar



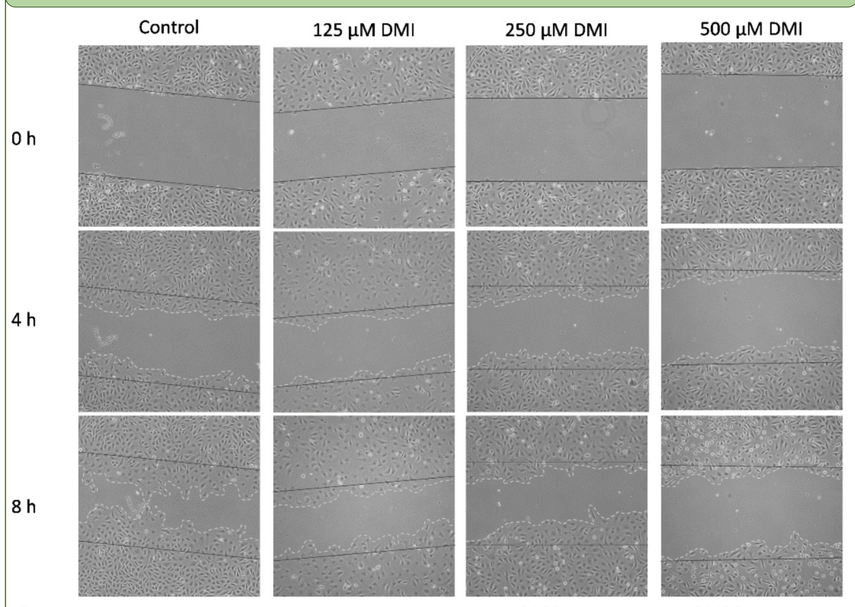
Growth of BAECs and HUVECs after 72 h treatment. Concentrations are represented in logarithmic scale. IC_{50} obtained for BAEC is 276 ± 53 μM, and 491 ± 76 μM for HUVEC for three independent experiments.

DMI does not affect cell cycle in endothelial cells

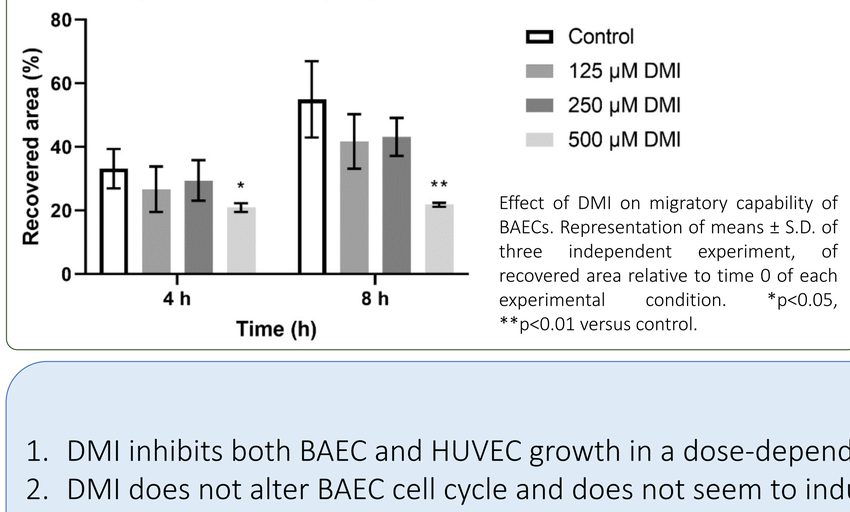


Effect of DMI on endothelial cell cycle. (A) Number of cells in each cell cycle phase after an incubation of 16 h with 10 μM 2-ME (C+), DMSO (C- or control) or DMI at the doses indicated. (B) Quantification of the percentage of cells in each cell cycle phase. Data are represented as means ± S. D. of four independent experiments. **p<0.01,*** p<0.001, ****p<0.0001.

DMI inhibits endothelial cell migration

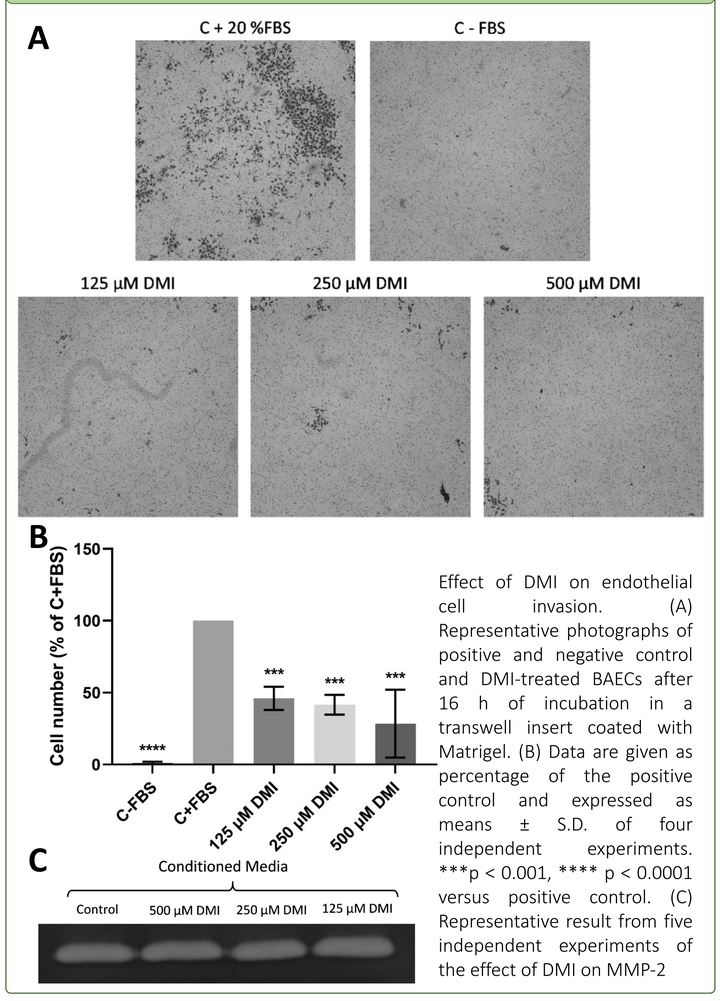


Representative images of wound-healing assays performed in BAECs in presence of DMSO (control) or DMI (125, 250 or 500 μM) at different time-points. Black lines represent the initial wound (t0) and discontinuous grey lines represent migrating front.



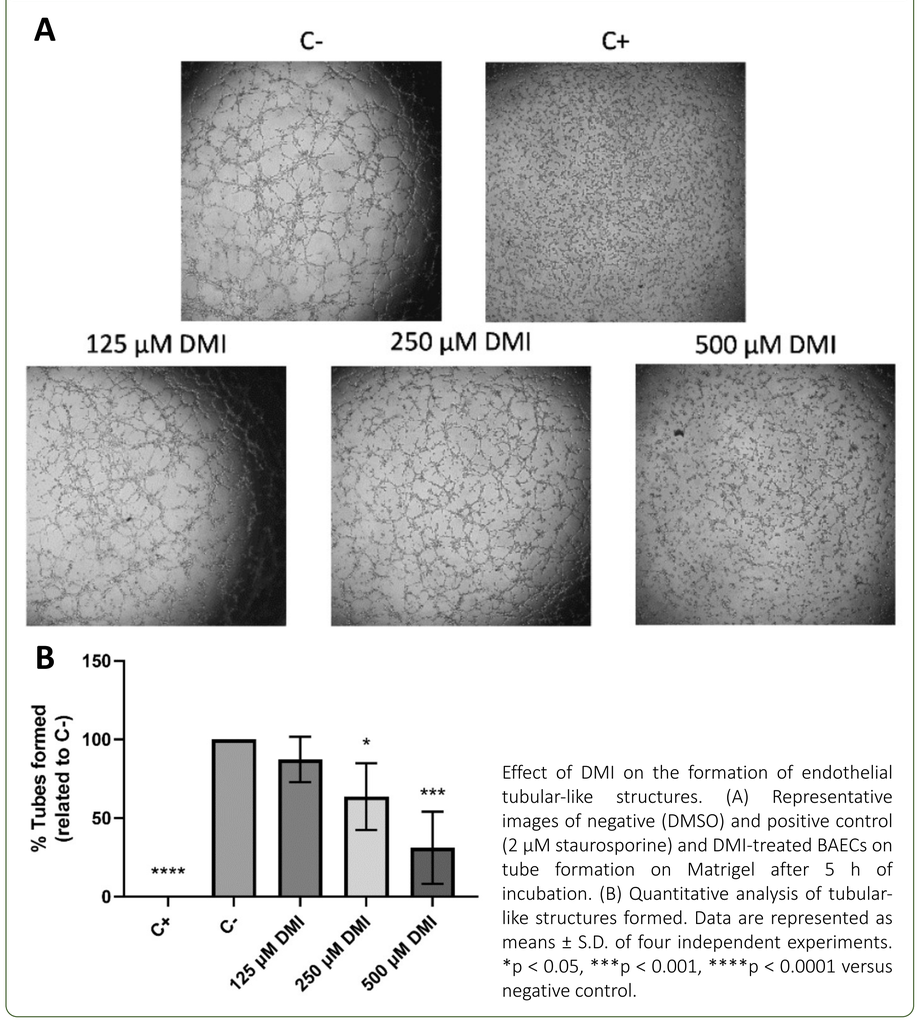
Effect of DMI on migratory capability of BAECs. Representation of means ± S.D. of three independent experiment, of recovered area relative to time 0 of each experimental condition. *p<0.05, **p<0.01 versus control.

DMI inhibits endothelial cell invasion without affecting MMP-2 secretion



Effect of DMI on endothelial cell invasion. (A) Representative photographs of positive and negative control and DMI-treated BAECs after 16 h of incubation in a transwell insert coated with Matrigel. (B) Data are given as percentage of the positive control and expressed as means ± S.D. of four independent experiments. ***p < 0.001, **** p < 0.0001 versus positive control. (C) Representative result from five independent experiments of the effect of DMI on MMP-2

DMI inhibits endothelial tubular-like structure formation on Matrigel



Effect of DMI on the formation of endothelial tubular-like structures. (A) Representative images of negative (DMSO) and positive control (2 μM staurosporine) and DMI-treated BAECs on tube formation on Matrigel after 5 h of incubation. (B) Quantitative analysis of tubular-like structures formed. Data are represented as means ± S.D. of four independent experiments. *p < 0.05, ***p < 0.001, ****p < 0.0001 versus negative control.

CONCLUSIONS:

1. DMI inhibits both BAEC and HUVEC growth in a dose-dependent manner with IC_{50} values in the submillimolar range of concentrations.
2. DMI does not alter BAEC cell cycle and does not seem to induce BAEC apoptosis.
3. The results obtained with DMI in the tubule formation assay on Matrigel, the wound healing assay and the invasion assay suggest that DMI can be considered a new anti-angiogenic compound.

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