ALDH2 Activator Prevents Myocardial Infarction Injury Caused by Nitroglycerin Tolerance

Supplementary Material

Figures

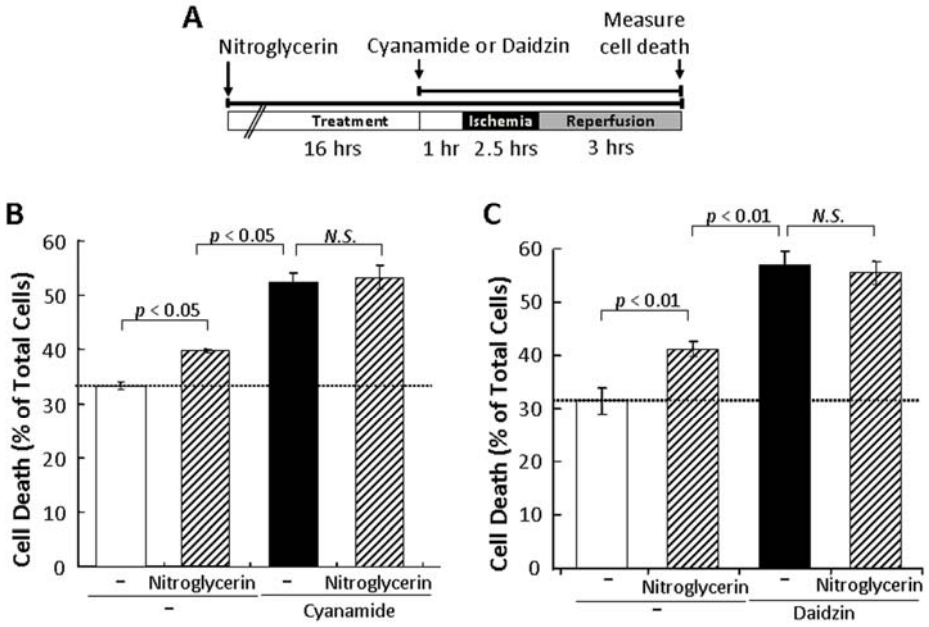


Fig. S1. Nitroglycerin did not induce increased cardiomyocyte death in the presence of an ALDH2 inhibitor, after hypoxia-reoxygenation, in culture. (**A**) Experiment protocol for ischemia-reoxygenation in cultured cardiomyocytes. (**B**) 16 hours of continuous treatment with nitroglycerin (100 μM) increased cardiomyocyte death after 2.5 hours of hypoxia and 3 hours of reoxygenation, in culture (p<0.05 *vs*. control). No increase in cell death was observed by the same nitroglycerin treatment after hypoxia-reoxygenation, when ALDH2 was inhibited by the ALDH2 inhibitor cyanamide (5mM, for 1 hour prior to hypoxia-reoxygenation) (*N.S.*; non significant difference compared with the group treated with cyanamide alone). Data are mean±SEM (n=3). (**C**) Similar results to these with cyanamide

were found with another ALDH2-selective inhibitor, daidzin (1μM, for 1 hour prior to hypoxia-reoxygenation) (*N.S.*; non significant difference compared with the group treated with daidzin alone). Data are mean±SEM (n=8).

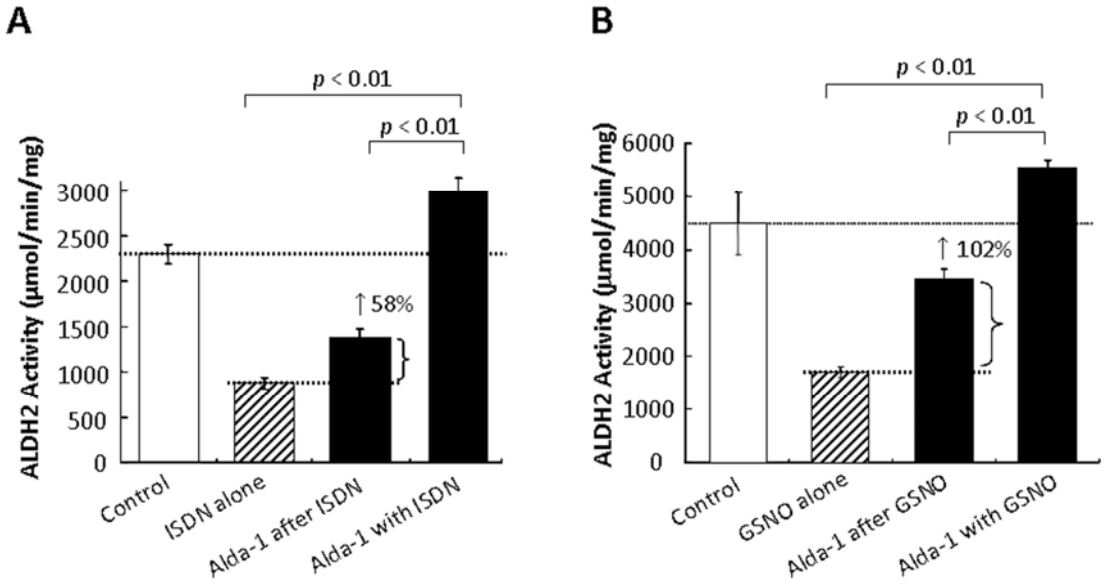


Fig. S2. Alda-1 prevented inactivation of ALDH2 caused by ISDN and GSNO, *in vitro*. (**A**) The effect of ISDN (protocol as in **Fig. 1A**). Treatment with ISDN (50 μM) for one hour inhibited ALDH2 activity by 59±3% (p<0.01) and subsequent Alda-1 (20 μM) treatment increased ALDH2 activity by 58±7% (p<0.01). Co-treatment with Alda-1 blocked ISDN-induced inactivation of ALDH2. Data are mean±SEM (n=3-6). (**B**) One-hour treatment with GSNO (40 μM; protocol as in **Fig. 1A**) inhibited ALDH2 activity by 49±6% (p<0.01). Subsequent addition of Alda-1 (20 μM) increased ALDH2 activity by 2 fold. In contrast, co-incubation of Alda-1 completely prevented GSNO-induced ALDH2 inactivation (p<0.01 *vs.* the group treated with GSNO alone). Data are mean±SEM (n=3).

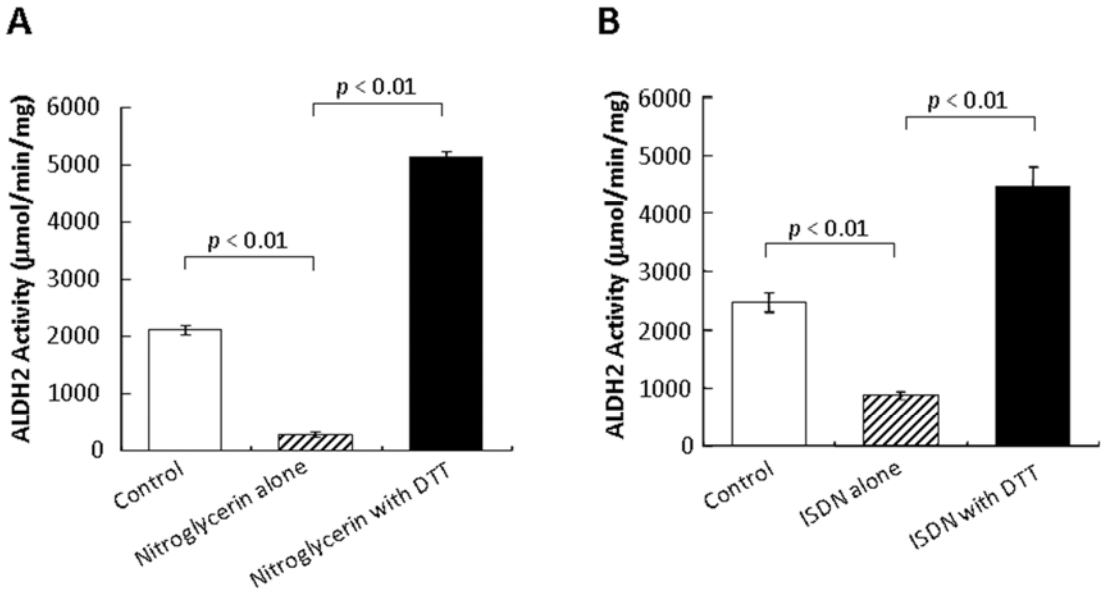
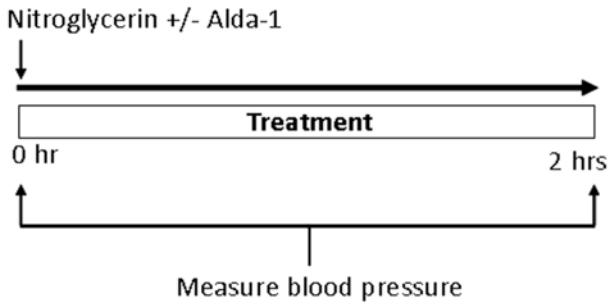


Fig. S3. DTT prevented inactivation of ALDH2 induced by nitroglycerin and ISDN, *in vitro*. (**A**) The effect of the reducing agent DTT on nitroglycerin-induced ALDH2 inactivation was determined (protocol as in **Fig. 1A**). Treatment with nitroglycerin (1 μM) for one hour inhibited ALDH2 activity by over 85%. DTT alone increases the catalytic activity of ALDH by 2.5 fold. Co-treatment of nitroglycerin with DTT prevented nitroglycerin-induced inactivation of ALDH2, bringing ALDH2 activity to levels similar to ALDH2 with DTT alone. (Data are mean±SEM (n=3). (**B**) One-hour treatment with ISDN (50 μM; protocol as in **Fig. 1A**) significantly inhibited the ALDH2 activity; the inhibition was prevented by co-incubation with ISDN and DTT. Data are mean±SEM (n=3).

Tables

A

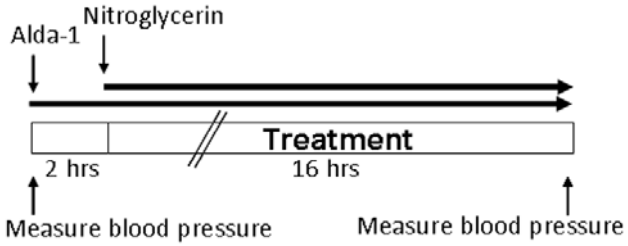


B

Time Point	N	Heart Rate	Systolic Pressure	Diastolic Pressure
Before NTG +/-Alda-1 Treatment	8	387 ± 8	108 ± 4	69 ± 5
Nitroglycerin		392 ± 7	89 ± 1*	48 ± 3*
Nitroglycerin + Alda-1		377 ± 8	82 ± 2*	46 ± 2*

Table S1. Acute treatment with nitroglycerin decreased blood pressure. (**A**) Protocol for acute treatment and blood pressure measurement. (**B**) Blood pressure and heart rate were measured before treatment and after 2 hours of nitroglycerin treatment (7.2 mg/kg/day) with or without Alda-1 (16 mg/kg/day) in rats. Systolic and diastolic blood pressures were decreased after 2 hours of treatment with nitroglycerin alone or with nitroglycerin and Alda-1, compared to those before treatment. Heart rate remained unchanged in all treatment groups. * $p<0.01$ *vs*. Before Treatment. Data are mean±SEM (n=8).

A

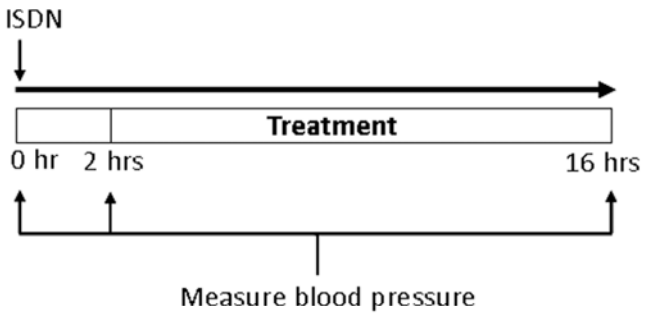


B

Treatment Group	N	Before Treatment			After Treatment		
		Heart Rate (beats/ min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)	Heart Rate (beats/ min)	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
Control	6	398 ± 11	110 ± 6	73 ± 7	388 ± 22	107 ± 5	75 ± 8
Alda-1	6	409 ± 9	113 ± 6	82 ± 7	401 ± 15	111 ± 9	74 ± 4
Nitroglycerin	8	405 ± 8	106 ± 3	75 ± 4	398 ± 5	113 ± 4	70 ± 5
Nitroglycerin+Alda-1	7	401 ± 7	118 ± 4	77 ± 2	399 ± 5	117 ± 5	75 ± 3

Table S2. Sustained treatment with nitroglycerin did not affect blood pressure. (**A**) Protocol for sustained treatment and blood pressure measurement. (**B**) Blood pressure and heart rate were measured before and after sustained treatment with nitroglycerin (7.2 mg/kg/day) and/or Alda-1 (16 mg/kg/day). Systolic and diastolic blood pressures remained unchanged before and after nitroglycerin treatment with or without Alda-1, compared to the control. Heart rate remained unchanged in all treatment groups. Data are mean±SEM (n=4-8).

A



B

Time Point	N	Heart Rate	Systolic Pressure	Diastolic Pressure
Before Treatment	6	389 ± 6	118 ± 3	78 ± 2
ISDN (2 hrs)		389 ± 7	90 ± 3*	46 ± 3*
ISDN (16 hrs)		398 ± 5	103 ± 1*	66 ± 4#

Table S3. Acute and sustained treatment with ISDN decreased blood pressure. (**A**) Protocol for acute treatment and blood pressure measurement with ISDN treatment. (**B**) Blood pressure and heart rate were measured before treatment and after 2 and 16 hours of ISDN treatment (16 mg/kg/day). Systolic and diastolic blood pressures were decreased after 2 and 16 hours of treatment with ISDN, compared to those before treatment. Heart rate remained unchanged in all treatment groups. * $p<0.01$ *vs*. Before Treatment. # $p<0.05$ *vs*. Before Treatment. Data are mean±SEM (n=6).