

Supplementary materials

Endothelial to mesenchymal transition contributes to arsenic-trioxide-induced cardiac fibrosis

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Supplementary Tables

Supplementary Table 1. Primers used for real-time qPCR (h and r indicate human and rat species, respectively).

Gene	Primer Sequences (forward, reverse)
VE-cadherin (h)	F: 5'-CAGCCCAAAGTGTGTGAGAA-3' R: 5'-TGTGATGTTGGCCGTGTTAT-3'
VE-cadherin (r)	F: 5'-ACCAGTGACAGAGGCCAATACT-3' R: 5'-GGCCTCCACAGTCAGGTTATAC-3'
CD31(h)	F: 5'-GAGTCCAGCCGCATATCC-3' R: 5'-TGACACAATCGTATCTTCCTTC-3'
CD31(r)	F: 5'-CTCCTAAGAGCAAAGAGCAACTTC-3' R: 5'-TACACTGGTATTCCATGTCTCTGG-3'
α -SMA(h)	F: 5'-TGACAATGGCTCTGGGCTCTGTAA-3' R: 5'-TTCGTCACCCACGTAGCTGTCTTT-3'
α -SMA(r)	F: 5'-CTGTGCTATGTCGCTCTGGA-3' R: 5'-CTGTGCTATGTCGCTCTGGA-3'
FSP1(h)	F: 5'-GTCCACCTTCCACAAGTAC-3' R: 5'-TGTCCAAGTTGCTCATCAG-3'
FSP1(r)	F: 5'-AGGACAGACGAAGCTGCATT-3' R: 5'-CTCACAGCCAACATGGAAGA-3'
Snail(h)	F: 5'-GCCTTCAACTGCAAATACTGC-3' R: 5'-CTTCTTGACATCTGAGTGGGTC-3'
Col1a(h)	F: 5'-CTTGCTTGAAGACCCATGCG-3' R: 5'-TTGGCAGTCTGAGAACCCCA-3'
Col1a(r)	F: 5'-GGTCAGACCTGTGTGTTCCC-3' R: 5'-GGTCCATGTAGGCTACGCTG-3'
Col3a(h)	F: 5'-AACACGCAAGGCTGTGAGACT-3' R: 5'-TTTTGTCGGTCACTTGCACTG-3'
Col3a(r)	F: 5'-CAATGTAAAGAAGTCTCTGAAG-3' R: 5'-CAAACAGGGCCAATGTCCAC-3'
Mmp2(h)	F: 5'-CCGTCGCCCATCATCAA-3' R: 5'-AGATATTGCACTGCCAACTCT-3'
Mmp2(r)	F: 5'-TCCCGAGATCTGCAAGCAAG-3' R: 5'-AGAATGTGGCCACCAGCAAG-3'
Mmp9(h)	F: 5'-GTGCTGGGCTGCTGCTTTGCTG-3' R: 5'-GTCGCCCTCAAAGGTTTGAAT-3'
Mmp9(r)	F: 5'-TGGAACACACAACGTCTTTCA-3' R: 5'-TCACCCGGTTGTGGAAACTC-3'
Fibronectin(h)	F: 5'-GATAAATCAACAGTGGGAGC-3' R: 5'-CCCAGATCATGGAGTCTTTA-3'
Fibronectin(r)	F: 5'-TCAGCTGTACCATTGCAAATC-3' R: 5'-TGGTGTCCCTGATCATTGCAT-3'
Vimentin(h)	F: 5'-GAGAAGTTTGCCGTTGAAGC-3'

	R: 5'-TCCAGCAGCTTCCTGTAGGT-3'
Vimentin(r)	F: 5'-TCCCTGAACCTGAGAGAAAC-3' R: 5'-ATCGTGGTGCTGAGAAGTC-3'
GAPDH(h, r)	F: 5'-AAGGTCGGAGTCAACGGATTT-3' R: 5'-AGATGATGACCCTTTTGGCTC-3'

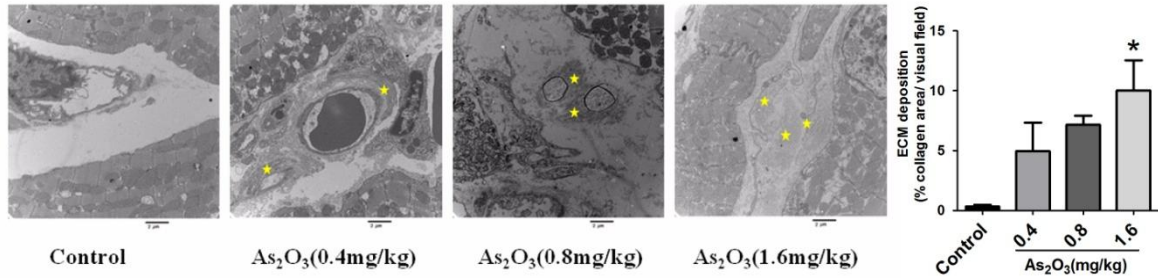
Supplementary Table 2. The parameters of echocardiography of rats after exposure to As₂O₃.

	Control	As ₂ O ₃ (0.4 mg/kg)	As ₂ O ₃ (0.8 mg/kg)	As ₂ O ₃ (1.6 mg/kg)
IVS;d(mm)	1.84 ± 0.10	1.89 ± 0.05	1.99 ± 0.04	2.23 ± 0.14*
IVS;s(mm)	2.72 ± 0.09	2.90 ± 0.09	3.16 ± 0.10	3.21 ± 0.28
LVID;d(mm)	5.07 ± 0.09	6.72 ± 0.18**	6.74 ± 0.38**	6.88 ± 0.31**
LVID;s(mm)	2.69 ± 0.09	3.84 ± 0.14*	3.90 ± 0.44*	4.46 ± 0.25**
LVPW;d(mm)	1.79 ± 0.06	2.03 ± 0.06	2.17 ± 0.10*	2.39 ± 0.11**
LVPW;s(mm)	2.80 ± 0.03	2.93 ± 0.08	3.00 ± 0.09	3.18 ± 0.07**
LV mass(mg)	666.5 ± 43.40	932.6 ± 31.77	975.1 ± 60.64*	1039.0 ± 120.70*
LVEDV(μL)	145.5 ± 8.53	238.1 ± 10.26	238.2 ± 32.13*	244.0 ± 27.15*
LVESV(μL)	35.17 ± 11.66	77.20 ± 4.05	83.43 ± 14.34*	100.50 ± 13.21**

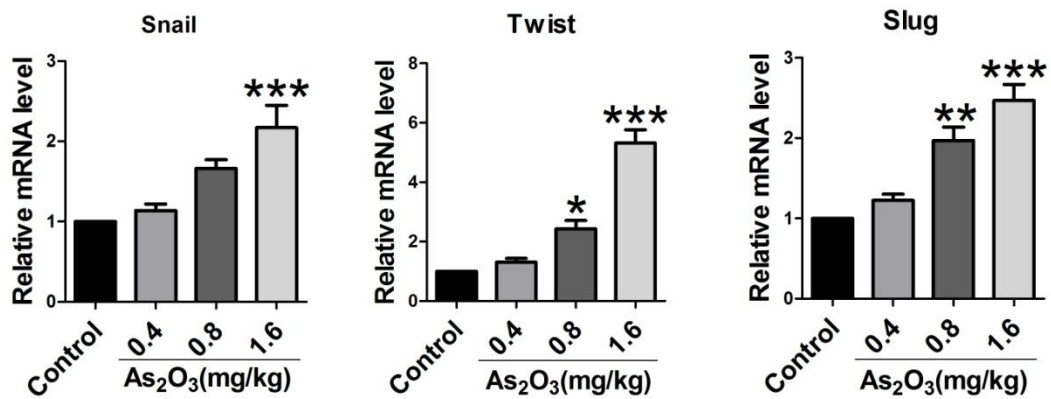
Results represent mean ± S.E.M. n = 4–5 rats in each group. IVS, interventricular septum; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall; LV mass, left ventricular mass; d, diastole; s, systole; LVEDV, left ventricular end-diastolic volume; LVESV, Left ventricular end-systolic volume.

Supplementary Figures

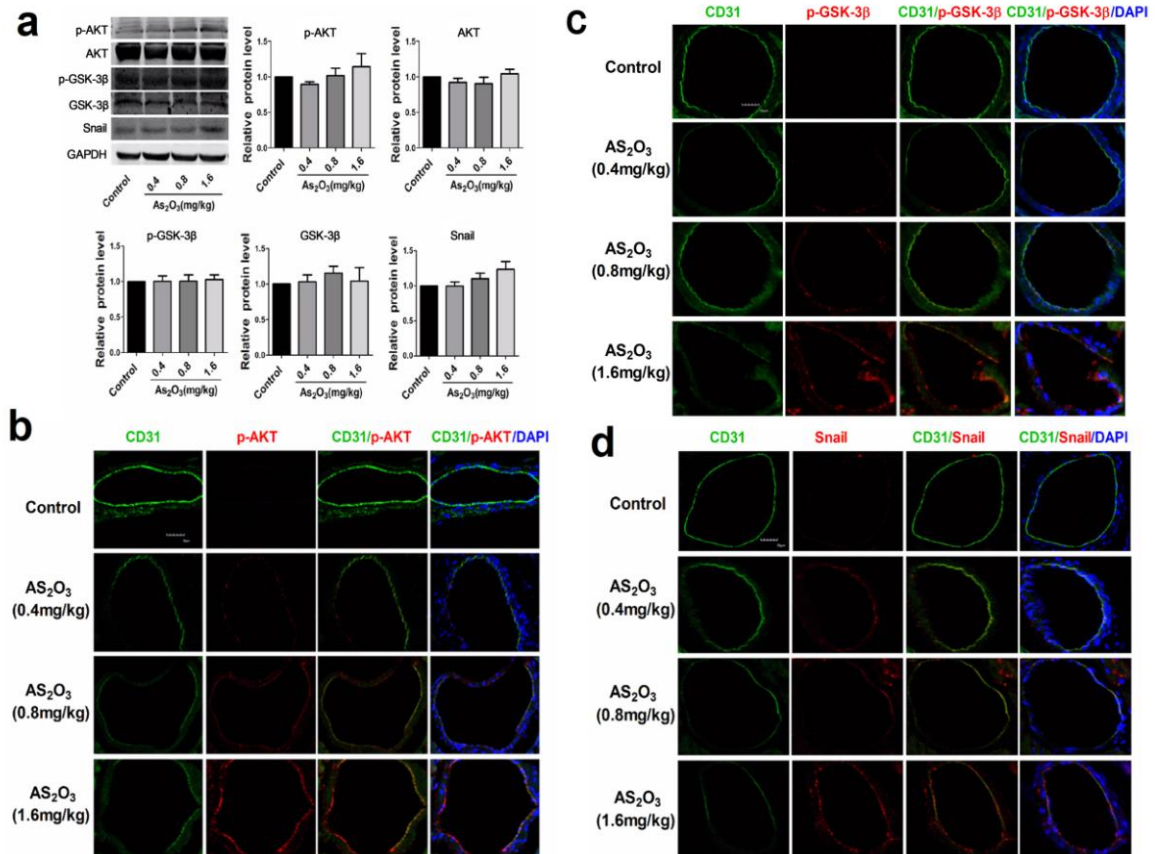
Supplementary Figure 1. Collagen production in the control group and As₂O₃-treated groups by transmission electron microscopy. Collagens are indicated by yellow stars. Scale bar=2 μm. *p < 0.05 vs Control. n=3.



Supplementary Figure 2. Relative mRNA expression of EndMT marker genes SNAIL, TWIST and SLUG in heart tissue. *p<0.05, **p<0.01, ***p<0.001 vs Control. n=3.



Supplementary Figure 3. Effects of As₂O₃ on the AKT/GSK-3β/Snail signaling pathway in vivo. (a) The protein levels of p-AKT, AKT, p-GSK-3β, GSK-3β and Snail were detected by western blotting in heart tissues after exposure to As₂O₃. n=3. (b) Co-immunostaining of CD31 and p-AKT in cardiac sections of rats treated with or without As₂O₃, nuclei are stained with DAPI (blue). Scale bar=30 μm. (c) Co-immunostaining of CD31 and p-GSK-3β in cardiac sections of rats treated with or without As₂O₃, nuclei are stained with DAPI (blue). Scale bar=30 μm. (d) Co-immunostaining of CD31 and snail in cardiac sections of rats treated with or without As₂O₃, nuclei are stained with DAPI (blue). Scale bar=30 μm.



Supplementary Figure 4. Inhibitory effect of NAC on intracellular ROS accumulation and on the activation of AKT/GSK-3 β /Snail pathway when exposed to As₂O₃. (a) Confocal images of 2',7'-dichlorofluorescein (DCF) staining showed ROS level in control endothelial cells, cells incubated with As₂O₃ and cells in the presence of As₂O₃ (8 μ M) and NAC (5 mM). (b) Representative western blotting bands and analyzed data for the expression of p-AKT, AKT, p-GSK-3 β , GSK-3 β and snail in endothelial cells in different groups. *p<0.05, **p<0.01, ***p<0.001 vs Control, #p<0.05, ##p<0.01, ###p<0.001 vs As₂O₃. n=3.

