

# Changes of Gene Expressions in Monocrotaline Induced Pulmonary Hypertension Rats after Bone Marrow Cell Transfusion

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## Background

- **Pulmonary artery hypertension (PAH)** causes right ventricular failure and possibly death by a progressive increase in pulmonary vascular resistance. With a progressive loss of pulmonary microvasculature, it later becomes refractory to traditional therapies.
- **Bone marrow-derived mesenchymal stem cell therapy** has provided an alternative for ailments of various organs by regeneration at the site of a lesion.

## Material & Method

200-250 gm Sprague-Dawley rat

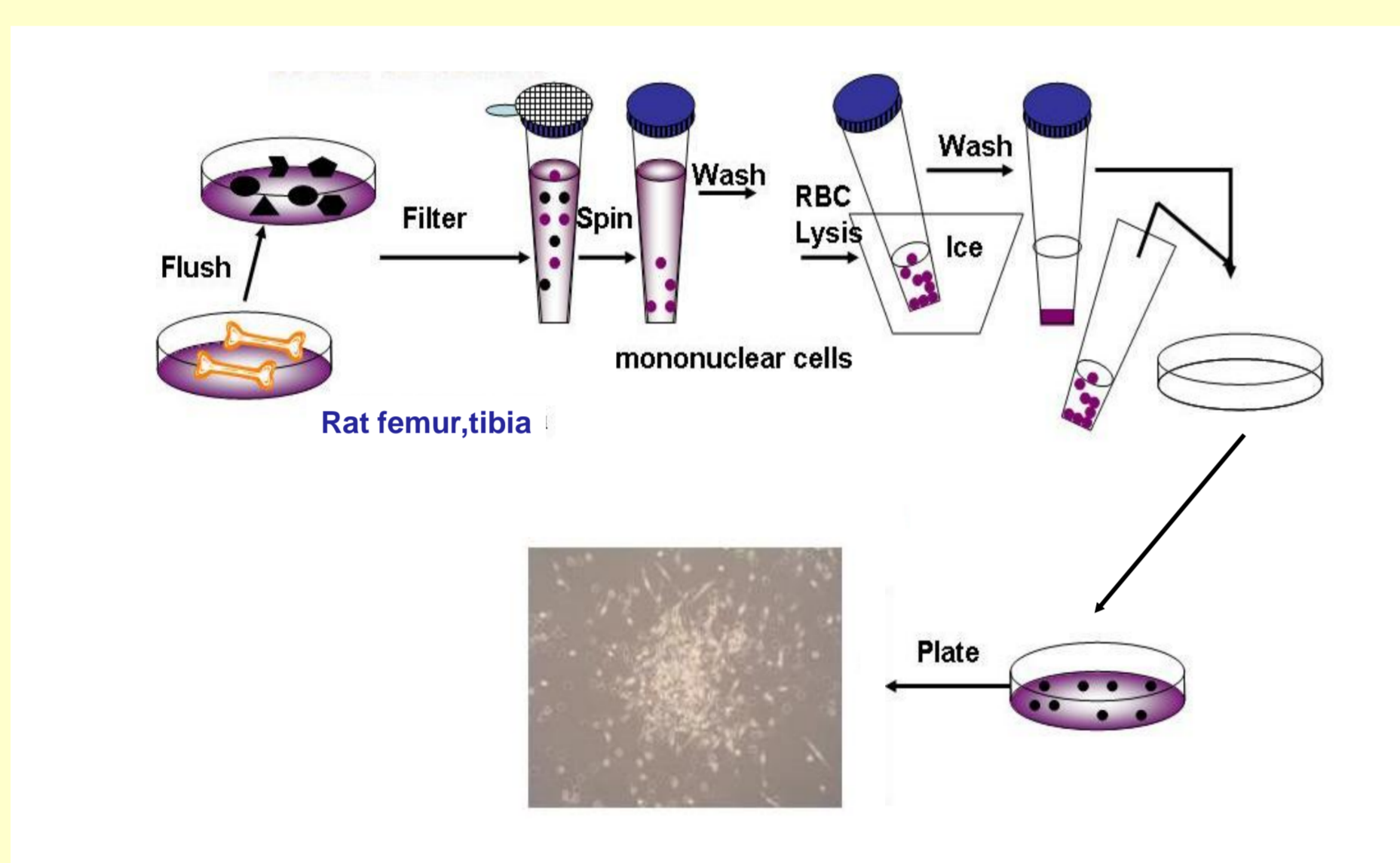
**Control group (N=9)** Normal saline  
0.1 cc/kg S.Q

**Monocrotaline group (N=9)** Monocrotaline  
0.1 cc/kg S.Q (60 mg/kg)

**Treatment group (N=9)** Monocrotaline  
0.1 cc/kg S.Q +  
**Bone marrow cell transfusion**  
3X10<sup>7</sup> colony

Investigation  
3wk, 4wk

### Bone Marrow Cell Isolation



### Changes in Body Weight, RV, LV+S, RV/LV+S and Lung

Group	Weight(g)	RV(g)	LV+ S(g)	RV/LV+S	Lung (g)
<b>C</b>					
3wk	384.2±32.6	0.27±0.01	0.86±0.05	0.31±0.02	1.54±0.09
4wk	442.0±31.5	0.24±0.01	0.87±0.09	0.28±0.02	1.53±0.15
<b>M</b>					
3wk	277.0±43.0*	0.38±0.04*	0.71±0.02	0.53±0.04*	2.22±0.19
4wk	241.3±87.9*	0.59±0.02*	0.66±0.10	0.89±0.17*	2.34±0.34
<b>B</b>					
3wk	328.2±20.6†	0.36±0.09	0.74±0.05	0.48±0.12	2.12±0.22
4wk	313.0±25.5†	0.44±0.12†	0.80±0.08†	0.56±0.07†	2.54±0.44

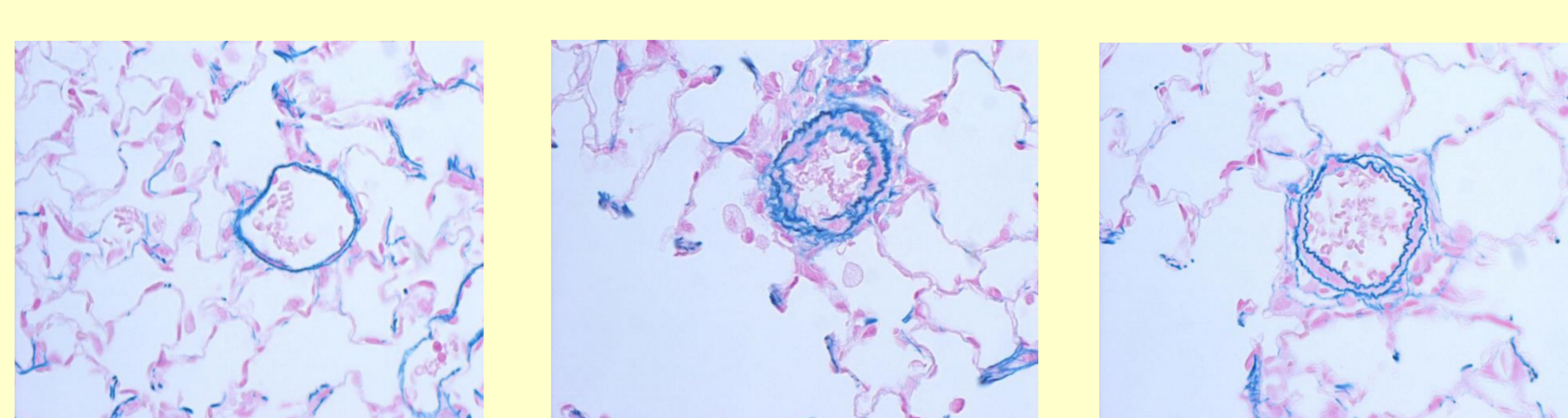
\*P < 0.05 vs. the corresponding value in the C group, †P < 0.05 vs. the corresponding value in the M group.  
RV: right ventricle, LV: left ventricle, S: septum, C: control, M: monocrotaline, B: bone marrow cell

### Mean RV Pressure in Each Group

Group	mmHg
<b>C group</b>	9.5±0.78
<b>M group</b>	34.0±7.1*
<b>B group</b>	15.5±4.9†

\*P < 0.05 vs. the corresponding value in the C group.  
†P < 0.05 vs. the corresponding value in the M group.  
C: control, M: monocrotaline, B: bone marrow cell

### Victoria Blue Staining in Lung Tissues



C group M group B group  
C: control, M: monocrotaline, B: bone marrow cell

### Medial Thickening of Pulmonary Arteriole in Each Group (%)

Group	Medial Thickening (%)
<b>C group</b>	4.4 ± 0.8
<b>M group</b>	11.1 ± 0.9*
<b>B group</b>	10.7 ± 2.8

C: control, M: monocrotaline, B: bone marrow cell  
\*P < 0.05 vs. the corresponding value in the C group

### Number of Muscular Pulmonary Arteriole in Each Group

Group	Number of Muscular Pulmonary Arteriole
<b>C group</b>	0.88 ± 0.46
<b>M group</b>	2.50 ± 0.52*
<b>B group</b>	1.90 ± 0.66

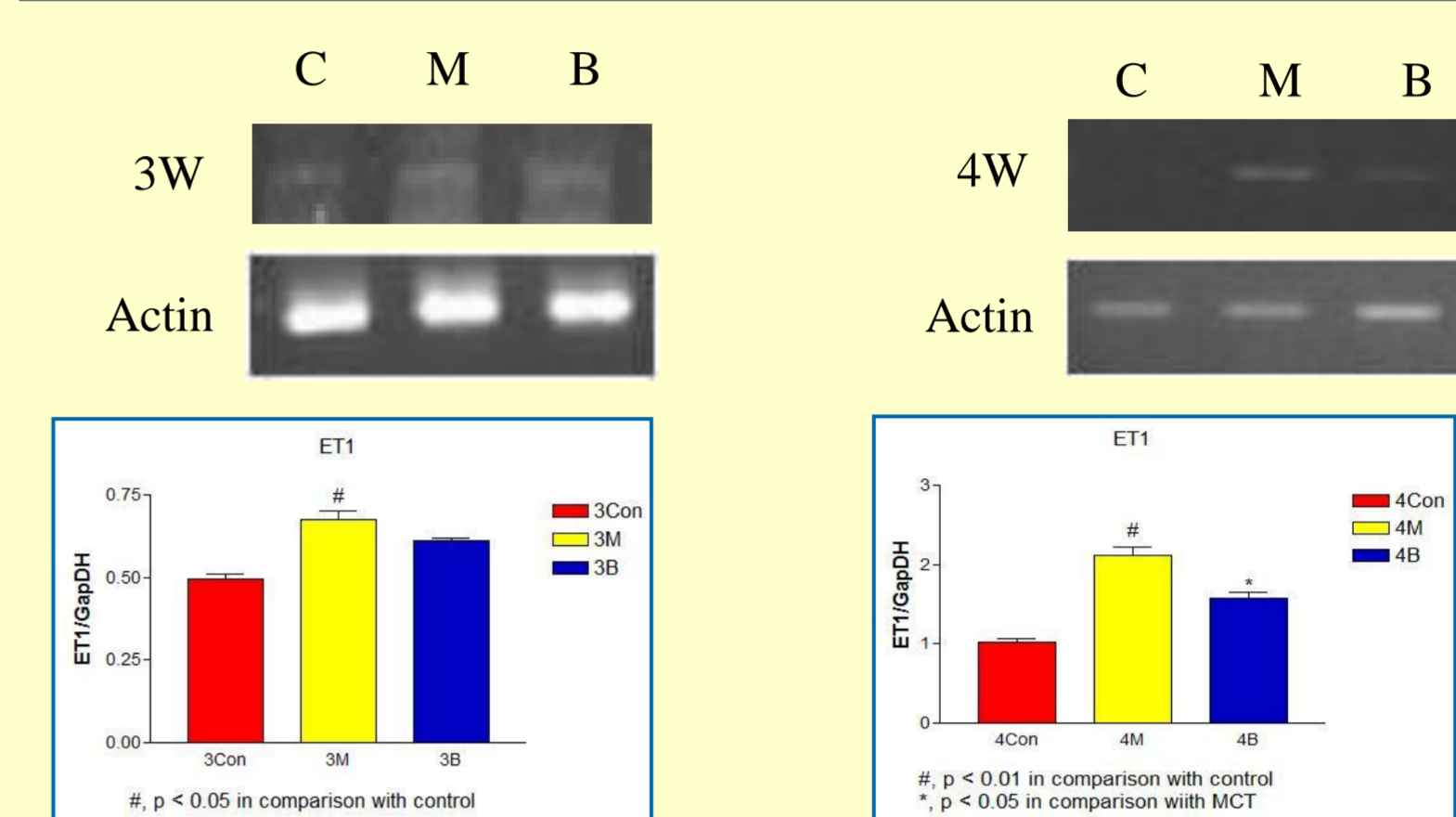
C: control, M: monocrotaline, B: bone marrow cell  
\*P < 0.05 vs. the corresponding value in the C group

## Gene Sequence of Primer of RT-PCR

Gene	Forward primer sequence	Reverse primer sequence	Size
ET-1	TCT CGG AGA GCA GAG ACA CA	TGG ACT TTG GAG TTT CTC CCT	156 bp
ERA	CAC AGG CTT CAG TGT GCA TT	CAA CAC AGG CCC TTA GCT TC	118 bp
NOS3	CTG CGG TGA TGT CAC TAT GG	AAA TGT CCT CGT GGT AGC GT	140 bp
MMP2	AAG AGG CCT GGT TAC CCT GT	AAG TAG CAC CTG GGA GGGAT	137 bp
TIMP1	GAC CTA TAG TGC TGG CTG TG	GATC GCT CTG GTA GCC CTT CT	133 bp

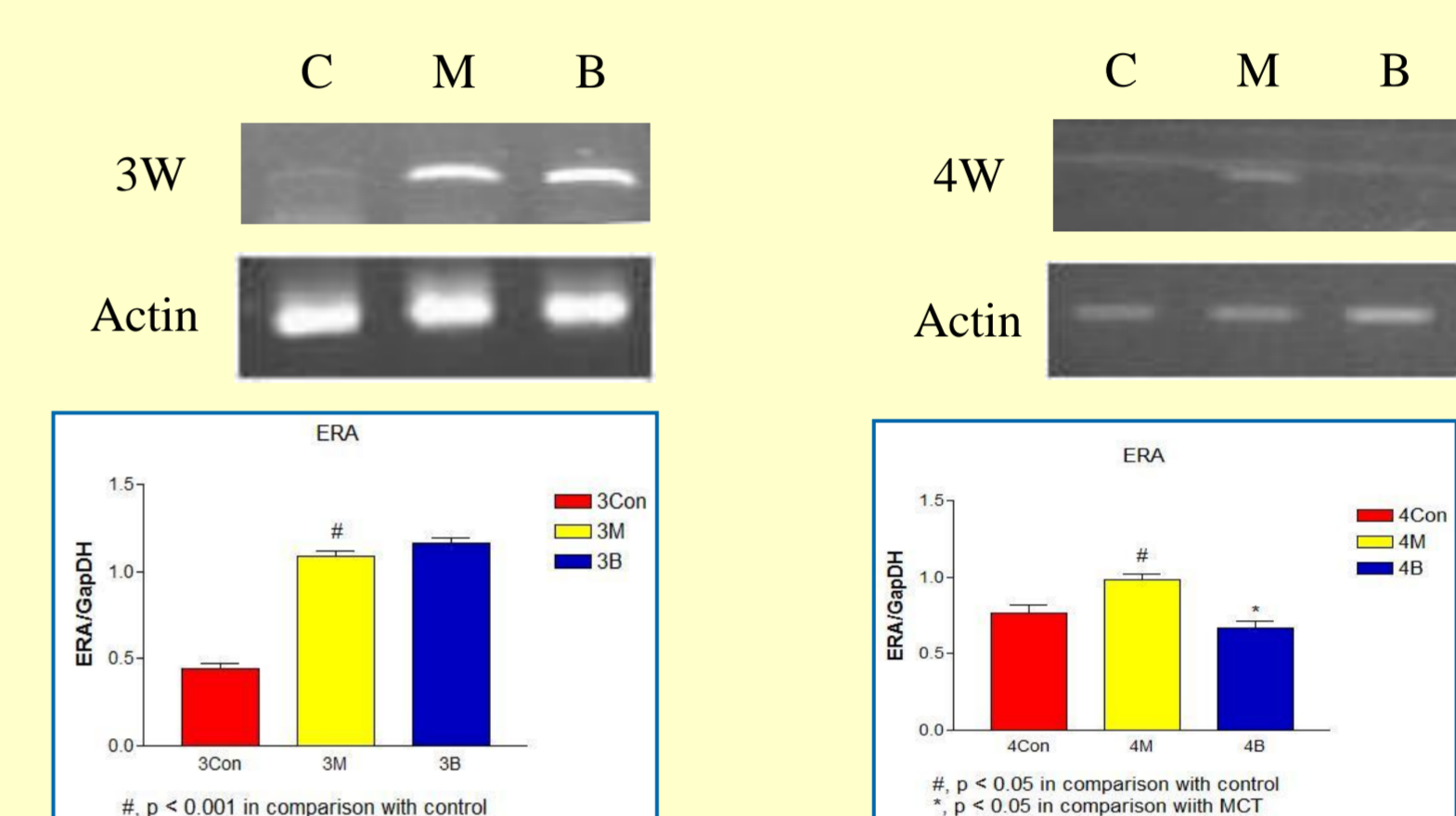
RT-PCR: reverse transcription-polymerase chain reaction, ET-1: endothelin-1,  
ERA: endothelin receptor A, NOS-3: endothelial nitric oxide synthase,  
MMP-2: matrix metalloproteinase 2, Timp, tissue inhibitor of matrix metalloproteinases

### Gene Expressions of ET1



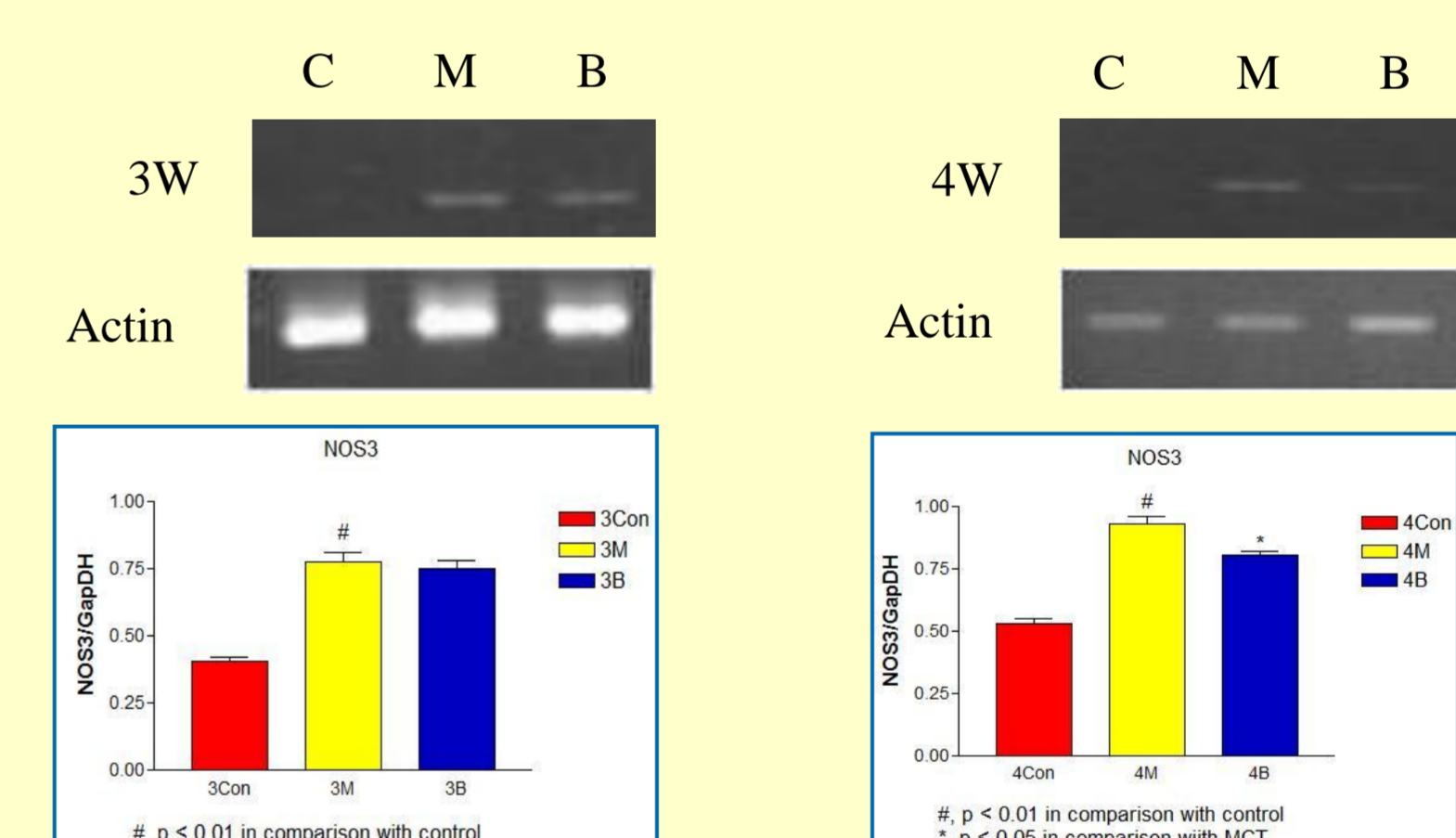
C: control, M: monocrotaline, B: bone marrow cell  
ET: endothelin

### Gene Expressions of ERA



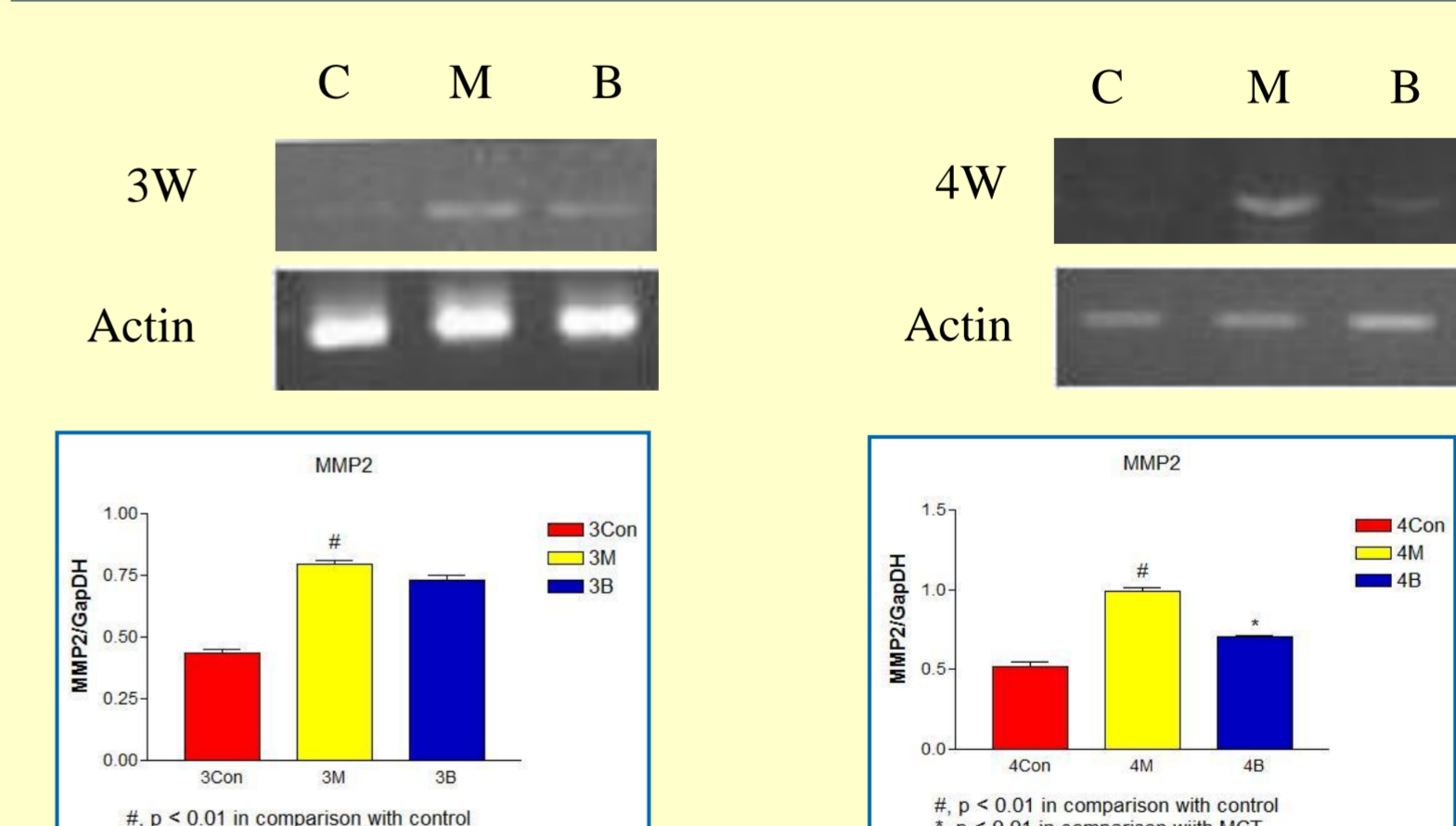
C: control, M: monocrotaline, B: bone marrow cell  
ERA: endothelin receptor A

### Gene Expressions of NOS3



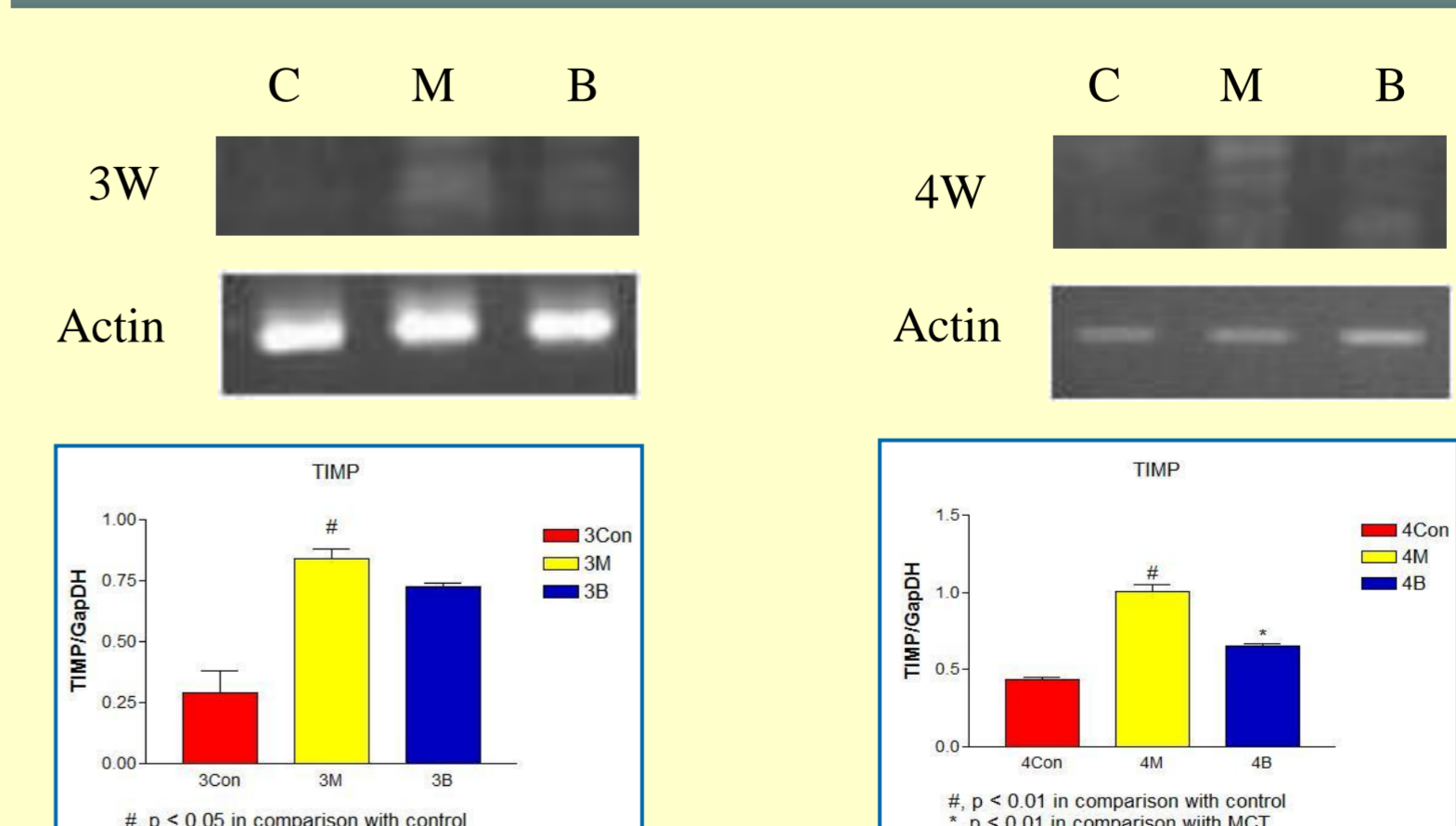
C: control, M: monocrotaline, B: bone marrow cell  
NOS: nitric oxide synthase

### Gene Expressions of MMP2



C: control, M: monocrotaline, B: bone marrow cell  
MMP: matrix metalloproteinase

### Gene Expressions of TIMP



C: control, M: monocrotaline, B: bone marrow cell  
TIMP: tissue inhibitor of matrix metalloproteinases

## Conclusion

- After **bone marrow cell infusion**, improvement of RVH and mean RV pressure and decrease in several gene expressions were observed.
- Additional research on dosage of **bone marrow cell** is needed to determine the appropriate amount of bone marrow cell required for PAH treatment.