

# Gene Expressions of Apoptosis in Monocrotaline-Induced Pulmonary Hypertension Rat Model after Bosentan Treatment

Ji Young Jung, M.D.<sup>1</sup>, Kwan Chang Kim, M.D.<sup>2</sup>, Young Mi Hong, M.D.<sup>1</sup>  
 Department of Pediatrics<sup>1</sup>, Department of Cardiovascular Surgery<sup>2</sup>,  
 School of Medicine, Ewha Womans University, Seoul, Korea

## Introduction

- Vascular wall remodeling in pulmonary hypertension is contributed to by an aberration in the normal balance between proliferation and apoptosis of smooth muscle.
- Endothelin-1 prevents up-regulation of pro-apoptosis and activates caspase-3.
- Endothelin (ET)-1, a potent endothelium-derived vasoconstrictor peptide, has several properties suggestive of its potential pathophysiological role in pulmonary hypertension.

## Purposes

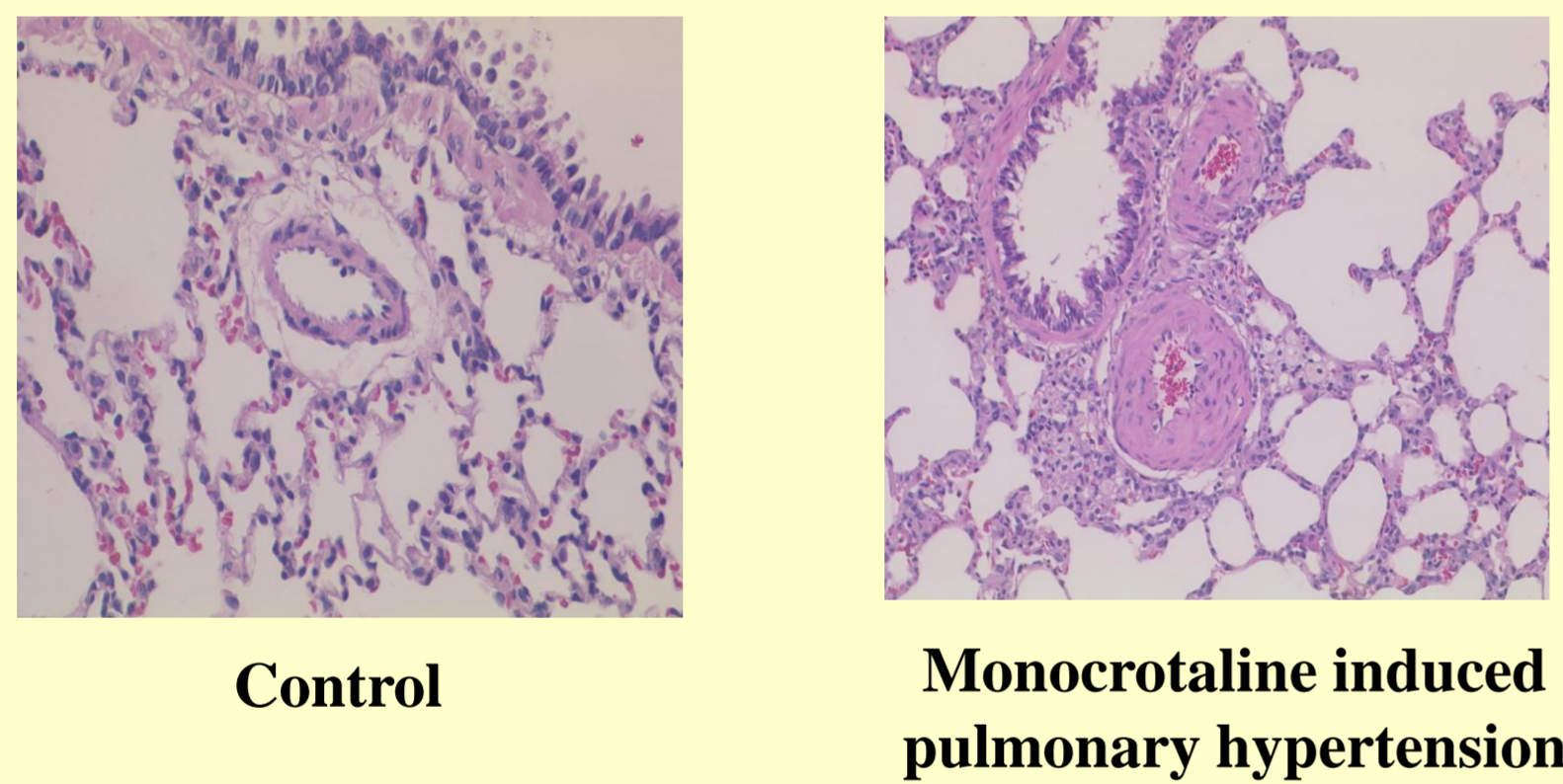
- To evaluate the effect of bosentan on Bcl 2, caspase 3, vascular endothelial growth factor (VEGF), interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$  in monocrotaline (MCT)-induced pulmonary hypertension and to investigate the correlation between caspase 3 gene expression and other associated genes.

## Methods

200-250 gm Sprague-Dawley rat

<b>Control group</b> (N=36)	<b>Normal saline</b> 0.1 cc/kg S.Q	<b>Investigation</b> day 1, 5, 7, 14, 28
<b>Monocrotaline group</b> (N=36)	<b>Monocrotaline</b> 0.1 cc/kg S.Q (60 mg/kg)	
<b>Treatment group</b> (N=36)	<b>Monocrotaline</b> 0.1 cc/kg S.Q + <b>Bosentan</b> 25mg/kg/D	

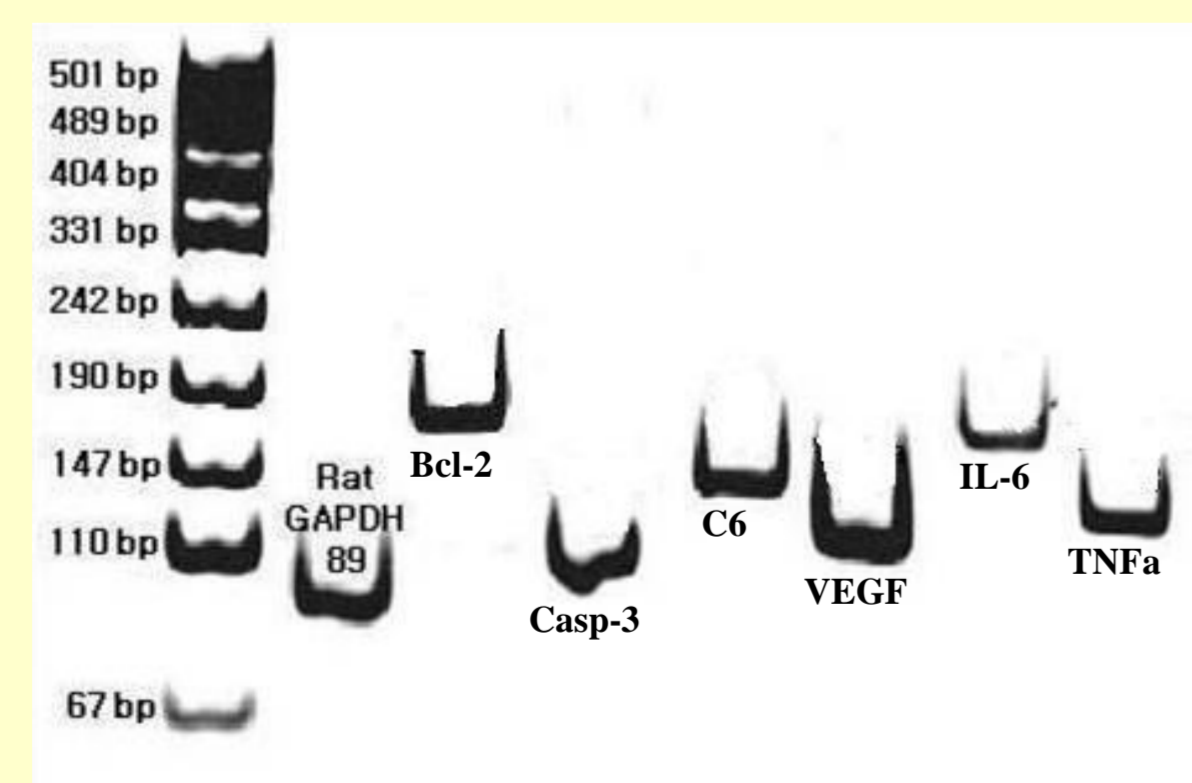
## H-E staining



## Primer Design

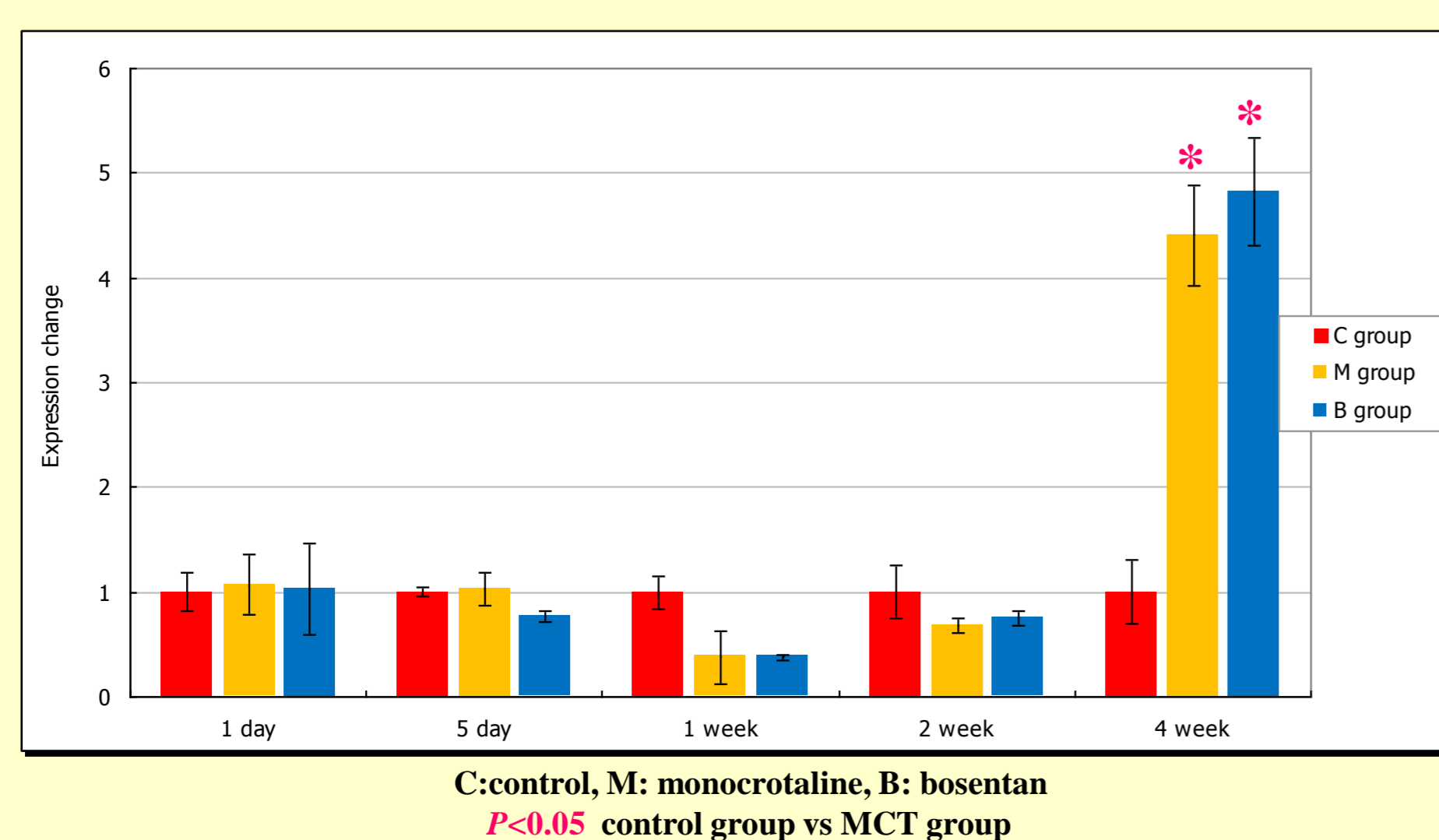
Gene	Forward primer sequence	Reverse primer sequence	Size
Bcl-2	CTC AGC CAG CCA GTG ACA TA	CCG TGC TCC TCC AGA TAC AT	177 bp
Caspase 3	GAA AGC ATC CAG CAA TAG GC	TAA GGA AGC CTG GAG CAC AG	100 bp
C6	ACT TTG TCC ATG CTG GTT CC	GAG GTG TGG GCT GAA CAT TT	145 bp
VEGF	GGA GGA TGT CCT CAC TTG GA	CAA ACA GAC TTC GGC CTC TC	100 bp
IL-6	CCG GAG AGG AGA CTT CAC AG	ACA GTG CAT CAT CGC TGT TC	161 bp
TNF	ACT TGA TTT CTG GGC CCT TT	CCA CTG TTC TGT GCT TGC C	113 bp

Bcl-2 : B-cell lymphoma 2  
 VEGF : Vascular endothelial growth factor  
 TNF : Tumor necrosis factors

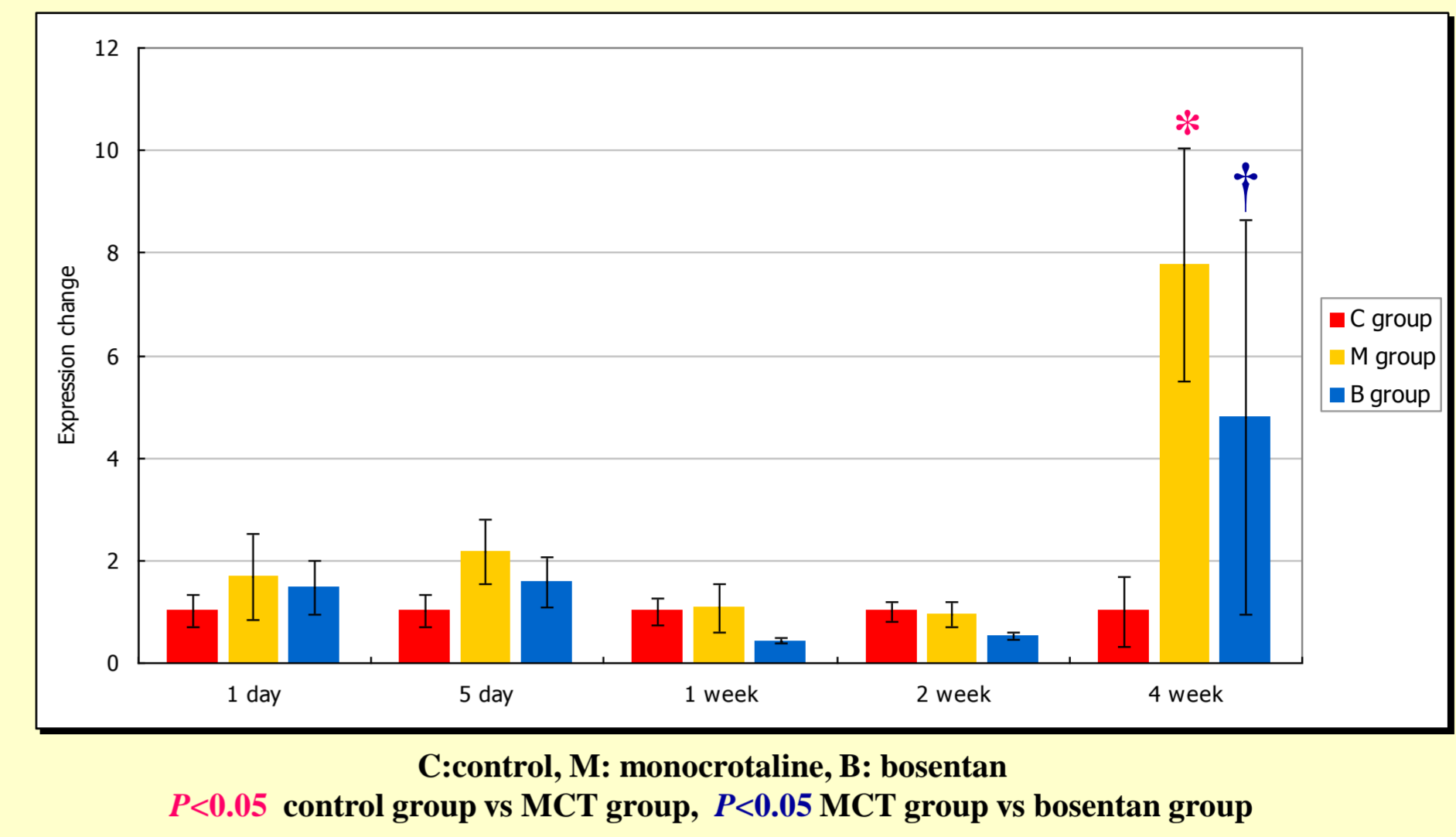


Typical example of RT-PCR products are shown for the level of Bcl2, caspase 3, C6, Vegf, IL6, Tnf mRNA in lung tissue. The RT-PCR products from the transcripts of Bcl2, caspase 3, C6, Vegf, IL-6, Tnf were 177 bp, 100 bp, 145 bp, 100 bp, 161 bp and 113 bp.  
 NOS: inducible nitric oxide synthase, Vegf: vascular endothelial growth factor  
 C6: complement 6, IL-6: interleukin-6 Tnf: tumor necrosis factor

## Gene Expression of Bcl-2 by RT PCR

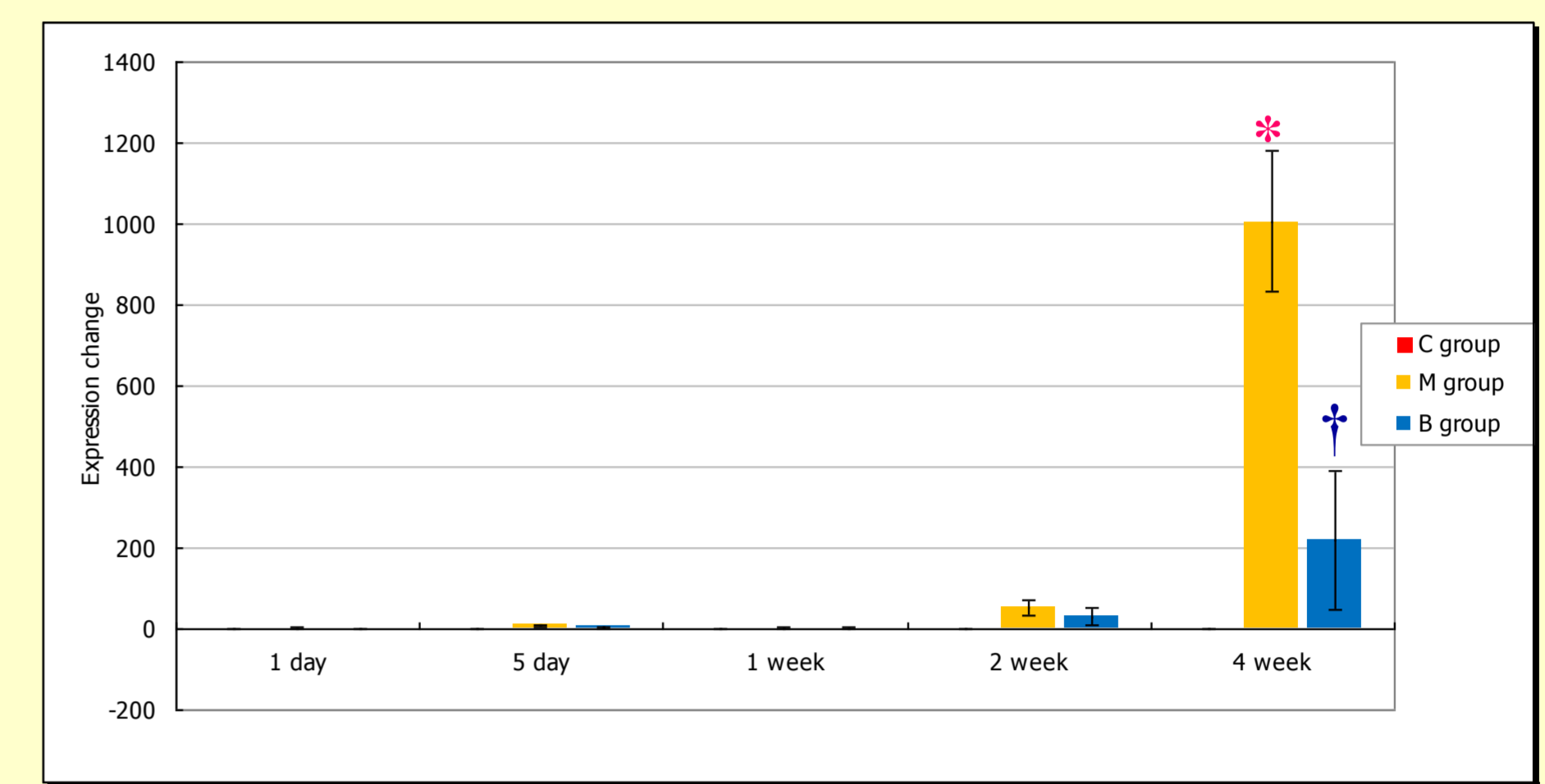


## Gene Expression of Caspase 3 by RT PCR



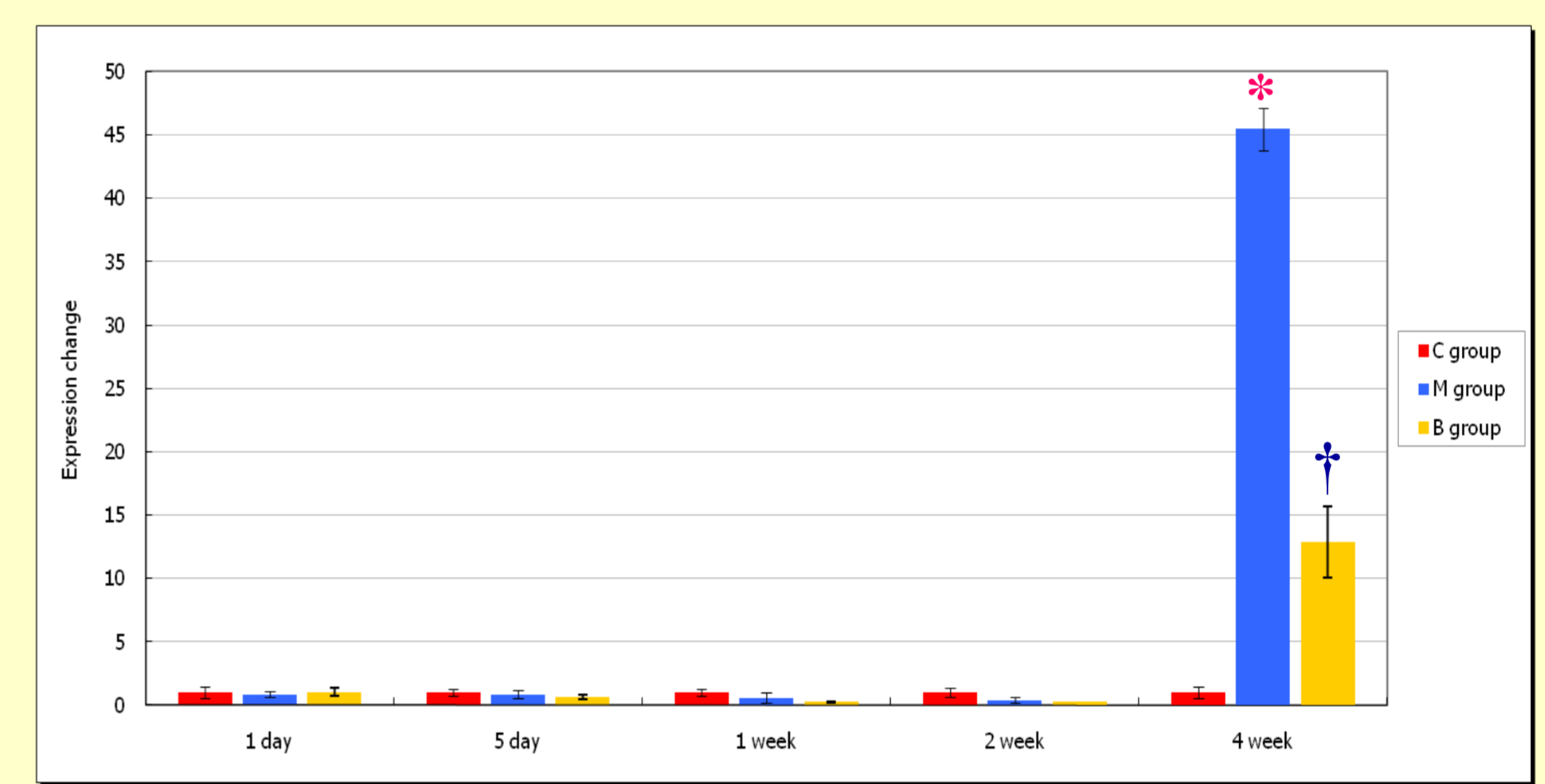
C:control, M: monocrotaline, B: bosentan  
*P*<0.05 control group vs MCT group, *P*<0.05 MCT group vs bosentan group

## Gene Expression of C6 by RT PCR



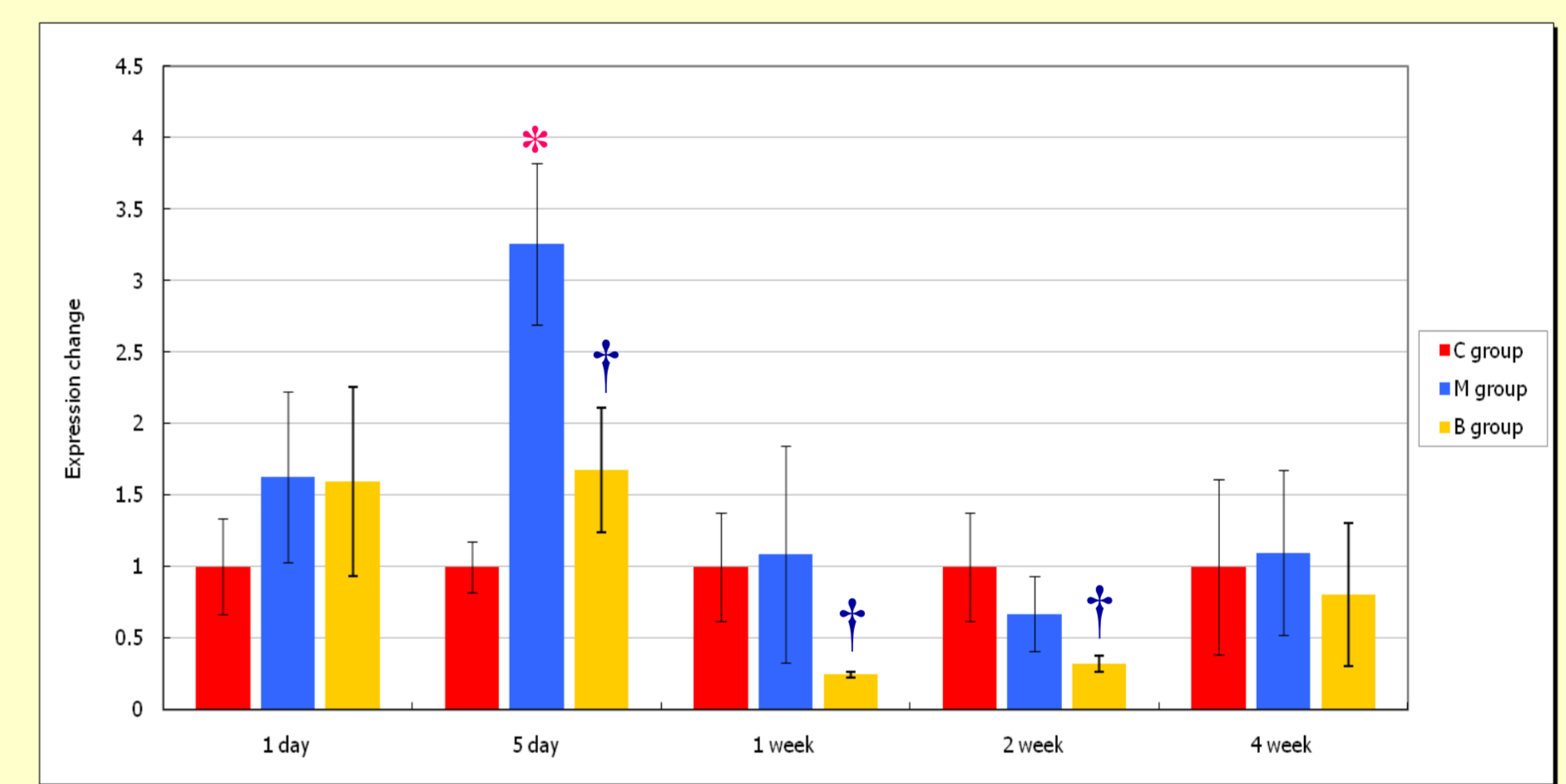
C:control, M: monocrotaline, B: bosentan  
*P*<0.05 control group vs MCT group, *P*<0.05 MCT group vs bosentan group

## Gene Expression of VEGF by RT PCR



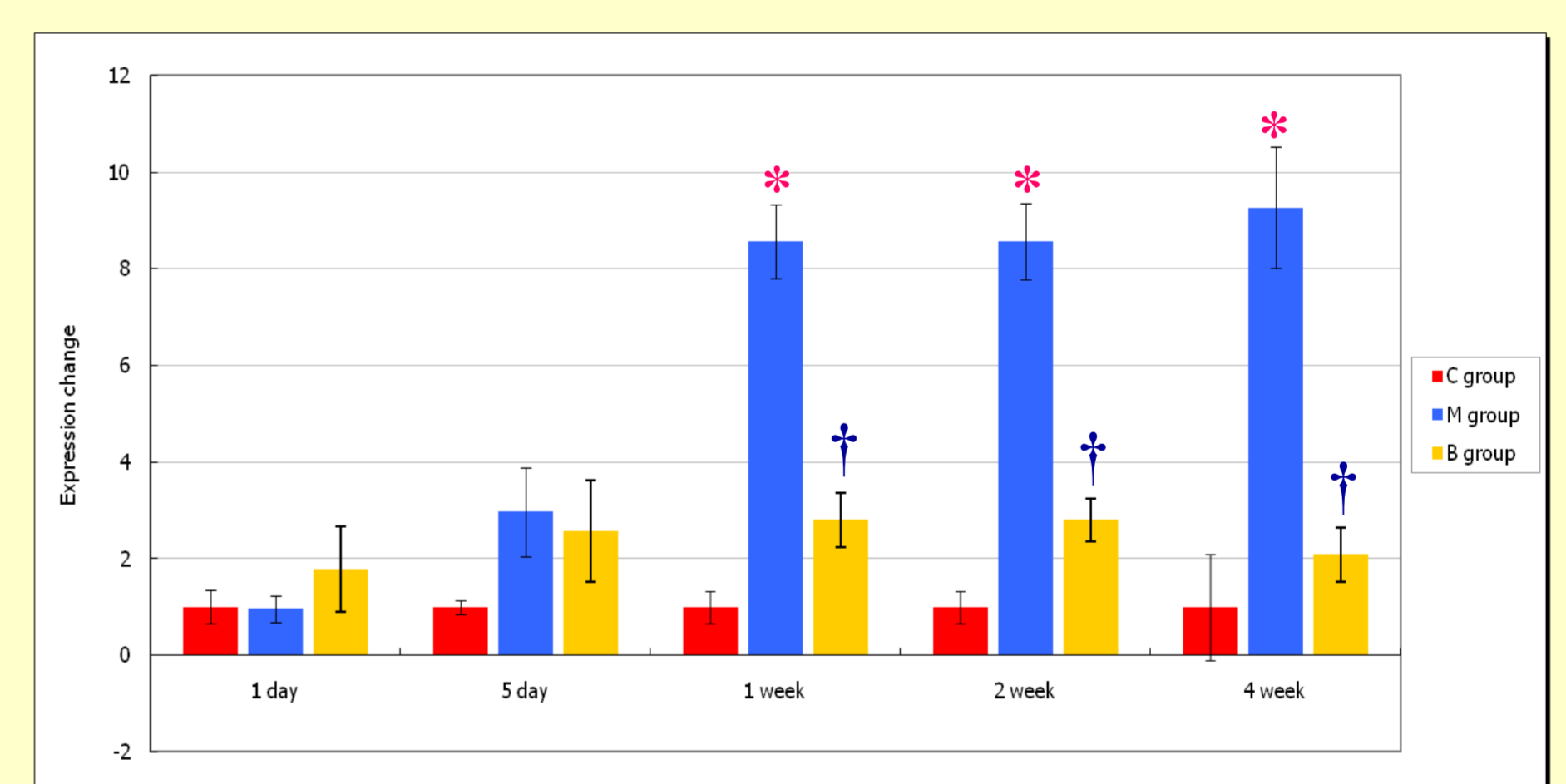
C:control, M: monocrotaline, B: bosentan  
*P*<0.05 control group vs MCT group, *P*<0.05 MCT group vs bosentan group

## Gene Expression of TNF- $\alpha$ by RT PCR



C:control, M: monocrotaline, B: bosentan  
*P*<0.05 control group vs MCT group, *P*<0.05 MCT group vs bosentan group

## Gene Expression of IL-6 by RT PCR



C:control, M: monocrotaline, B: bosentan  
*P*<0.05 control group vs MCT group, *P*<0.05 MCT group vs bosentan group

## Summary

### MCT induced pulmonary hypertension

Gene expression of Caspase 3, C6, VEGF ↑	4 wk
Gene expression of Bcl-2 ↓	4 wk
Gene expression of IL-6 ↑	1, 2, 4 wk
Gene expression of TNF- $\alpha$ ↑	5 day

### Bosentan effect

Gene expression of Caspase 3, C6, VEGF ↓	5 day
Gene expression of IL-6 ↓	1, 2, 4 wk
Gene expression of TNF- $\alpha$ ↓	5day, 1, 2 wk
Gene expression of Bcl-2 ↑	4 wk

## Conclusion

- Bosentan may hold considerable potential for preventing apoptosis and inflammation.