

EFFECT OF OMEGA-3 FATTY ACIDS ON NRF-2 EXPRESSION, APOPTOSIS, INFLAMMATION AND FIBROSIS IN CYCLOSPORINE INDUCED RAT MODEL

Won Suk An¹, Hye Won Lee¹, Dongyeol Lee¹, Su Mi Lee¹, Young Ki Son¹, Seong Eun Kim¹, You Jeong Oh²

¹Internal Medicine, Dong-A University, Busan, Republic of Korea, ²Dong-Eui Medical Center, Busan, Republic of Korea.

Introduction & Aims

- ◆ Cyclosporine (CsA)-induced kidney injury is characterized by renal dysfunction with inflammatory cell infiltrations, apoptosis, fibrosis, and long-standing hypoxic injury
- ◆ The Nrf-2 regulates antioxidant and anti-inflammatory process in kidney injury model
- ◆ It is not clear that omega-3 fatty acid (FA) with anti-inflammatory and antioxidant role affect on Nrf-2 expression
- ◆ The aim of this study is to investigate whether omega-3 FA affects the Nrf-2 expression and has anti-inflammatory, anti-apoptotic, and anti-fibrotic processes in CsA-induced nephropathy

Methods

- ◆ Male Sprague-Dawley rats fed a low-sodium diet were divided into three treatment groups
- ✓ **Control group** (n = 5) ; rats received saline (1mL/kg /day by subcutaneous injection)
- ✓ **CsA group** (n = 6) → rats received CsA (15mg/kg/day by subcutaneous injection)
- ✓ **CsA with Omega-3 FA group** (n = 6) → rats received CsA and omega-3 FA (300mg/kg/day by gastric gavage)
- ◆ **Measurements**
- Kidney function was measured at the end of 4 weeks
- The expression of Iκ-b, ED-1, transforming growth factor-β1 (TGF-β1), α-smooth muscle actin (α-SMA), E-cadherin, Smads for inflammation and fibrosis, caspase-3, caspase-7, and BAX/Bcl-2 ratio for apoptosis, and Nrf2 were examined by Western blot analysis

Results

- ◆ Kidney function was decreased in CsA-treated rats compared with controls and histologic findings were more comparable with acute and chronic cyclosporine toxicity
- ◆ Compared with control, CsA group significantly upregulated ED-1 protein expression and Iκ-b
- ◆ Omega-3 FA supplementation attenuated increased ED-1 expression and Iκ-b
- ◆ We found that caspase-3, caspase-7, Bax/Bcl2 ratio, TGF-β1, Smad-2/3 and Smad-4 were activated in CsA group and that omega-3 FA prevented these activation related with apoptosis and renal fibrosis
- ◆ The expression of Nrf2 was decreased in CsA-treated rats but Nrf-2 was increased by omega-3 FA

Table.1 Expression of markers associated with apoptosis, inflammation, and fibrosis

	Control	CsA	CsA with Omega3 FA
Caspase3	0.10±0.07	5.07±1.62 [*]	0.95±0.25 [#]
Caspase7	0.07±0.06	3.58±0.38 [*]	0.76±0.35 [#]
BAX	0.48±0.56	2.85±0.87 ^{**}	1.64±1.34 ^{**}
Bcl2	3.05±0.82	0.26±0.14 [*]	1.33±0.69 [#]
BAX/Bcl2	0.10±0.06	9.84±3.79 [*]	0.74±1.00 [#]
Ecadherin	0.30±0.42	2.16±0.27 [*]	1.31±0.50 [#]
aSMA	1.31±1.10	2.36±1.70	1.05±1.07
P_Erk	1.19±1.58	2.33±1.78	1.55±2.14
P62	1.68±0.91	0.89±0.62	0.94±0.67
Beclin1	1.41±1.40	2.60±1.81	1.80±0.97
ATG5	0.70±0.22	1.15±0.48	0.92±0.36
ED1	0.07±0.07	2.61±0.15 [*]	1.45±0.20 [#]
IκB	0.60±0.17	1.84±0.97 ^{***}	0.69±0.29 ^{***##}
TGF_β	0.07±0.05	3.91±0.33 [*]	0.54±0.62 ^{***}
Smad23	0.69±0.26	2.42±0.21 [*]	1.55±0.15 [#]
Smad4	0.73±0.25	2.05±0.11 [*]	1.47±0.16 [#]
Smad6	1.01±0.27	2.21±0.04 [*]	1.45±0.28 [#]
Smad7	0.63±0.32	2.42±0.42 [*]	1.61±0.26 [#]
CYP27B	1.16±0.26	1.31±0.61	1.22±0.29
CYP24	0.98±0.32	1.27±0.32	0.86±0.42
Nrf2	1.20±0.17	0.47±0.13 ^{**}	0.77±0.08 ^{***##}

*P < 0.05, compared to control, **P < 0.01, compared to control, ***P < 0.001, compared to control, #P < 0.05, compared to CsA, ##P < 0.01, compared to CsA

Figure.1 Expression of ED-1 and Iκ-B

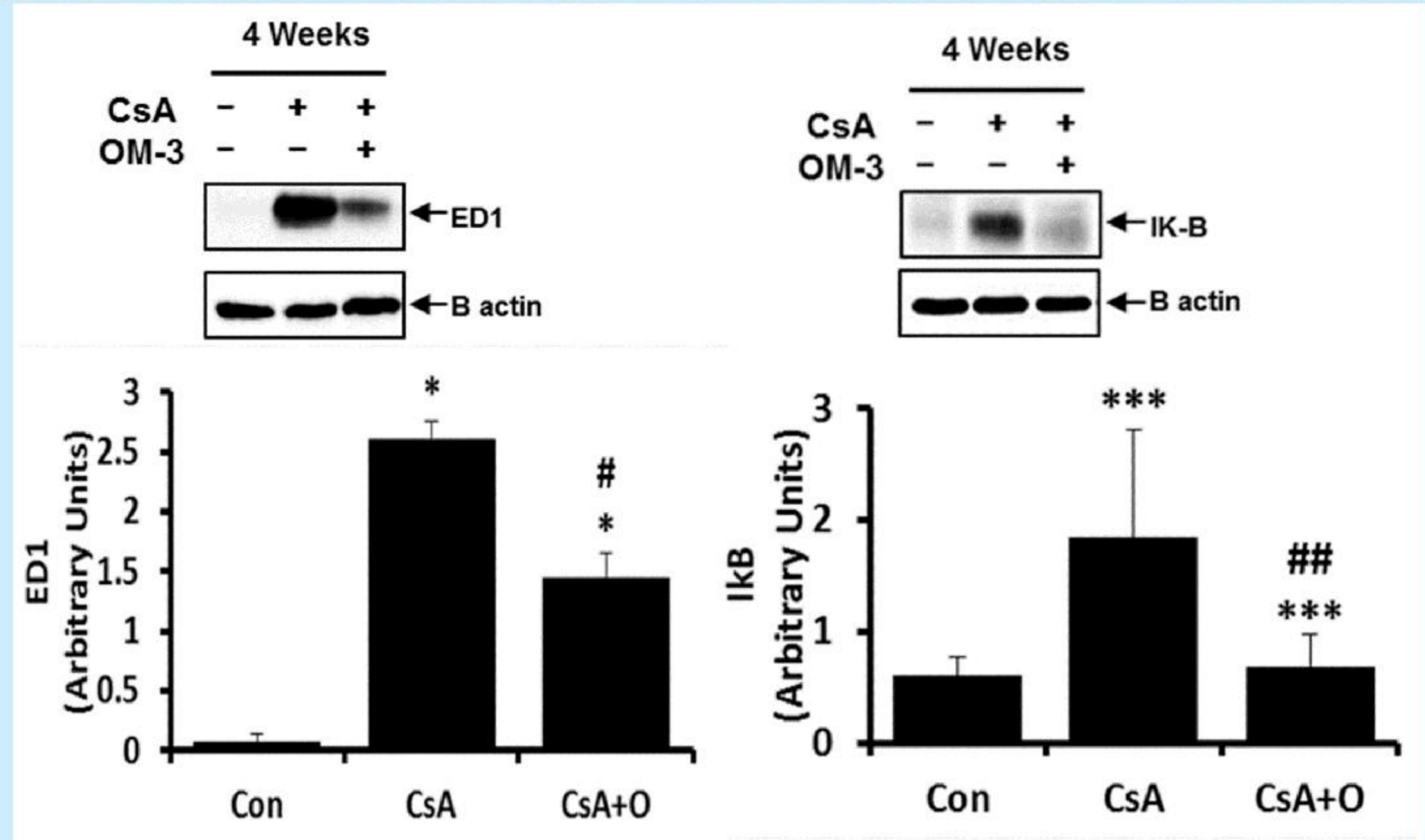


Figure.2 Expression of Caspase 3 and Caspase 7

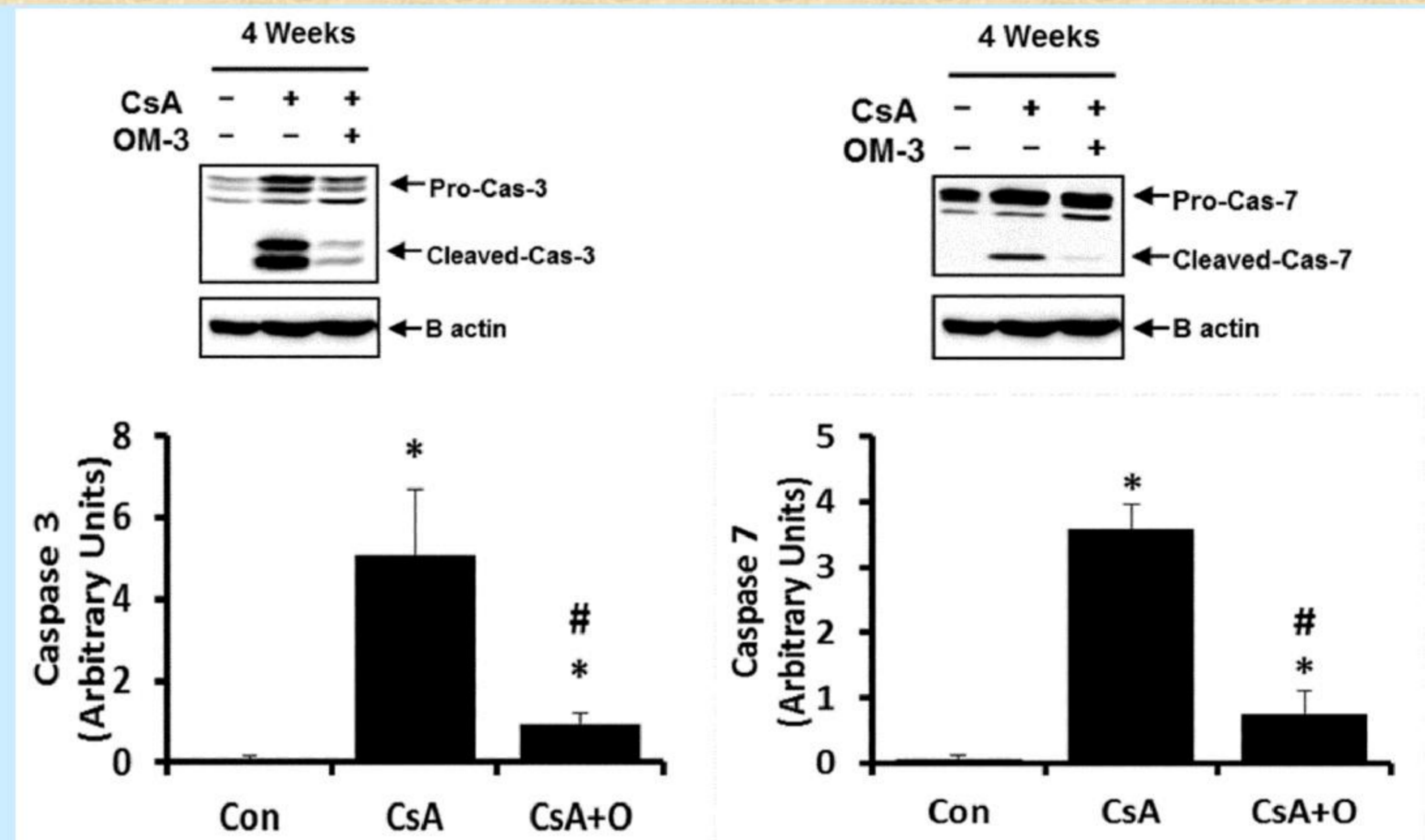


Figure.3 Expression of BAX and Bcl2

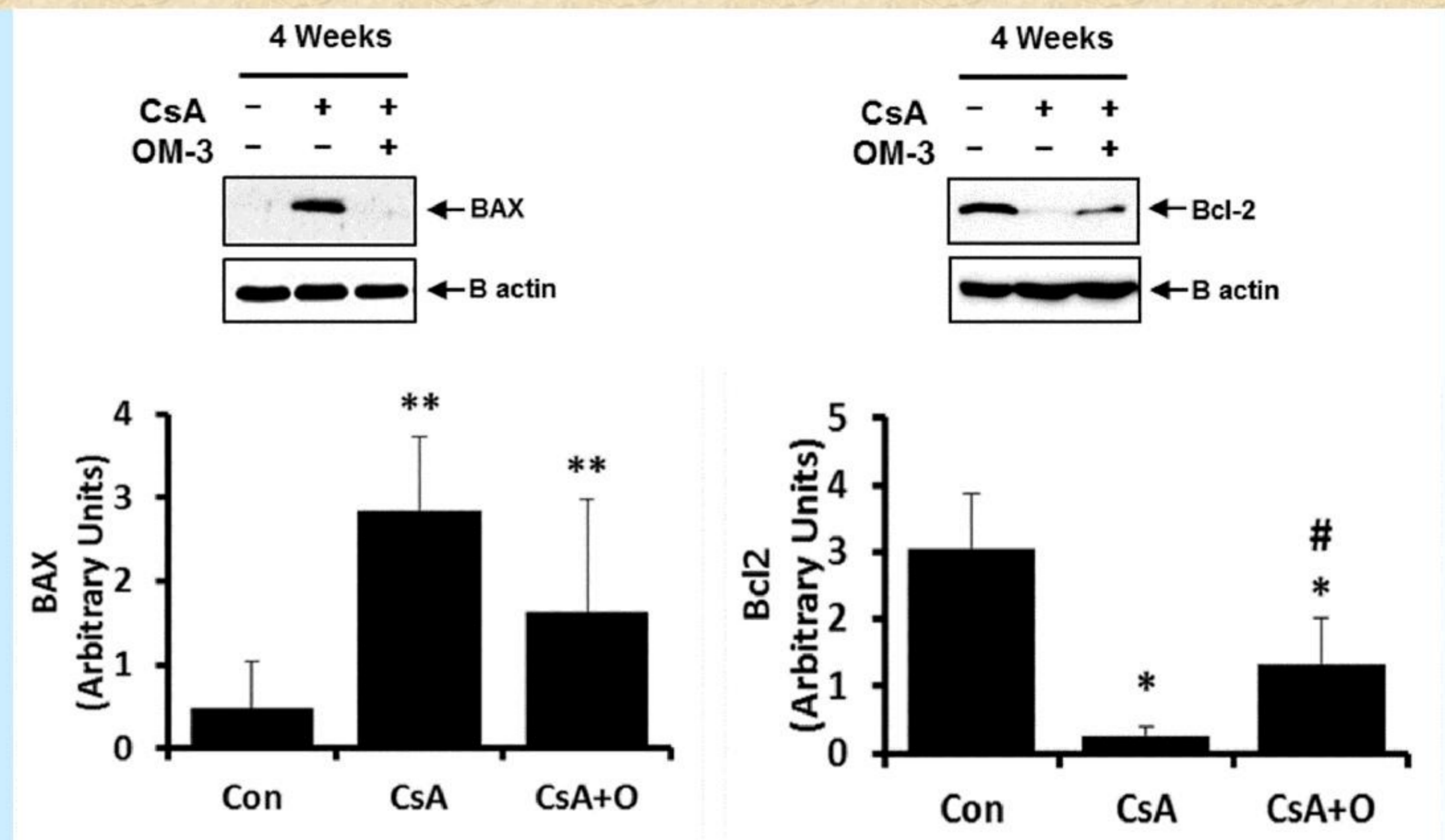
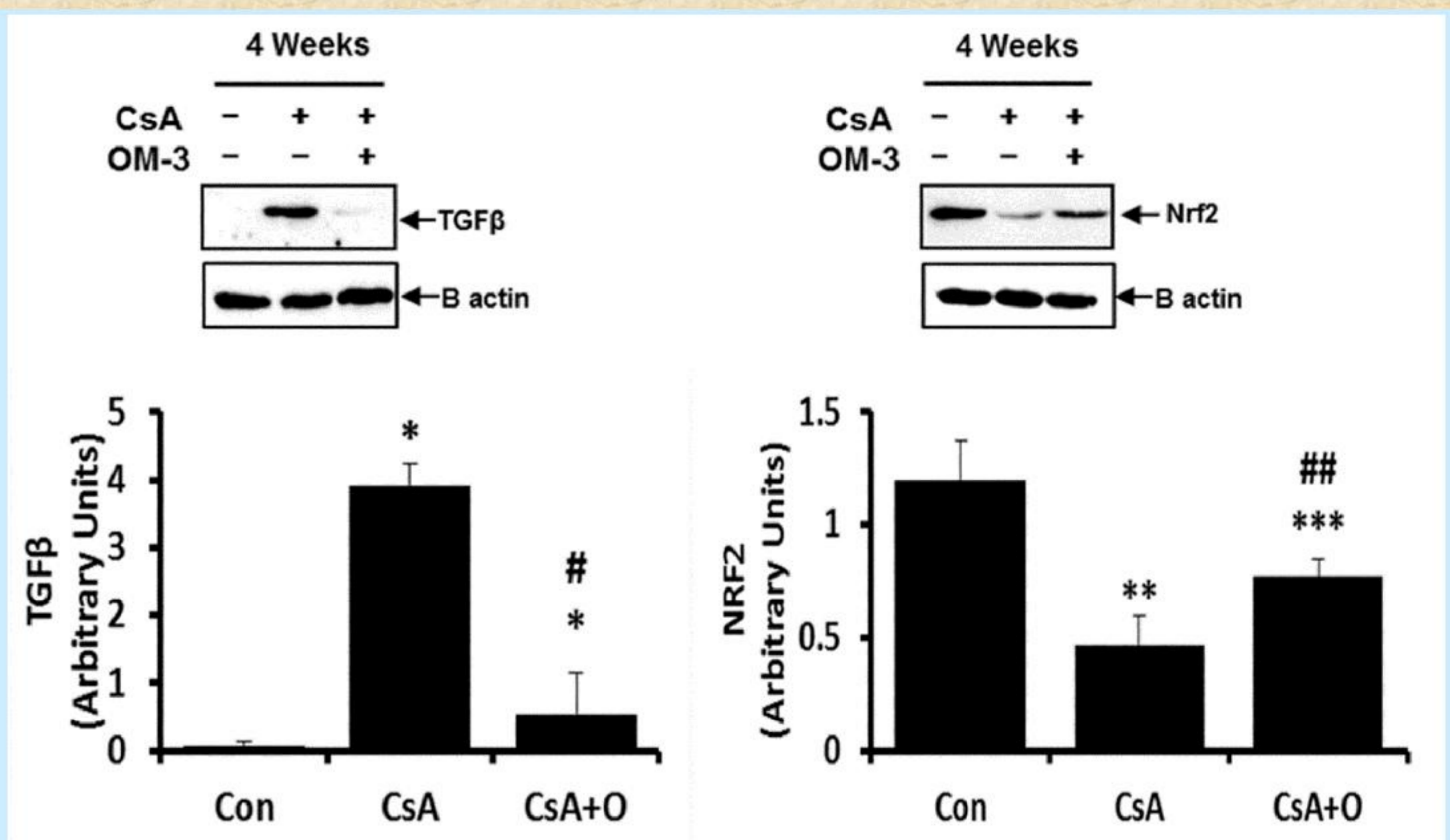


Figure.4 Expression of TGF-β and Nrf2



Conclusions

- ◆ We suggest that Nrf-2 is one of potential mediators induced by omega-3 FA supplementation attenuating pro-inflammatory pathway, fibrotic processes and apoptosis
- ◆ Further studies are necessary to elucidate cross-talks between Nrf2 expression and related signals of omega-3 FA