The Study for the Anti-Oxidant Effects of Dr.coa

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The Report of Dr. coa

Researchers: Drs. SeungBaek Lee and JungJin Kim (Mayo Clinic, USA)

A. Summarizing the specific aims

Reactive oxygen (ROS) is a substance produced during the respiration process and plays an important role in maintaining homeostasis of various cells. However, the abnormally increased free radicals may cause various diseases such as cancer, aging, diabetes, osteoporosis, and heart disease (Fig. 1). Therefore, a study on antioxidants that remove free radicals in the body is an important part of human disease research and has been studied by many basic/clinical fields. ROS is also closely related to TNF-α, a major inflammation mediator. Cytokines such as TNF-α trigger inflammatory reactions, exacerbating asthma, organ inflammation, respiratory diseases, diabetes complications, heart disease, etc. ROS formation triggered by TNF-α is one of the main bridges between TNF-α and abnormal inflammatory signaling.

Recently, we found that Dr.coa is effective for various diseases including Diabetes. Also proinflammatory cytokines such as IL-1β , TNF-α, and IL-6 levels were significantly decreased in cell growth media using Dr.coa instead of normal water. Since ROS is the main cause of various diseases and TNF-α-induced inflammation signaling, we hypothesized that Dr.coa could have an anti-oxidant effect. To test our hypothesis, we studied the following Specific Aims:

Aim 1. Study the effect of Dr.coa on ROS production

Aim 2. Study the role of Dr.coa in TNF-α-induced ROS

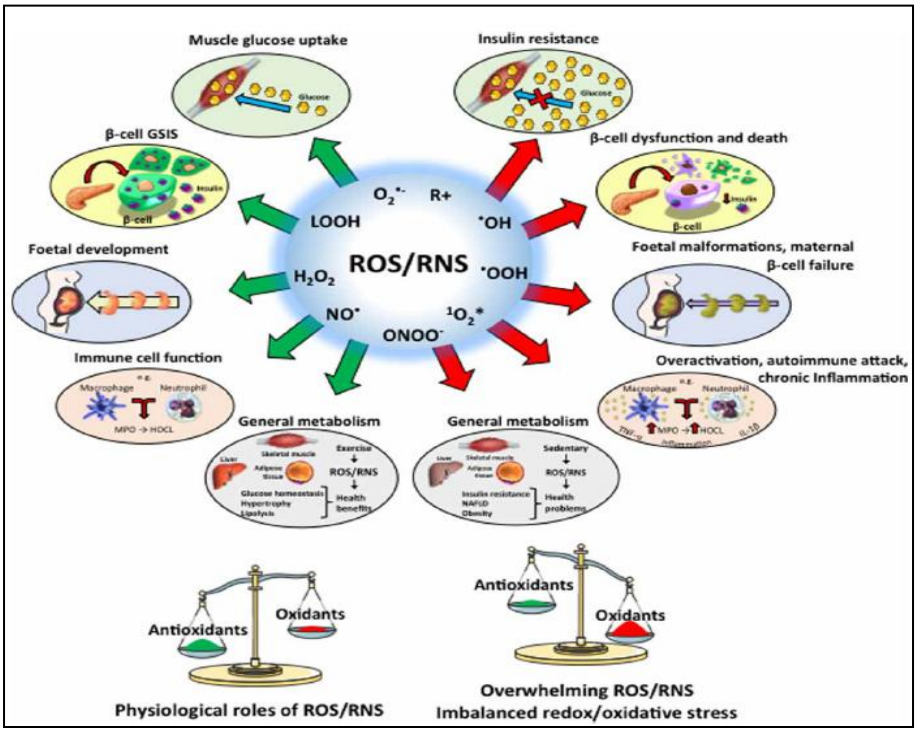

Fig. 1. Dual involvement of ROS/RNS in physiology and disease.
(Biochemical Journal (2016) 473 4527–4550)

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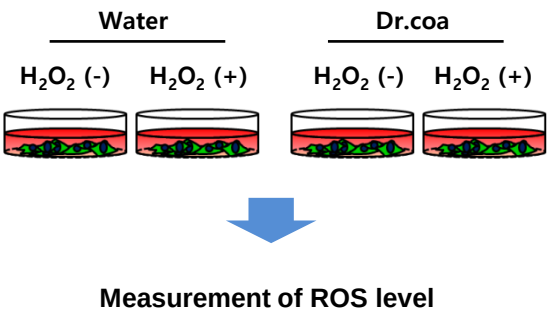
B-1. Methods



To study the anti-oxidant effect of Dr.coa, we treated H_2O_2 (200μM), ROS inducer, to BEAS-2B (human normal bronchial epithelial cells) cell lines for 1 hour. BEAS-2B cells were cultured in working medium that was solved in general water or Dr. coa for two days before H_2O_2 treatment. All results were performed in triplicate of at least three independent experiments. H_2O_2- induced ROS was detected by H_2-DCFDA, an indicator for ROS. When H_2-DCFDA is oxidized, it shows a green fluorescent. ROS (green) detection was done by using a fluorescent microscope.

C-1. Results

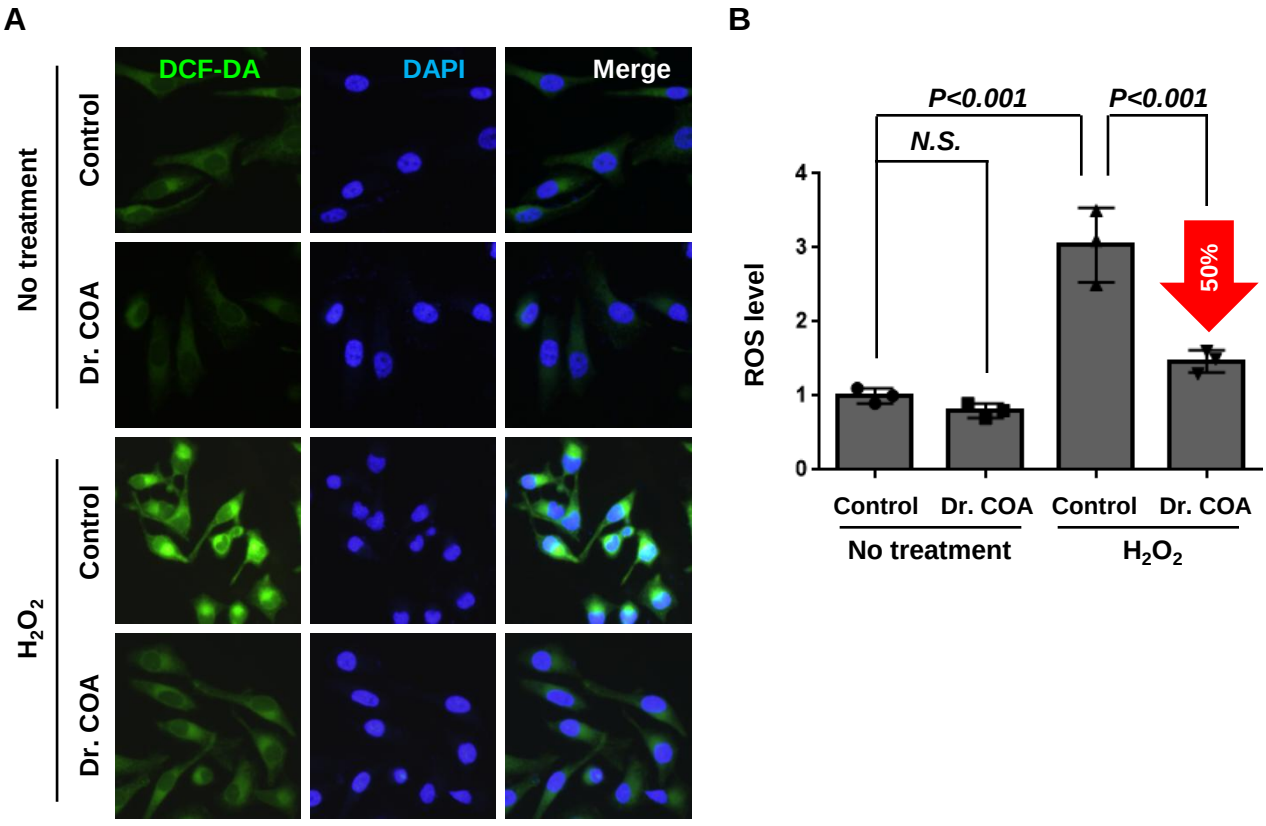


Fig. 2. Anti-oxidant Effects of Dr. coa

BEAS-2B cells were incubated with H_2O_2 (200μM) for 1 hour. The cells were stained with CM-H2DCFDA (5 mg/ml) for 30 min and then observed through a fluorescence microscopy. The cells placed in Dr.coa showed ***-50% reduced ROS production*** and supported the maintenance of ***good cell condition***.

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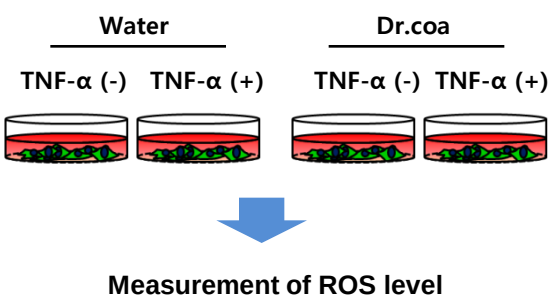
B-2. Methods



To study the anti-oxidant effect of Dr.coa during TNF-α-induced ROS production that is also the cause of inflammation, we treated TNF-α for 15, 30, and 120 minutes to BEAS-2B cells. Cells were cultured in working medium that was solved in general water or Dr.coa during two days before TNF-α treatment. All results were performed in triplicate of at least three independent experiments. ROS was detected by H_2-DCFDA, an indicator for ROS. When H_2-DCFDA is oxidized, it shows a green fluorescent. ROS (green) was detected by using a fluorescent microscope.

C-2. Results

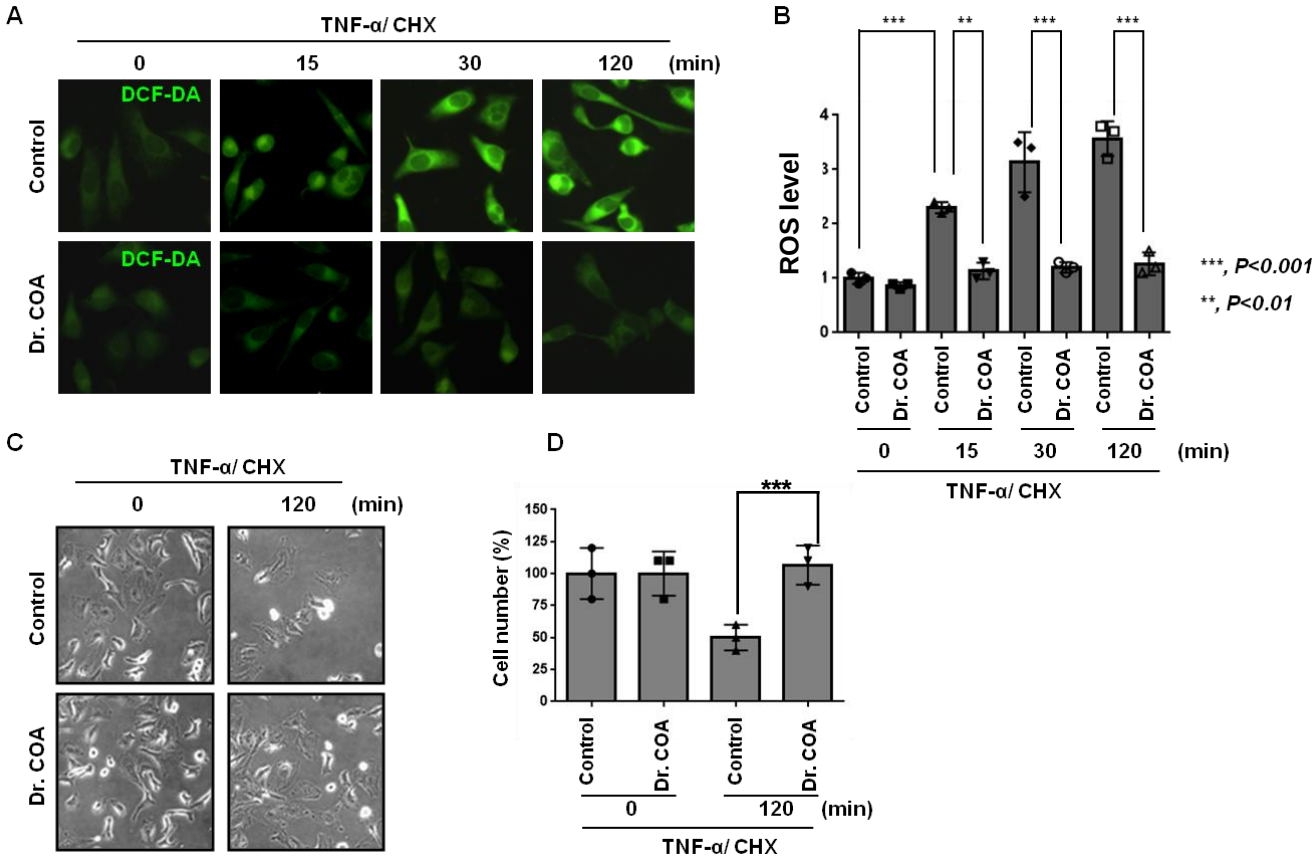


Fig. 3. The Effects of Dr.coa on TNF-α – induced ROS and cell death

BEAS-2B cells were incubated with TNF-α (20 ng/ml) and CHX (10 mg/ml) in working medium that was solved in general water or Dr.coa for the indicated times. (A and B) The cells were stained with CM-H2DCFDA (5 mg/ml) for 30 min and then observed by confocal microscopy. In human bronchial epithelial cells cultured in normal medium, ROS was increased time-dependent manner after TNF-α treatment, whereas in the cells in ***Dr.coa, TNF-α-induced ROS was significantly decreased*** (C and D). TNF-α-induced ***apoptotic cell death was also significantly inhibited in the cell that cultured in Dr.coa.***

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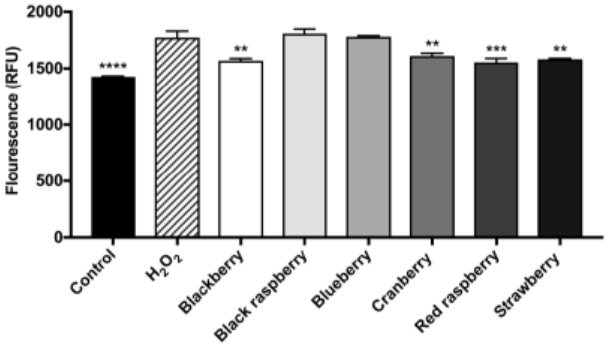
D. Conclusion

1. **Dr.coa has an *anti-oxidant effect*.**
2. **Dr. coa has an effect of *suppressing ROS* directly as well as *TNF-α-induced ROS and apoptosis*.**

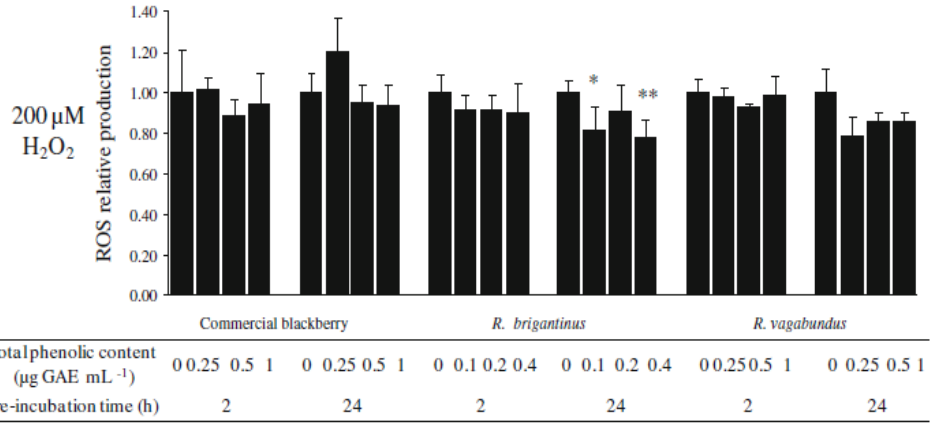
E. Etc - The anti-oxidant effect of Berries



International J of Molecular Sciences (2018)

In the International Journal of Molecular Sciences, Hang et al studied that Berry extracts were evaluated for their inhibitory effects on the ROS production using H_2O_2 treatment. The berry extracts reduced H_2O_2 –induced ROS production up to 12%.

European Journal of Nutrition (2012)



Lucelia et al also studied the anti-oxidant effect of Berries. They found that just *R.brigantinus* (a kind of black berry) reduced 20% of ROS levels after H_2O_2 treatment. However none of the other black berry could lower ROS levels.

F. Discussion

Table 1. The anti-oxidant effect

	International J of Molecular Sciences (2018)						*European Journal of Nutrition (2012)*		Dr. coa
	Black-berry	Black rasp-berry	Blue-berry	Cran-berry	Red rasp-berry	Straw-berry	Black-berry	*R. brig-antinus*	Dr. coa
Anti-oxidant Effect (%)	11.5	*n.s.*	n.s.	9.2	12.3	10.8	*n.s.*	20	**50**