Supporting Information to:

Involvement of p38 MAPK Phosphorylation and Nitrate Formation in Aristolochic Acid-Mediated Antiplatelet Activity

Ming-Yi Shen[1], Chien-Liang Liu[3], Geroge Hsiao[2], Chiung-Yueh Liu[1], Kuang-Hung Lin[1], Duen-Suey Chou[2], Joen-Rong Sheu[1,2]

Affiliation

[1] Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan

[2] Department of Pharmacology, Taipei Medical University, Taipei, Taiwan

[3] Department of Surgery, Mackay Memorial Hospital, Taipei, Taiwan

Correspondence

Dr. Joen-Rong Sheu

Graduate Institute of Medical Sciences

Taipei Medical University

250 Wu-Hsing Street

Taipei 110

Taiwan

R.O.C.

Tel: +886/2/2736/1661 ext 3199

Fax: +886/2/2739/0450

sheujr@tmu.edu.tw

© Georg Thieme Verlag KG · DOI 10.1055/s-2008-1074560 · Planta Med 2008; 74: e707–e709 · Shen M-Y et al.

Materials and Methods

Measurement of free radicals by electron spin resonance (ESR) spectrometry

For the ESR method, we used a Bruker EMX ESR spectrometer as described previously [1]. In brief, washed platelets (3.6×10^8/mL) were preincubated with AsA (115 and 150 μM) or an isovolumetric solvent control (0.5% DMSO) for 3 min before the addition of collagen (1 μg/mL). The reaction was allowed to proceed for 5 min, followed by the addition of DMPO (100 μM) for the ESR study. The rate of free radical-scavenging activity is defined by the following equation: inhibition rate = 1 – [signal height (AsA)/signal height (control)] [1].

Results

In this study, a typical ESR signal of hydroxyl radical ($OH^{\bullet}$) formation was induced by collagen (1 μg/mL) in platelets compared with resting platelets (Fig. **1S, A, B**); pretreatment with AsA (115 and 150 μM) did not significantly reduce hydroxyl radical formation stimulated by collagen (1 μg/mL) (Fig. **1S, C, D**). The antioxidant, catalase (1000 U/mL), markedly suppressed hydroxyl radical formation by about 79% (data not shown).

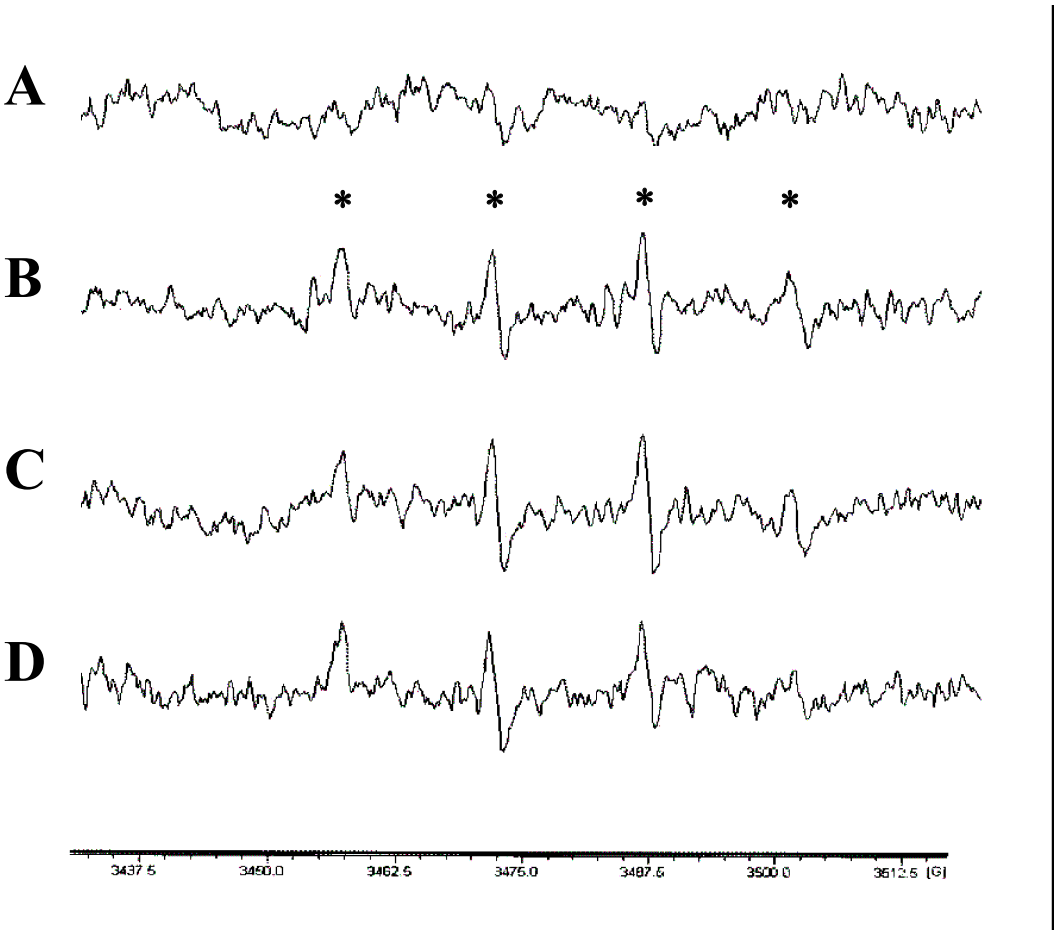

Fig. 1S. Electron spin resonance (ESR) spectra of aristolochic acid in hydroxyl radical ($OH^{\bullet}$) formation in collagen-activated platelets. Washed platelets (3.6×10^8/mL) were preincubated with (**A**) Tyrode's solution (resting group), or (**B**) the solvent control (0.5% DMSO) and aristolochic acid (**C**) (115 μM) and (**D**) (150 μM), followed by the addition of collagen (1 μg/mL) to trigger hydroxyl radical formation. Spectra are representative examples of four similar experiments.

References

1 *Chou DS, Hsiao G, Shen MY, Tsai YJ, Chen TF, Sheu JR.* ESR spin-trapping of a carbon-centered free radical from agonist-stimulated human platelets. Free Radic Biol Med 2005; 39: 237-48.