

Resveratrol, an extract of red wine, inhibits lipopolysaccharide induced airway neutrophilia and inflammatory mediators through an NF- κ B-independent mechanism

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 To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.04-2691fje>; doi: 10.1096/fj.04-2691fje

SPECIFIC AIMS

Consumption of resveratrol, particularly through drinking moderate amounts of red wine, has been suggested to have beneficial health effects. Over the last decade a great deal of research has been performed on this naturally occurring compound and it has been shown to possess anti-inflammatory properties *in vitro*.

The initial aim of this study was to determine what effect resveratrol would have in a preclinical *in vivo* model of airway neutrophilia. The secondary aim was to attempt to elucidate how this molecule was affecting cellular inflammation by measuring a range of reported “resveratrol-sensitive” mediators and parameters known to be involved in the inflammatory response.

PRINCIPAL FINDINGS

1. Resveratrol inhibited airway tissue neutrophilia

Administration of resveratrol into the airway before exposure to aerosolized LPS resulted in a dose-related reduction in airway tissue neutrophilia to a similar extent as that produced by the clinically relevant glucocorticoid, budesonide (**Fig. 1**).

2. Inflammatory mediators were inhibited by resveratrol

The reduction in airway neutrophilia by resveratrol treatment was associated with a reduction in some LPS-induced inflammatory mediators such as TNF- α , IL-1 β , CINC-1, and PGE₂ (**Fig. 2**), suggesting a causative link.


3. The inhibition of inflammatory mediators by resveratrol was independent of effects on NF- κ B activity or gene expression

Recent *in vitro* studies have suggested that the effect of resveratrol on inflammation is due to its impact on the

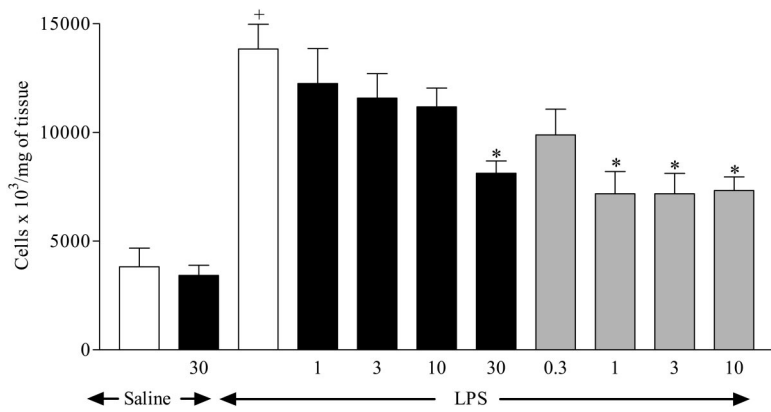
NF- κ B pathway and subsequent reduction in gene expression. In this preclinical model, it was shown that resveratrol did not reduce LPS-induced increase in p65 levels, a marker of NF- κ B activation, or expression of genes linked to the activity of NF- κ B, iNOS, and COX-2, which suggests resveratrol was not affecting NF- κ B activation. Indeed, none of the inflammatory proteins that were reduced by resveratrol had altered gene expression levels, suggesting the effect of resveratrol is posttranscriptional on these proteins.

CONCLUSIONS AND SIGNIFICANCE

This is the first study to demonstrate anti-inflammatory effects of resveratrol in an *in vivo* model of airway inflammation. We have also shown that the inhibition of inflammatory mediators is through an NF- κ B-independent mechanism. These results suggest that this naturally occurring molecule may possess anti-inflammatory properties via a novel mechanism (**Fig. 3**). Elucidation of this mechanism of action may lead to potential new therapies for the treatment of chronic inflammation.

Recently, there have been many well-publicized links between drinking moderate amounts of red wine and beneficial health effects. This study reveals an exciting and highly promising prospect for a possible new anti-inflammatory agent. These findings are doubly exciting because resveratrol is a naturally occurring molecule that is ingested by many, which suggests the possibility of minimal toxic side effects. This study also may have highlighted a compound with a potential novel mechanism of action. This in turn may lead to new avenues of research in the quest for novel therapies for chronic inflammation. 

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the saline-challenged vehicle group; * $P < 0.05$, denoting significant difference to the LPS-challenged vehicle group).

Figure 1. Effect of resveratrol and budesonide on inflammatory cell numbers in the lung tissue. Rats were dosed with vehicle (0.5% methylcellulose and 0.2% Tween 80 in saline, 1 mL/kg), resveratrol (1, 3, 10, or 30 mg/kg) or budesonide (0.3, 1, 3, and 10 mg/kg) intratracheally under halothane (4% in oxygen for 4 min). 90 min later, the rats were exposed to aerosolized saline or LPS (0.3 mg/mL). 6 h later, challenge lung samples were collected and white cells extracted by enzymatic digest. Numbers of neutrophils in the cell pellet were determined by differential counting under light microscopy. Results are expressed as mean \pm SEM of 8 animals. Statistical analysis was assessed using one-way ANOVA and the appropriate post-test. ($^+P < 0.05$, denoting significant difference to the saline-challenged vehicle group; * $P < 0.05$, denoting significant difference to the LPS-challenged vehicle group).

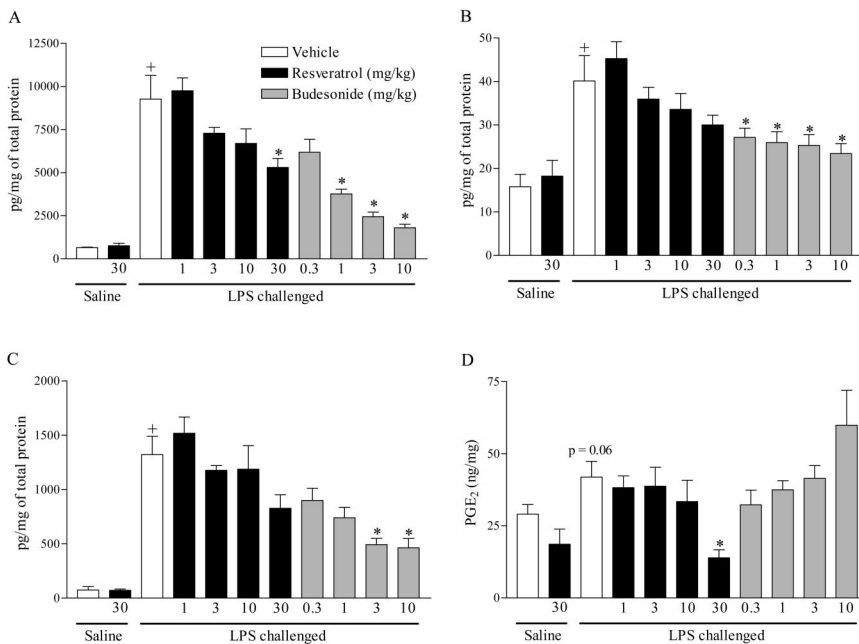


Figure 2. Effect of resveratrol and budesonide on levels of mediators in the lung tissue. Rats were dosed with vehicle (0.5% methylcellulose and 0.2% Tween 80 in saline, 1 mL/kg), resveratrol (1, 3, 10, or 30 mg/kg) or budesonide (0.3, 1, 3, and 10 mg/kg) intratracheally under halothane (4% in oxygen for 4 min). 90 min later, the rats were exposed to aerosolized saline or LPS (0.3 mg/mL). 6 h later, lung tissue samples were collected. Levels of mediators in the lung tissue homogenate were determined by ELISA-IL-1 β (A), TNF- α (B) and CINC-1 (C) or by PGE₂ RIA (D). Results are expressed as mean \pm SEM of 8 animals. Statistical analysis was assessed using one-way ANOVA and the appropriate posttest. ($^+P < 0.05$, denoting significant difference to the saline-challenged vehicle group; * $P < 0.05$, denoting significant difference to the LPS-challenged vehicle group).

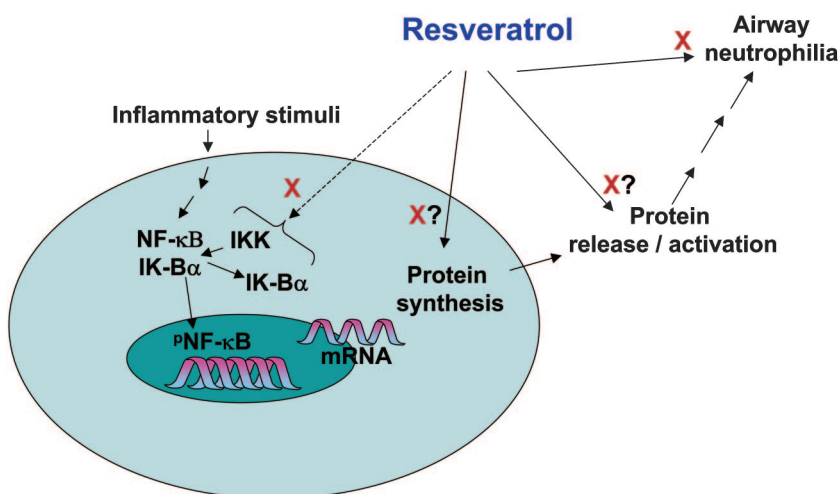


Figure 3. Schematic diagram illustrating how resveratrol could be exerting its anti-inflammatory effects in this preclinical model. This diagram depicts the simplified production of inflammatory mediators from stimuli to the recruitment of airway neutrophilia. The dotted line indicates previously hypothesized in vitro mechanisms of action of this naturally occurring compound. In this preclinical in vivo model, it appears that resveratrol has not affected NF- κ B activity or gene expression to reduce the production of inflammatory mediators. The unbroken arrows indicate possible mechanisms of action of the molecule.