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Metabolic Inactivation of Resolvin E1 and Stabilization of Its Anti-inflammatory Actions*

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The resolvins (Rv) are lipid mediators derived from omega-3 polyunsaturated fatty acids that act within a local inflammatory milieu to stop leukocyte recruitment and promote resolution. Resolvin E1 (RvE1; (5*S*,12*R*,18*R*)-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eicosapentaenoic acid) is an oxygenase product derived from omega-3 eicosapentaenoic acid that displays potent anti-inflammation/pro-resolution actions *in vivo*. Here, we determined whether oxidoreductase enzymes catalyze the conversion of RvE1 and assessed the biological activity of the RvE1 metabolite. With NAD⁺ as a cofactor, recombinant 15-hydroxyprostaglandin dehydrogenase acted as an 18-hydroxyl dehydrogenase to form 18-oxo-RvE1. In the murine lung, dehydrogenation of the hydroxyl group at carbon 18 position to form 18-oxo-RvE1 represented the major initial metabolic route for RvE1. At a concentration where RvE1 potently reduced polymorphonuclear leukocyte (PMN) recruitment in zymosan-induced peritonitis, 18-oxo-RvE1 was devoid of activity. In human neutrophils, carbon 20 hydroxylation of RvE1 was the main route of conversion. An RvE1 analog, *i.e.* 19-(*p*-fluorophenoxy)-RvE1, was synthesized that resisted rapid metabolic inactivation and proved to retain biological activity reducing PMN infiltration and pro-inflammatory cytokine/chemokine production *in vivo*. These results established the structure of a novel RvE1 initial metabolite, indicating that conversion of RvE1 to the oxo product represents a mode of RvE1 inactivation. Moreover, the designed RvE1 analog, which resisted further metabolism/inactivation, could be a useful tool to evaluate the actions of RvE1 in complex disease models.

Most inflammatory processes are self-limiting (1), implicating the existence of endogenous circuits for anti-inflammation and/or pro-resolution mediators that are operative during the temporal events of host defense and inflammation (recently reviewed in Refs. 2 and 3). We identified and characterized lipid mediators that are generated during spontaneous resolution

phase and that possess anti-inflammatory and/or pro-resolving properties; these include lipoxins, resolvins, and protectins (2). Like other autacoids, these lipid mediators are generated in response to stimuli, act locally, and may be rapidly inactivated by further metabolism via enzymatic pathways (4–6). Resolution of inflammation is an active process governed by timely and spatially regulated formation and inactivation of local lipid mediators and termination of pro-resolving signals, so that tissues can return to homeostasis (4). Thus, it is important to identify the further metabolic products of these pro-resolving lipid mediators and evaluate their bioactivities *in vivo*.

The resolvins and protectins are new families of lipid mediators derived from omega-3 polyunsaturated fatty acids, namely eicosapentaenoic acid and docosahexaenoic acid, that are generated and act locally at sites of inflammation, where they counterregulate polymorphonuclear leukocyte (PMN)² infiltration and promote resolution (for recent reviews, see Refs. 2 and 3, and references therein). They are generated during multicellular responses such as inflammation and microbial infections, a unique pathway that involve cell-cell interactions and transcellular biosynthetic routes. When aspirin is given during inflammation, resolvin E1 (RvE1) is formed from eicosapentaenoic acid via cell-cell interactions involving cells bearing cyclooxygenase-2 that has been acetylated at Ser⁵¹⁶ by aspirin and cells that possess 5-lipoxygenase (7, 8). These newly produced resolvins may be responsible for some of the beneficial effects of taking omega-3 eicosapentaenoic acid and aspirin (9, 10). RvE1 is also formed by microbial cytochrome P450 monooxygenase in an aspirin-independent manner (11), which can contribute to its production *in vivo* that is enhanced with aspirin treatment in both human (8) and mouse (9). The complete stereochemistry of bioactive RvE1 was established and demonstrated to be (5*S*,12*R*,18*R*)-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eicosapentaenoic acid (8).

RvE1 is active in both *in vitro* and *in vivo* systems, where it displays potent counterregulatory and tissue-protective roles.

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² The abbreviations used are: PMN, polymorphonuclear leukocyte; resolvin, resolution phase interaction product; RvE1, resolvin E1; PGDH, prostaglandin dehydrogenase; MS/MS, tandem mass spectrometry; IL, interleukin; ESI, electrospray ionization; LC, liquid chromatography; GC, gas chromatography; LXA₄, lipoxin A₄; ATL_a, 15-epi-16-(*p*-fluorophenoxy)-LXA₄ methyl ester (LXA₄ analog); LTB₄, leukotriene B₄; PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography; RANTES, regulated on activation normal T cell expressed and secreted.

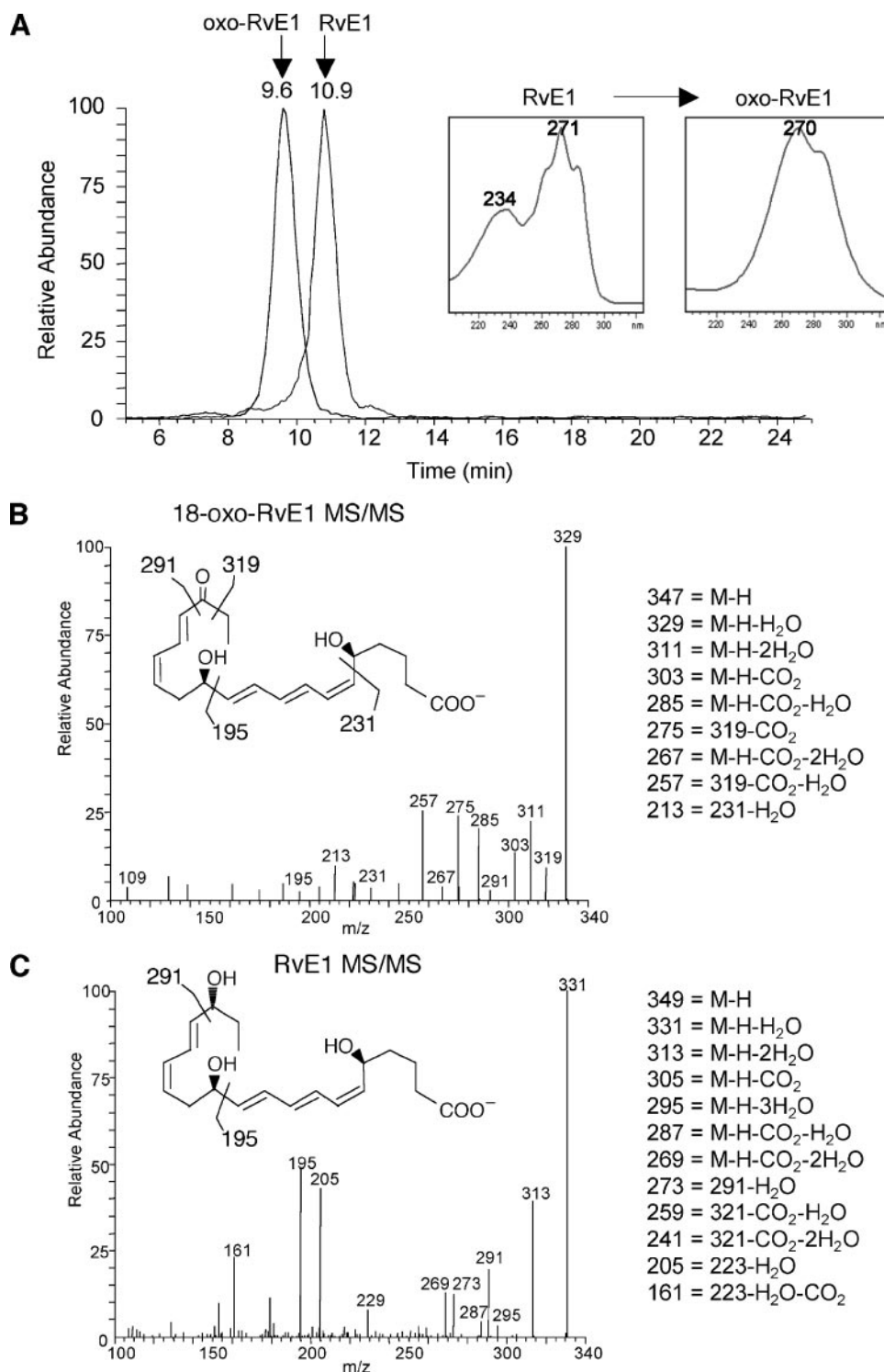


FIGURE 1. LC-ESI-MS/MS chromatograms and spectra of RvE1 and 18-oxo-RvE1. A, RvE1 (5 μ g, $[M - H]^- = m/z$ 349, retention time = 10.9 min) was incubated with recombinant human 15-PGDH (1 μ g) and NAD⁺ (1 mM) in a 100- μ l reaction volume. The major product, oxo-RvE1 ($[M - H]^- = m/z$ 347, retention time = 9.6 min), was 2 atomic mass units lower in mass. UV absorbance spectra of RvE1 and oxo-RvE1 are provided in the insets. B, ESI-MS/MS spectrum of oxo-RvE1. The molecular anion of oxo-RvE1 ($[M - H]^- = m/z$ 347) was further fragmented to yield diagnostic product ions (see "Results" for further details). C, ESI-MS/MS spectrum of RvE1. The molecular anion of RvE1 ($[M - H]^- = m/z$ 349) was further fragmented to yield diagnostic product ions.

RvE1, for example, reduces neutrophil transendothelial migration *in vitro* in the nanomolar concentration range (7). These anti-inflammatory actions are also demonstrable *in vivo*, where

RvE1 blocks PMN infiltration both in peritonitis (8) and in inflamed colon tissues during colitis (9). RvE1 also attenuates antigen-presenting cell functions such as dendritic cell migration and interleukin (IL)-12 production *in vivo* (8).

Here, we report that enzymatic conversion of RvE1 specifically generates 18-oxo-RvE1 resulting in its inactivation. An RvE1 stable analog was designed that resisted rapid conversion by dehydrogenation and retained biological activity that reduced both PMN infiltration and pro-inflammatory cytokines and chemokines *in vivo*.

EXPERIMENTAL PROCEDURES

Materials—Synthetic RvE1 was prepared by total organic synthesis and matched with the published criteria (8). The RvE1 analog (19-(*p*-fluorophenoxy)-RvE1 methyl ester) was prepared in a stereochemically pure form by total organic synthesis (structure and olefinic region NMR spectrum is shown in Fig. 6A). Female C57BL/6J mice (7–8 weeks old) were from The Jackson Laboratory (Bar Harbor, ME). Recombinant human 15-PGDH was isolated as described previously (6). NAD⁺, diethyl ether, and methoxamine hydrochloride were from Sigma. *N,O*-Bis(trimethylsilyl)trifluoroacetamide was from Fisher.

LC-UV-MS/MS Analysis—Liquid chromatography-ultraviolet-tandem mass spectrometry (LC-UV-MS/MS) results were acquired with the LCQ (Thermo Finnigan) quadrupole ion trap mass spectrometer system equipped with an electrospray ionization probe. RvE1 and its enzymatic products were suspended in mobile phase and injected into the HPLC component with a LUNA C₁₈-2 (100 $\times$ 2 mm $\times$ 5 μ m) column and a UV diode array detector. The column was eluted with methanol/water/acetic acid (58/42/0.01 (v/v/v)) at 0.2 ml/min.

GC-MS Analysis—Carboxylic acid was methylated with excess ethereal diazomethane for 1 h at room temperature. The RvE1 metabolic product was derivatized with hydromethoxamine in pyridine to form methoxime for 2 h at room temperature, and

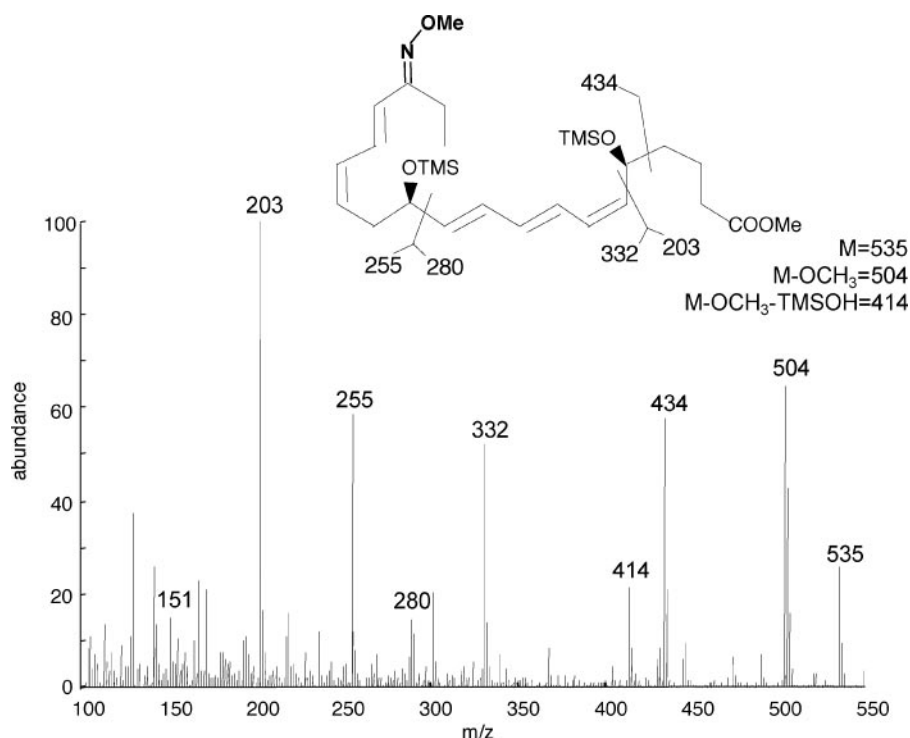


FIGURE 2. GC-MS spectrum of the derivatized methoximation product of 18-oxo-RvE1. Dehydrogenase product of RvE1 was analyzed by GC-MS after methylation, trimethylsilylation (TMS), and methoximation.

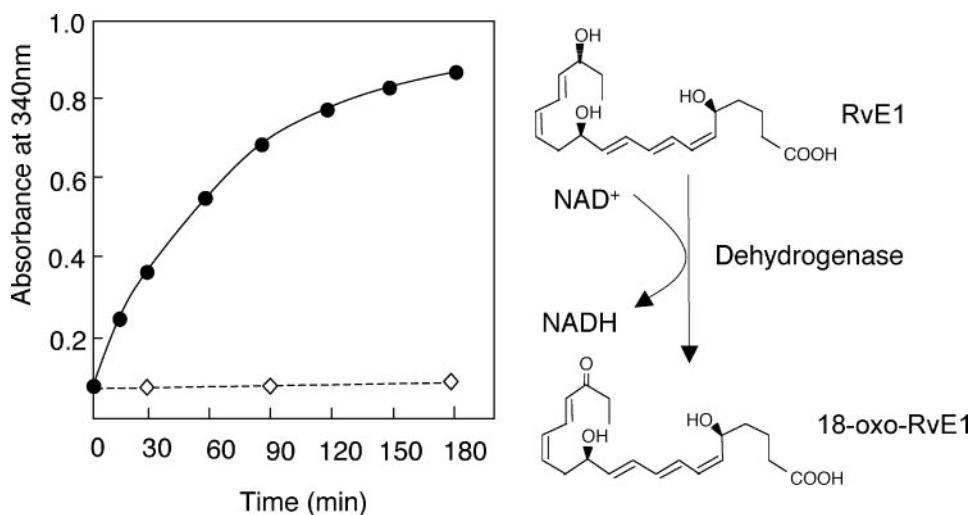


FIGURE 3. Time course of RvE1 conversion. The time course of NADH formation was monitored at 340 nm absorption in a system containing RvE1 and recombinant human enzyme as described in the legend for Fig. 1. Closed circles, with enzyme; open diamonds, without enzyme.

hydroxyl groups were derivatized by *N,O*-bis(trimethylsilyl)tri-fluoroacetamide to form *O*-trimethylsilyl ether (12). GC-MS analysis was performed with a Hewlett-Packard 5971A mass selective detector quadrupole equipped with a HPG1030A work station and GC 5890. The column was a HP-5MS (5% diphenyl-95% dimethylpolysiloxane). The temperature program was initiated at 150 °C for 2 min, reaching 230 °C at 10 min and then 280 °C at 20 min. Reference saturated fatty acid methyl esters carbons C_{16} – C_{24} gave the following retention times (min): C_{16} , 8.80; C_{18} , 10.64; C_{20} , 12.54; C_{22} , 14.70; C_{24} , 17.06; these were used to calculate C-values.

Enzymatic Conversion of RvE1—RvE1 (5 μ g) was incubated with recombinant human 15-PGDH (1 μ g) and NAD^+ (1 mM) in 100 μ l of Tris-HCl buffer (50 mM, pH 9.0) at 37 °C. Increments in absorption at 340 nm were recorded, and reaction rates were calculated via first order regression curves computer-fitted to the change in absorption as a function of time. The product of this reaction was purified by solid extraction (Sep-Pak C_{18}) and further purified by reverse-phase HPLC with a LUNA C_{18} -2 (100 $\times$ 2 mm $\times$ 5 μ m) column eluted with methanol/water/acetic acid (58/42/0.01 (v/v/v)) at 0.2 ml/min.

RvE1 Metabolism in Cells and Tissues—Female C57BL/6J mice at 7–8 weeks were euthanized by isoflurane, and lungs (150–200 mg) were isolated and washed with ice-cold phosphate buffered saline (PBS). Dissected organs were freeze-thawed three times and then suspended in 200 μ l of PBS (pH 7.4) and 1 mM NAD^+ . Incubations were initiated by adding 1 μ g of RvE1 at 37 °C, and after 2 h, 2 volumes of ice-cold methanol was added to terminate the reactions. Human PMN were isolated from healthy volunteers (Brigham and Women's Hospital protocol 88-02642) by Ficoll gradient as described previously (7). Freshly isolated PMNs were suspended at 25×10^6 cells in 0.5 ml of PBS, incubated with 1 μ g of RvE1 for 20 min at 37 °C, and the reaction was stopped with 2 volumes of ice-cold methanol.

Inflammation—Murine peritonitis was carried out as described previously (13), and 100 ng/mouse of RvE1 sodium salt or 18-oxo-RvE1 was injected into the tail vein

followed by 1 ml of zymosan A (1 mg/ml) injected into the peritoneum. For experiments with the RvE1 analog, RvE1 methyl ester or 19-(*p*-fluorophenoxy)-RvE1 methyl ester was each given intravenously at 1.0 μ g/mouse. Peritoneal lavages were collected at 2 h, and cells were enumerated. For differential leukocyte counts, 100 μ l of the lavaged cells were added to 100 μ l of 30% bovine serum albumin and centrifuged onto microscope slides at 2,200 rpm for 4 min using a Cytofuge (StatSpin, Norwood, MA). The slides were allowed to air-dry, and cells were visualized using Wright Giemsa stain. Chemokine and cytokine levels were determined from

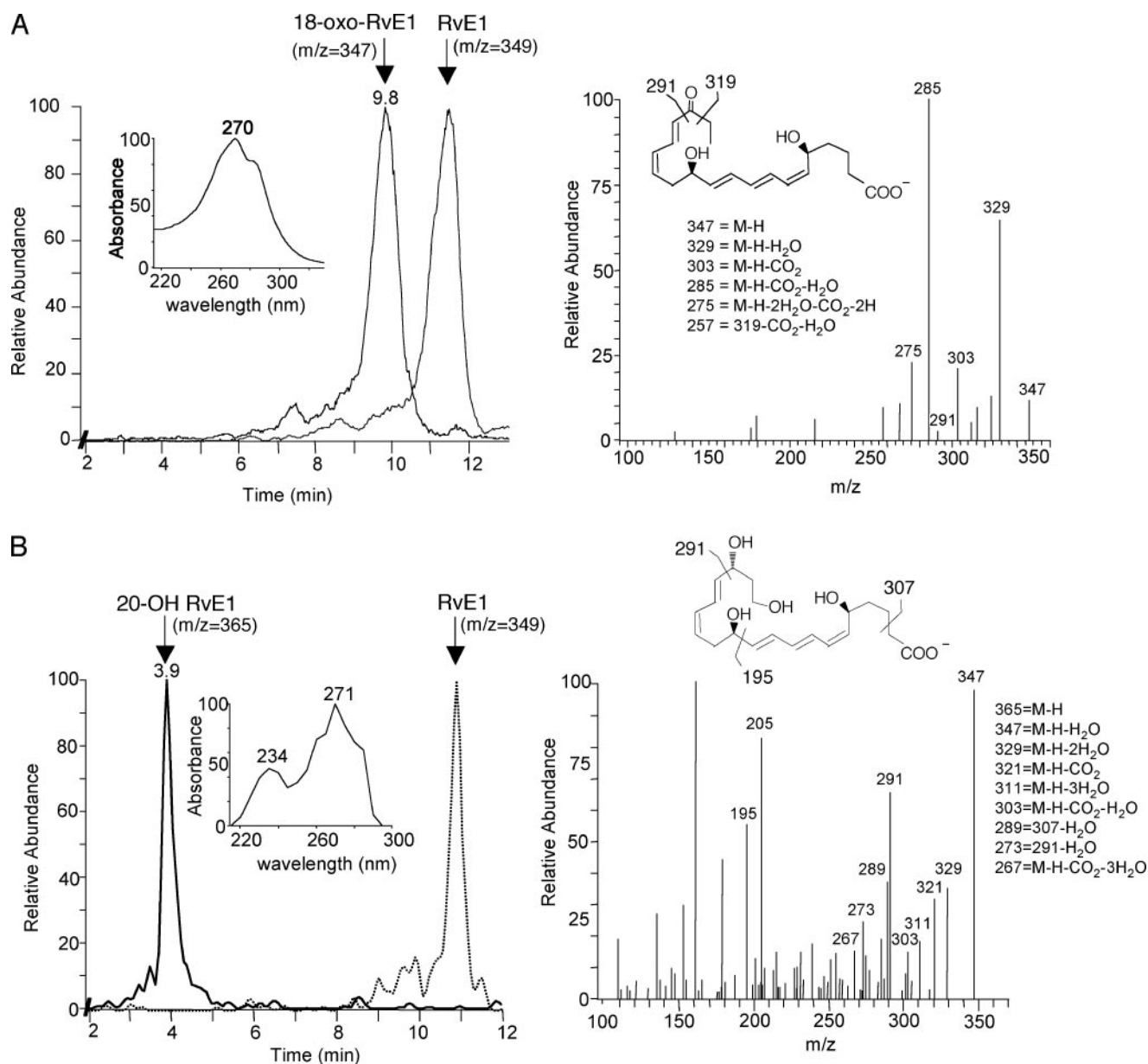


FIGURE 4. **RvE1 metabolism in tissue or cell incubations.** A, RvE1 (1 μ g) was incubated with mouse lungs (150–200 mg, freeze-thawed) and NAD⁺ (1 mM) in a 200- μ l volume of PBS (pH 7.4) for 2 h at 37 °C. RvE1 (m/z = 349) was eluted at 11.5 min, and the main oxidation product (m/z 347, retention time at 9.8 min) gave a UV absorbance spectrum as provided in the *inset*. The molecular anion of the component beneath a peak (m/z 347) was further fragmented to yield diagnostic ions of 18-oxo-RvE1 in ESI-MS/MS spectrum (see “Results” for further details). B, RvE1 (1 μ g) was incubated with human PMN (25×10^6 cells in 0.5 ml of PBS (pH 7.4)) for 20 min at 37 °C. RvE1 (m/z = 349) was eluted at 10.9 min, and the main product (m/z 365, retention time at 3.9 min) gave a UV absorbance spectrum as provided in the *inset*. The molecular anion of the peak (m/z 365) was further fragmented to yield diagnostic product ions of 20-hydroxy-RvE1 in the ESI-MS/MS spectrum (see “Results” for further details). Results are representative of $n = 3$ separate experiments.

peritoneal exudates using a SearchLight mouse chemokine array (Pierce).

RESULTS

Enzymatic Conversion of RvE1—LC-MS/MS-based analyses were employed to determine whether 15-PGDH could catalyze the conversion of RvE1 to oxo metabolites. RvE1 eluted from the LC system at 10.9 min and gave a molecular anion of 349 atomic mass units ($[M - H]^- = m/z$ 349), which was rapidly and enzymatically converted to a product upon addition of recombinant human 15-PGDH and NAD⁺. The main product derived from RvE1 gave a retention time

of 9.6 min and a molecular anion of 347 atomic mass units ($[M - H]^- = m/z$ 347) (Fig. 1A). The reduction in mass of 2 atomic mass units corresponded to the loss of two hydrogen atoms that could occur with the oxidation of one of the alcohol groups within RvE1 to give rise to an oxo group. The UV absorption spectrum of RvE1 shows a maxima at 271 and 234 nm consistent with the reported characteristics (8), indicative of the presence of both conjugated triene and diene chromophores. Conversion of an alcohol of RvE1 to an oxo group also switched the chromophore from diene to dienone (comprising a ketone in conjugation with the diene). This extension of conjugation lowered the difference in energy

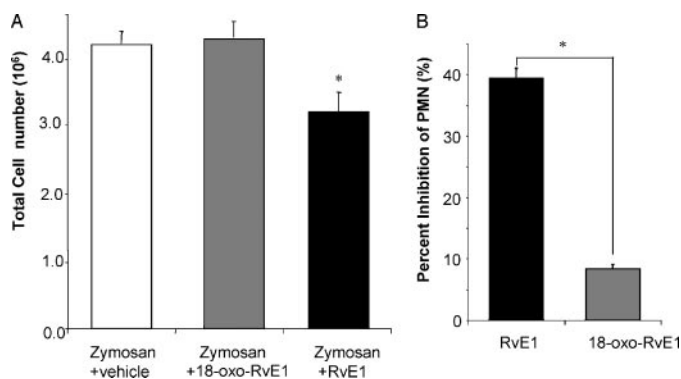


FIGURE 5. **RvE1 but not 18-oxo-RvE1 reduces PMN infiltration *in vivo*.** RvE1 and 18-oxo-RvE1 were injected intravenously at 100 ng/mouse followed by 1 ml of zymosan A (1 mg/ml) injected into the peritoneum. After 2 h, total leukocytes (A) and PMN (B) were enumerated. Values represent mean $\pm$ S.E. from four different mice. *, $p < 0.001$.

between ground and excited electronic states and consequently the absorption maximum was red-shifted from 234 to 270 nm (Fig. 1A).

To gain evidence for the proposed oxo-RvE1 structure and the carbon position, the product ion mass spectrum (MS/MS) was analyzed. Oxo-RvE1 fragmented via: (i) neutral loss of H₂O and CO₂ to yield the product ions m/z 329 ([M - H]⁻-H₂O), 311 ([M - H]⁻-2H₂O), 303 ([M - H]⁻-CO₂), 285 ([M - H]⁻-CO₂-H₂O), and 267 ([M - H]⁻-CO₂-2H₂O); and (ii) cleavages of the 17,18 C-C bond to yield m/z 291, 18,19 C-C bond to yield m/z 319, and 5,6 C-C bond to yield m/z 231 (Fig. 1B). The product ion derived from cleavage of the 17,18 C-C bond (m/z 291) implicated that the oxo group was at the carbon 18 position rather than the possible carbon 12 position or even the 5 position.

To further confirm the oxo group at the carbon 18 position, the isolated compound was treated with diazomethane followed by methoxyamine to form the methoxime and analyzed using GC-MS. Selective ion monitoring at m/z = 535 (expected molecular weight of 18-oxo-RvE1 methyl ester in the presence of 2 trimethylsilane ether and 1 methoxime) identified a single peak at 18.6 min (C-value of 26.3). The MS spectrum of the trimethylsilane-derivatized methoxime product gave diagnostic fragments as reported and assigned in Fig. 2 (see *inset*). Therefore, the structure of RvE1 conversion product was confirmed as 18-oxo RvE1.

Next, the time course of RvE1 conversion to 18-oxo-RvE1 by the dehydrogenase was monitored by the formation of NADH from NAD⁺ as increase in absorbance at 340 nm (Fig. 3). The initial conversion rate for RvE1 was 0.43 ± 0.07 μ mol of NADH/min/mg of enzyme. Using essentially identical reaction conditions, prostaglandin E₂ was converted at a rate of 1.43 ± 0.26 μ mol of NADH/min/mg of enzyme. In addition, almost 50% less conversion was observed using the 6,7,14,15-diacetylenic derivative of RvE1 ($n = 3$, data not shown). Taken together, these results emphasize the structural recognition required for the dehydrogenase reaction.

RvE1 Metabolism in Cells and Tissues—To address whether oxidation of the alcohol group at carbon 18 position represents a major pathway of RvE1 metabolism in cells or tissues, we performed RvE1 incubations with isolated mouse lung (Fig. 4A)

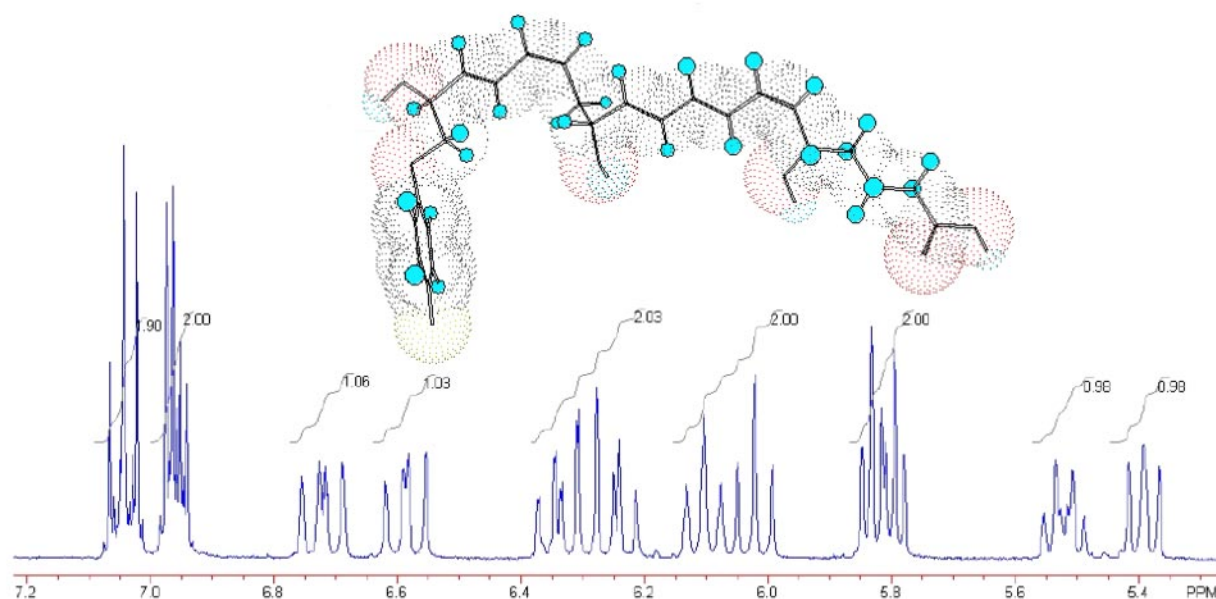
and human PMN (Fig. 4B). As shown in Fig. 4A, lung incubations gave a single component beneath a peak of m/z = 347 at 9.8 min on LC-UV-MS chromatogram. This peak had a unique UV absorbance with a maximum at 270 nm (Fig. 4A, *inset*) and co-eluted with the enzymatically prepared 18-oxo-RvE1 by the recombinant 15-PGDH as described above. Tandem MS spectrum at m/z 347 further supported the identity of the lung incubation product as 18-oxo-RvE1 with the corresponding fragments at m/z 347 (M - H), 329 (M - H-H₂O), 311 (M - H-2H₂O), 303 (M - H-CO₂), 285 (M - H-H₂O-CO₂), 275 (M - H-2H₂O-CO₂-2H), and diagnostic ions at m/z 291 and 257 (319-H₂O-CO₂). Approximately 25–30% of the added RvE1 was converted to 18-oxo-RvE1, and no other components were apparent at m/z 347 that corresponded to either 5-oxo or 12-oxo-RvE1. These results demonstrate that 18-oxo-RvE1 is the major initial oxidation product of RvE1 in the lung.

On the other hand, RvE1 incubations with isolated human PMN gave a major single component beneath a peak of m/z = 365 at 3.9 min with UV chromophores at 234 and 271 nm, suggesting an oxidation product retaining diene and triene structures in the molecule (Fig. 4B and *inset*). The tandem mass spectrum of the material beneath this peak at m/z = 365 supported an ω -oxidation product 20-hydroxy-RvE1 structure with corresponding fragments at m/z 365 (M - H), 347 (M - H-H₂O), 329 (M - H-2H₂O), 321 (M - H-CO₂), 311 (M - H-3H₂O), 303 (M - H-H₂O-CO₂), 285 (M - H-2H₂O-CO₂), and 267 (M - H-3H₂O-CO₂) as well as diagnostic ions at m/z 291, 289 (307-H₂O), 273 (291-H₂O), and 195. Therefore, RvE1 metabolism was tissue- and cell-type specific, in that oxidation at the carbon 18 position was the major initial metabolism in the lung, and ω -oxidation to form 20-hydroxy-RvE1 was the major pathway in peripheral blood human PMN.

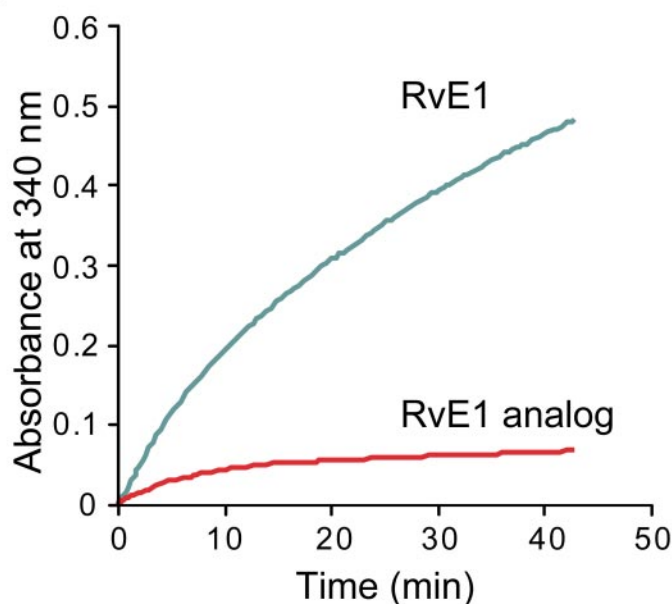
Anti-inflammatory Actions of 18-Oxo-RvE1—To determine whether 18-oxo-RvE1 retained the bioactivities of RvE1 (8), the isolated 18-oxo-containing compound was assessed for its ability to regulate leukocyte infiltration *in vivo* using the zymosan-induced peritonitis. Intravenous administration of RvE1 at 100 ng reduced total leukocyte numbers by ~25% in the inflamed peritoneal cavity and, specifically, stopped PMN infiltration by ~40%. By direct comparison, 18-oxo-RvE1 at the same dose was essentially devoid of anti-inflammatory activity (Fig. 5). These results demonstrate that the conversion of RvE1 to its 18-oxo-product represents a mode of RvE1 inactivation.

Blocking Metabolic Conversion of RvE1 Retains Function—Because RvE1 conversion to 18-oxo-RvE1 by dehydrogenation at carbon 18 represents biological inactivation (Fig. 5), an analog was designed with bulky substitutions placed adjacent to the 18 position on the ω -end of the molecule to resist both ω -oxidation and carbon 18 position oxidation, namely 19-(*p*-fluorophenoxy)-RvE1 methyl ester (Fig. 6A). This RvE1 analog was designed using an approach and rationale similar to that used for ATLa, a stable analog structure of aspirin-triggered lipoxin (14, 15). Here, a *para*-fluorophenoxy group substitution at carbon 19 was used in place of the remaining carbons at the ω -end (Fig. 6A). This novel RvE1 analog was resistant to rapid oxidation by the recombinant human dehydrogenase (Fig. 6, B and C). To determine

A



B



C

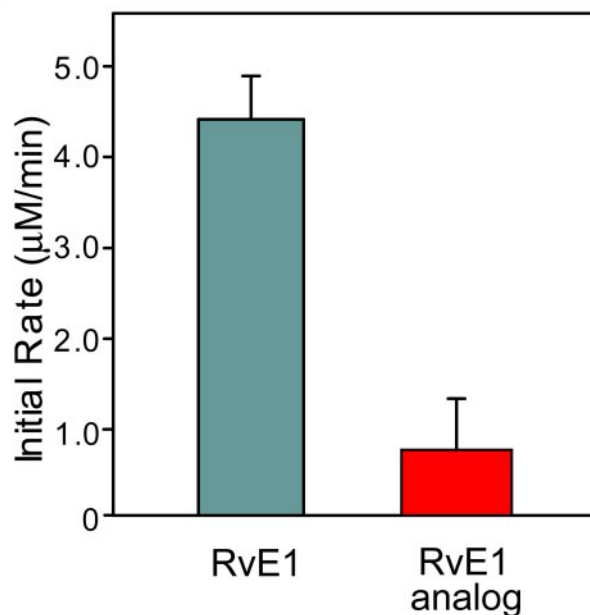


FIGURE 6. **RvE1 analog.** A, structure and 400 MHz NMR spectrum of the olefinic region of the RvE1 analog, 19-(*p*-fluorophenoxy)-RvE1 methyl ester. B and C, conversion of RvE1 and synthetic analog by recombinant 15-PGDH. The time course of NADH formation was monitored at A_{340} using 5 μ g of substrate in a 100- μ l incubation volume as described under "Experimental Procedures." Values are expressed as mean $\pm$ S.E. of $n = 3$ separate experiments.

whether the designed RvE1 substitution retained the anti-inflammatory activity of native RvE1, we evaluated this analog directly compared with the actions of RvE1 *in vivo*. This RvE1 analog, 19-(*p*-fluorophenoxy)-RvE1 methyl ester, proved to be as potent as native RvE1 in stopping total leukocyte infiltration and specifically PMN infiltration into murine peritonium (Table 1). Also the key cytokines and chemokines associated with leukocytes and inflammation were monitored (Table 2). At 2 h, both RvE1 and its analog markedly inhibited pro-inflammatory cytokines/chemokines (e.g. tumor necrosis factor- α , IL-1 β , IL-6, IL-12, KC,

RANTES, and MCP-5). Thus these results indicate that the metabolically resistant RvE1 analog depicted in Fig. 6A retained the bioactivity of native RvE1.

DISCUSSION

The results presented here are the first to demonstrate that RvE1 is enzymatically further metabolized via NAD $^{+}$ -dependent dehydrogenation that is regiospecific at carbon 18 to generate 18-oxo-RvE1. In the lung, dehydrogenation of the hydroxyl group at carbon 18 to form 18-oxo-RvE1 represented the major initial metabolic route for RvE1. The 18-oxo-RvE1

was devoid of the potent anti-inflammatory actions of its precursor RvE1 *in vivo*. A metabolically resistant RvE1 analog, namely 19-(*p*-fluorophenoxy)-RvE1 methyl ester, was prepared that proved to retain the anti-inflammatory actions of native RvE1 to reduce PMN infiltration and pro-inflammatory cytokine/chemokine productions *in vivo*.

RvE1 has potent actions both *in vitro* and *in vivo* within the nanomolar range to stop leukocyte migration and protect tissues from leukocyte-mediated damage (8, 9). In general, eicosanoids such as prostaglandins, leukotrienes, and lipoxins are generated from stored precursors, exert their bioactions at specific receptors (16), and are rapidly inactivated via local enzymatic catabolism in target tissues. Several enzyme pathways are described that are involved in eicosanoid metabolism via each of the main pathways, including β -oxidation, ω -oxidation, dehydrogenation, and/or reduction (5). Leukotriene B₄ (LTB₄), for example, is converted by ω -oxidation in human PMN by a cytochrome P450 enzyme (17) or by dehydrogenation at the 12-hydroxyl group to form 12-oxo-LTB₄ in kidney (18). LXA₄ is converted by sequential dehydrogenation and reduction in macrophages by 15-PGDH and 15-oxoprostaglandin 13-reductase/LTB₄ 12-hydroxydehydrogenase (PGR/LTB₄DH) (6). 15-PGDH activity is found in high levels in lung, liver, kidney, placenta, spleen, and immune cells and is involved in the metabolic inactivation of prostaglandins E₂ and F₂ and LXA₄ (5, 19). Here, we demonstrate that RvE1 undergoes initial meta-

bolic pathways that are tissue- and/or cell-type specific, in that oxidation of alcohol group at the carbon 18 position was the major route in the lung, and ω -oxidation to form 20-hydroxy-RvE1 was a major route in human PMN. Moreover, in addition to the ω -6 carbon, *i.e.* C-15 hydroxyl dehydrogenase activities, we report here that 15-PGDH also serves as ω -3 C-18 hydroxyl dehydrogenase for RvE1 to form 18-oxo-RvE1, which results in inactivation of native RvE1.

The inflammatory response of the body constitutes a recognition of invading pathogens, and chemicals, and/or tissue injury followed by recruitment of inflammatory cells clears the original stimulus and promotes resolution of inflammation, which allows tissue to return to homeostasis (1). Many inflammatory processes are self-limiting and self-resolving systems, suggesting the existence of endogenous anti-inflammatory and/or pro-resolution mediators that are operative during the course of inflammation (7). RvE1 is a potent anti-inflammatory lipid mediator derived from ω -3 eicosapentaenoic acid, which is generated during the spontaneous resolution phase of acute inflammation where specific cell-cell interactions occur (7, 8). Here we have described that the counterregulatory activity of RvE1 on leukocyte migration is subject to enzymatic inactivation. This system may contribute in part to the return of inflamed tissue to homeostasis associated with a temporal switch of the families of lipid mediators generated during the course of inflammation and resolution (2, 4).

An RvE1 analog was designed and synthesized by chemical modification at the ω -end as with the aspirin-triggered LXA₄ analog, ATLa (14, 15). ATLa protects tissues from leukocyte-mediated damage such as dermatitis (14), periodontitis (20), nephritis (21), colitis (22), airway inflammations (23, 24), and microbial infections (25). In many disorders associated with inflammation, uncontrolled neutrophil infiltrations and local pro-inflammatory cytokine productions such as tumor necrosis factor- α play critical roles in pathogenesis and tissue damage (26–28). In the present experiments, a RvE1 analog, namely 19-(*p*-fluorophenoxy)-RvE1 methyl ester (Fig. 6A), was designed to protect from both ω -oxidation and/or rapid dehydrogenation at carbon 18 and was found to be essentially equipotent to native RvE1 *in vivo* in stopping leukocyte infiltration

TABLE 1

RvE1 and RvE1 stable analog stop PMN infiltration *in vivo*

RvE1 methyl ester and 19-(*p*-fluorophenoxy)-RvE1 methyl ester were injected intravenously at 1 μ g/mouse followed by 1 ml of zymosan A (1 mg/ml) into the peritoneum. After 2 h, total leukocytes and PMN numbers were enumerated. Values represent mean $\pm$ S.E. from four or five different mice.

Injection	n	No. of leukocytes		
		Total cells	Neutrophils	Monocytes
			$\times 10^6$	
Zymosan A	4	5.0 $\pm$ 0.3	3.6 $\pm$ 0.2	1.4 $\pm$ 0.2
+RvE1	4	3.8 $\pm$ 0.3 ^a	2.2 $\pm$ 0.2 ^a	1.5 $\pm$ 0.1
		(21.7 $\pm$ 7.0%) ^b	(35.0 $\pm$ 5.2%) ^b	
+RvE1 analog	5	4.0 $\pm$ 0.2 ^a	2.5 $\pm$ 0.1 ^a	1.6 $\pm$ 0.1
		(18.0 $\pm$ 6.4%) ^b	(31.1 $\pm$ 4.8%) ^b	

^a $p < 0.001$.

^b Values in parentheses indicate percent inhibition.

TABLE 2

RvE1 and RvE1 stable analog reduce cytokine/chemokine productions *in vivo*

Cell-free fluids from murine peritoneal lavages were collected as in Table 1 and amounts of selected chemokines and cytokines were measured by multiplex enzyme-linked immunosorbent assay. Results represent mean $\pm$ S.E. ($n = 3$). MIP, macrophage inflammatory protein; TNF, tumor necrosis factor; SDF, stromal cell-derived factor; IFN, interferon; MCP, monocyte chemoattractant protein; GM-CSF, granulocyte/macrophage colony-stimulating factor.

Cytokine/chemokine	Zymosan A	Zymosan A + RvE1	% inhibition from zymosan A	Zymosan A + RvE1 analog	% inhibition from zymosan A
IL-1 β (pg/ml)	16.7 $\pm$ 3.5	13.7 $\pm$ 7.3		7.8 $\pm$ 3.6	
IL-4 (pg/ml)	8.6 $\pm$ 2.6	5.0 $\pm$ 1.3		4.2 $\pm$ 1.5	
IL-6 (ng/ml)	22.9 $\pm$ 1.9	17.8 $\pm$ 2.5		19.9 $\pm$ 2.1	
IL-10 (pg/ml)	206.3 $\pm$ 47.7	170.4 $\pm$ 27.2		177.4 $\pm$ 14.3	
IL-12 (pg/ml)	5.2 $\pm$ 2.3	4.5 $\pm$ 1.6		2.0 $\pm$ 1.0	
IL-13 (pg/ml)	29.7 $\pm$ 4.3	37.1 $\pm$ 11.9		25.4 $\pm$ 3.3	
KC (ng/ml)	5.8 $\pm$ 0.5	4.1 $\pm$ 0.5 ^a	29	3.8 $\pm$ 1.0 ^a	44.5
JE (ng/ml)	1.9 $\pm$ 0.2	1.4 $\pm$ 0.1 ^a	26	1.4 $\pm$ 0.4	
MIP-2 (ng/ml)	2.7 $\pm$ 0.3	2.2 $\pm$ 0.3		2.2 $\pm$ 0.3	
TNF- α (pg/ml)	310.3 $\pm$ 33.4	244.2 $\pm$ 3.4 ^a	21	187.2 $\pm$ 60.4 ^a	39.7
RANTES (pg/ml)	31.8 $\pm$ 4.9	23.6 $\pm$ 2.1		23.8 $\pm$ 1.8	
SDF-1 β (pg/ml)	550.2 $\pm$ 12.7	595.1 $\pm$ 113.2		524.2 $\pm$ 62.7	
IFN- γ (pg/ml)	67.6 $\pm$ 17.5	61.6 $\pm$ 15.0		62.8 $\pm$ 20.7	
GM-CSF (pg/ml)	14.5 $\pm$ 5.6	18.8 $\pm$ 3.1		11.5 $\pm$ 1.1	
MCP-5 (pg/ml)	85.4 $\pm$ 19.5	64.5 $\pm$ 5.2		50.0 $\pm$ 9.4	

^a $p < 0.05$.

and reducing pro-inflammatory cytokine/chemokine production in peritonitis. RvE1 oxidation at either the carbon 5 or 12 hydroxyl group is also possible, because 5- and 12-dehydrogenases have been shown to exist in some mammalian cells (18, 29), although those metabolites were not apparent in the present study. Thus designer resolvin mimetics that resist further metabolism/inactivation can be useful tools *in vivo* to evaluate the roles and actions of resolvins and their therapeutic potential for new treatment of human disorders associated with aberrant inflammation.

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Metabolic Inactivation of Resolvin E1 and Stabilization of Its Anti-inflammatory Actions

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