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EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: Evidence for a PPAR-γ–dependent mechanism

HANG LI, XIONG Z. RUAN, STEPHEN H. POWIS, RAY FERNANDO, WINT Y. MON, DAVID C. WHEELER, JOHN F. MOORHEAD, and ZAC VARGHESE

Centre for Nephrology, Royal Free and University College Medical School, University College London, London, United Kingdom; and Department of Nephrology, Peking Union Medical College Hospital, Beijing, People's Republic of China

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Background. Recent studies have shown that fish oil, containing ω-3 polyunsaturated fatty acids (ω-3 PUFAs) eicosapentaenoic acid (EPA) (C20:5 ω 3), and docosahexaenoic acid (DHA) (C22:6 ω 3) retard the progression of renal disease, especially in IgA nephropathy (IgAN). Despite increasing knowledge of the beneficial effects of fish oils, little is known about the mechanisms of action of ω-3 PUFAs. It has been reported that activation of peroxisome proliferator-activated receptors (PPARs) inhibits production of proinflammatory cytokines. Both EPA and DHA have been shown to activate PPARs. The aim of this study was to examine if ω-3 PUFAs have anti-inflammatory effects via activation of PPARs in human renal tubular cells.

Methods. An immortalized human proximal tubular cell line [human kidney-2 (HK-2) cells] was used in all experiments. Conditioned media was collected from ω-3 PUFAs- treated cells and subjected to enzyme-linked immunosorbent assay (ELISA). Total cellular RNA was isolated from the above cells for real-time quantitative polymerase chain reaction (PCR). Nuclear Extracts were prepared from the HK-2 cells for transcription factor activation assay.

Results. Both EPA and DHA at 10 μmol/L and 100 μmol/L concentrations effectively decreased lipopolysaccharide (LPS)-induced nuclear factor-kappaB (NF-κB) activation and monocyte chemoattractant protein-1 (MCP-1) expression. EPA and DHA also increased both PPAR-γ mRNA and protein activity (two- to threefold) in HK-2 cells. A dose of 100 μmol/L bisphenol A diglycidyl ether (BADGE) abolished the PPAR-γ activation induced by both EPA and DHA and removed the inhibitory effect of EPA and DHA on LPS-induced NF-κB activation in HK-2 cells. Overexpression of PPAR-γ further inhibited NF-κB activation compared to the control cells in the presence of EPA and DHA.

Conclusion. Our data demonstrate that both EPA and DHA down-regulate LPS-induced activation of NF-κB via a PPAR-γ–dependent pathway in HK-2 cells. These results suggest that PPAR-γ activation by EPA and DHA may be one of the underlying mechanisms for the beneficial effects of fish oil.

Key words: ω-3 polyunsaturated fatty acids, PPARγ, NF-κ B.

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Consumption of fish oil containing high levels of ω-3 polyunsaturated fatty acids(ω-3 PUFAs) such as eicosapentaenoic acid (EPA) (C20:5 ω 3) and docosahexaenoic acid (DHA) (C22:6 ω 3) has been reported to improve the prognosis of several chronic inflammatory diseases, including atherosclerosis, systemic lupus erythematosus, psoriasis, inflammatory bowel disease, and rheumatoid arthritis [1–4]. Recent studies have shown that administration of ω-3 PUFA reduces proteinuria and ameliorates renal injury in murine lupus nephritis, experimental focal segmental glomerulosclerosis, and other types of renal diseases [5–9]. In IgA nephropathy (IgAN), the most common glomerulonephritis worldwide, renal disease progression was markedly reduced in patients given a fish oil supplement containing EPA and DHA [10–12].

While the potential beneficial effects of dietary fish oil are reasonably well documented, the mechanism by which disease manifestations are suppressed remains unknown [13]. The accumulation of macrophages within the interstitial space of the renal cortex plays a pathogenic role in the development of tubular injury and interstitial fibrosis in progressive chronic renal diseases [14, 15]. Proximal tubular epithelial cells (PTC) are thought to mediate the interstitial macrophage infiltration because of their anatomic proximity and ability to produce proinflammatory mediators and chemotactic cytokines such as monocyte chemoattractant protein-1 (MCP-1), which have an important role in the regulation of interstitial inflammation and other processes related to matrix deposition [16]. In PTC, the ubiquitous proinflammatory transcription factor nuclear factor-kappaB (NF-κB) has a pivotal role in the regulation of chemokines (MCP-1), adhesion molecules [intracellular adhesion molecule-1 (ICAM-1)], cytokines [such as interleukin (IL)-1 and

tumor necrosis factor-α (TNF-α)] and many early response genes [17].

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-dependent transcription factors. They play an important role in the general transcriptional control of numerous cellular processes, including lipid metabolism, glucose homeostasis, cell cycle progression, cell differentiation, inflammation, and extracellular matrix remodeling [18]. Known PPAR isoforms include PPAR-α, implicated in fatty acid metabolism, PPAR-γ, important in lipoprotein metabolism, adipogenesis, and insulin sensitivity, and PPAR-β/δ about which least is known. Synthetic PPAR-α ligands such as fibrates, for example, fenofibric acid, is used therapeutically to lower triglyceride-rich lipoproteins [19]. Synthetic PPAR-γ ligands such as thiazolidinediones (for example, rosiglitazone) are insulin-sensitizing drugs used in the treatment of diabetes [20]. Recent studies suggest that PPAR ligands may reduce the inflammatory response by attenuating the production of proinflammatory cytokine [21]. ω-3 PUFAs such as EPA and DHA are known natural ligands of PPARs, although they are required in relatively high concentrations (approximately 100 μmol/L) for PPAR activation and are not selective for PPAR subtypes. Their derivatives can further activate PPAR. For example, a cyclooxygenase product of arachidonic acid, 15d-Δ12,14-prostaglandin J_2 (PGJ_2), is a selective PPAR-γ ligand, whereas its lipoxygenase product, 8(S)hydroxy-(5Z,9E, 11Z,14Z)-eicosatetraenoic acid (HETE), is a selective PPAR-α ligand [22–24].

Since fatty acids are natural PPAR ligands, we hypothesized that ω-3 PUFAs EPA and DHA may inhibit NF-κB activation and MCP-1 expression in a human PTC cells line through PPAR activation.

METHODS

Preparation of fatty acids

EPA and DHA were purchased from Sigma Chemical Co. Ltd (Dorset, UK), resuspended in absolute ethanol and added to a 0.3 mmol/L solution of essential fatty acid–free bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Solutions were aliquoted and frozen. The vehicle control for EPA and DHA was 0.3 mmol/L solution of essential fatty acid–free BSA in PBS plus absolute ethanol. Bisphenol A diglycidyl ether (BADGE) was purchased from Tocris (Ballwin, MO, USA).

Cell culture

Human kidney-2 (HK-2) cells, an immortalized human PTC line, was purchased from American Type Culture Collection (Mannasas, VA, USA) [25]. The cells were grown in keratinocyte serum-free media (K-SFM) (Invitrogen, Paisley, UK) supplemented with bovine pituitary extract (40 μg/mL) and epidermal growth factor (5 ng/mL). The cell lines were complemented with 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin and maintained in a 37°C, 5% CO_2 humidified atmosphere. Cultures were refed with fresh media every 2 to 3 days. Experiments were performed on cells following a 24-hour incubation in the medium used for experiments which consisted of keratinocyte serum-free medium without bovine pituitary extract (40 μg/mL) and epidermal growth factor (5 ng/mL). Cells were incubated with various concentrations of ω-3 PUFAs such as EPA and DHA in the presence or absence of lipopolysaccharide (LPS) for 24 hours. The conditioned media and cells were collected for analysis.

RNA isolation and real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured HK-2 cells by the guanidinium method. Total RNA (500 ng) was used as a template for RT using an RNA RT Kit from ABI (Applied Biosystems Ltd., Warrington, Cheshire, UK). The RT reaction was set up in a 20 μL mixture containing 50 mmol/L KCl, 10 mmol/L Tris/HCl, 5 mmol/L $MgCl_2$, 1 mmol/L of each deoxynucleoside triphosphate (dNTP), 2.5 μmol/L random hexamers, 20 U RNAsin, and 50 U of Moloney-murine leukemia virus (M-MLV) reverse transcriptase. Incubations were performed in a DNA Thermal Cycler 9700 (Applied Biosystems, Foster City, CA, USA) for 10 minutes at room temperature, followed by 30 minutes at 42°C and 5 minutes at 99°C. Real-time quantitative PCR was performed on a TaqMan ABI 7000 Sequence Detection System (Applied Biosystems) using TaqMan SybrGreen PCR Master Mix (Applied Biosystems). Thermal Cycler conditions contained holds for 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of 20 seconds at 95°C and 20 seconds at 55°C and 30 seconds at 72°C. Relative amount of mRNA was calculated using comparative Ct (ΔCt) method. β-actin served as the reference housekeeping gene. The amplification efficiencies of the target and reference were shown to be approximately equal with a slope of log input amount to Ct <0.1. Controls consisting of H_2O or samples that were not RT were negative for target and reference. The following oligonucleotide primers were used for MCP-1 sense (975–996) 5′-TGTTCT TCGAAAGCACATCAC-3′ and antisense (1023–1046), 5′-GTAGGTGACAGAGGGACATCT-TTT–3′, melting temperature (TM) = 79°C; PPAR-γ sense (807–824) 5′-GGGCCCTGGCAAAACATT-3′ and antisense (865–872) 5′-AAGATCGCCCTCGCCTTT-3′, TM = 80°C; and β-actin sense (1030–1047) 5′-CCTGG CACCCAGCACAAT-3′ and antisense (1080–1099) 5′-GCCG-ATCCACACGGAGTACT-3′, TM = 82°C.

Primers were designed with Primer Express Software version 2.0 System (Applied Biosystems, Foster City, CA, USA).

MCP-1 assays

Culture supernatant was collected from 24-well plates and concentrations of MCP-1 measured by enzyme-linked immunosorbent assay (ELISA) using protocols supplied by the manufacturer (R&D Systems, Abingdon, UK) and normalized to cell protein concentrations.

Preparation of nuclear extracts

Confluent HK-2 cells in T75 flasks were washed with 5 mL PBS/phosphatase inhibitors, the supernatant aspirated and 3 mL ice-cold PBS/phosphatase inhibitors added. Cells were removed by gently scraping with a cell lifter and transferred to a prechilled 15 mL conical tube. The cell suspension was centrifuged for 5 minutes at 500 rpm in a centrifuge precooled at 4°C and the supernatant discarded. A Nuclear Extract Kit (Active Motif, Rixensart, Belgium) was used for isolation of nuclear extracts from cell pellets, according to the manufacturer's instructions.

Trans-AM PPAR-γ and NF-κB p65 transcription factor assay

The Trans-AM PPAR-γ Kit contains a 96-well plate on which an oligonucleotide containing a peroxisome proliferator response element (PPRE) (5′-AACTA GGTCAAAGGTCA-3′) has been immobilized. PPAR-γ contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used in the Trans-AM PPAR-γ Kit recognizes an accessible epitope on PPAR-γ protein upon DNA binding. Addition of a secondary horseradish peroxidase (HRP)-conjugated antibody provides a sensitive colorimetric readout easily quantified by spectrophotometry. To quantify PPAR-γ activation, 5 μg of nuclear extract was measured using the Trans-AM PPARγ Kit according to the manufacturer's instructions (Active Motif).

The Trans-AM NF-κB p65 kits contain a 96-well plate on which oligonucleotide containing the NF-κB consensus site (5′-GGGACTTTCC-3′) has been immobilized. The active form of NF-κB contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used to detect NF-κB recognizes an epitope on p65 that is accessible only when NF-κB is activated and bound to its target DNA. A secondary HRP-conjugated antibody provides a sensitive colorimetric readout easily quantified by spectrophotometry. To quantify NF-κB activation, 5 μg of nuclear extract was measured using the Trans-AM NF-κB Kit according to the manufacturer's instructions (Active Motif).

Transient transfect for PPAR-γ

HK-2 cells were cultured and transfected with supercoiled PPAR-γ expressive vectors pSG5hPPAR-γ, which encode a constitutively active PPAR-γ (kindly provided by Professor B. Staels, Lille, France), by electroporation at 400 V and 100 μF with a Gene Pulser (Bio-Rad, Hemel Hempstead, Herefordshire, UK). pSG5 vector without insert of PPAR-γ (Stratagene Europe, Amsterdam, The Netherlands) was used as plasmid control. After electroporation, the cells were placed in a T75 flask with growth medium. Approximately 24 hours after replating, cells were replaced by fresh serum-free medium in the presence or absence of ω-3 polyunsaturated fatty acids (EPA or DHA) for 24 hours. The cells were then collected and nuclear extracts were isolated.

Statistical analyses

In all experiments, data were evaluated for significance by one-way analysis of variance (ANOVA) using Minitab software. Data were considered significant at $P < 0.05$.

RESULTS

Both EPA and DHA at 10 μmol/L and 100 μmol/L concentrations effectively decreased LPS-induced (10 μg/mL) NF-κB activation (Fig.1A). The specificity of the assay was confirmed by showing that 20 pmol/well of the wild-type consensus oligonucleotide prevented NF-κB activation, while the mutated consensus oligonucleotide had no effect on NF-κB activation (Fig. 1B). The MCP-1 concentration was examined by ELISA in the supernatant of LPS-stimulated HK-2 cells (10 μg/mL for 24 hours) in the absence or presence of various concentrations of EPA or DHA. Both EPA and DHA effectively decreased LPS induced MCP-1 production in a dose-dependent manner (Fig.2A). LPS-induced MCP-1 mRNA was also suppressed by EPA and DHA (Fig. 2B). We examined the cell viability under stimulation of high concentration of EPA and DHA (100 μmol/L) using trypan blue staining and lactate dehydrogenase (LDH) measurement. The concentrations of EPA and DHA used in this manuscript are not toxic for HK-2 cells during 24 to 48 hours of culture.

Both EPA and DHA increased PPAR-γ mRNA expression (Fig. 3A). A two- to threefold increased binding of PPAR-γ to PPRE was observed, with little difference between EPA and DHA (Fig. 3B). The specificity of the assay was demonstrated by showing that 40 pmol/well of the wild-type consensus oligonucleotide prevented PPAR-γ activation, while the mutated consensus oligonucleotide had no effect on PPAR-γ activation (Fig. 3C).

Next, we demonstrated that the PPAR-γ antagonist BADGE abolished PPAR-γ activation by EPA and DHA in HK-2 cells (Fig. 4A). BADGE (100 μmol/L) also

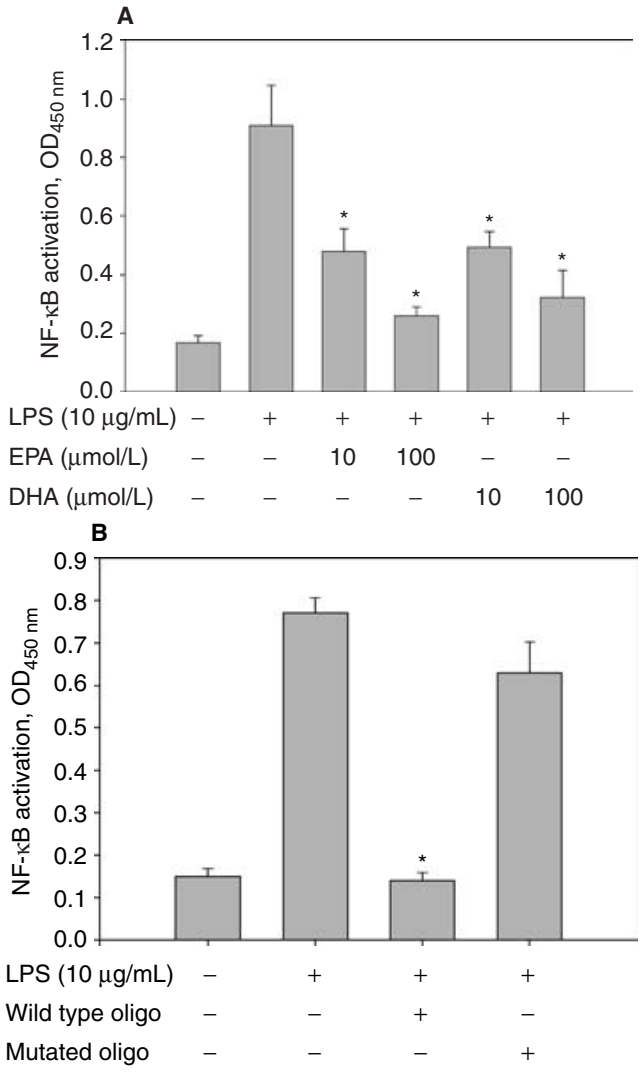

Fig. 1. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on nuclear factor-kappaB (NF-κB) activity in lipopolysaccharide (LPS)-stimulated human kidney-2 (HK-2) cells. (*A*) HK-2 cells were pretreated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of EPA, DHA for 23 hours, and then incubated for another 1 hour in the presence or absence of 10 μg/mL LPS. Consensus nuclear extracts were prepared and assayed using the Trans-AM enzyme-linked immunsorbent assay (ELISA) system as described in the **Methods** section. (*B*) The wild-type and mutated oligonucleotides were provided as a competitor for NF-κB binding in order to monitor the specificity of the assay. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. LPS induction group. OD is optical density.

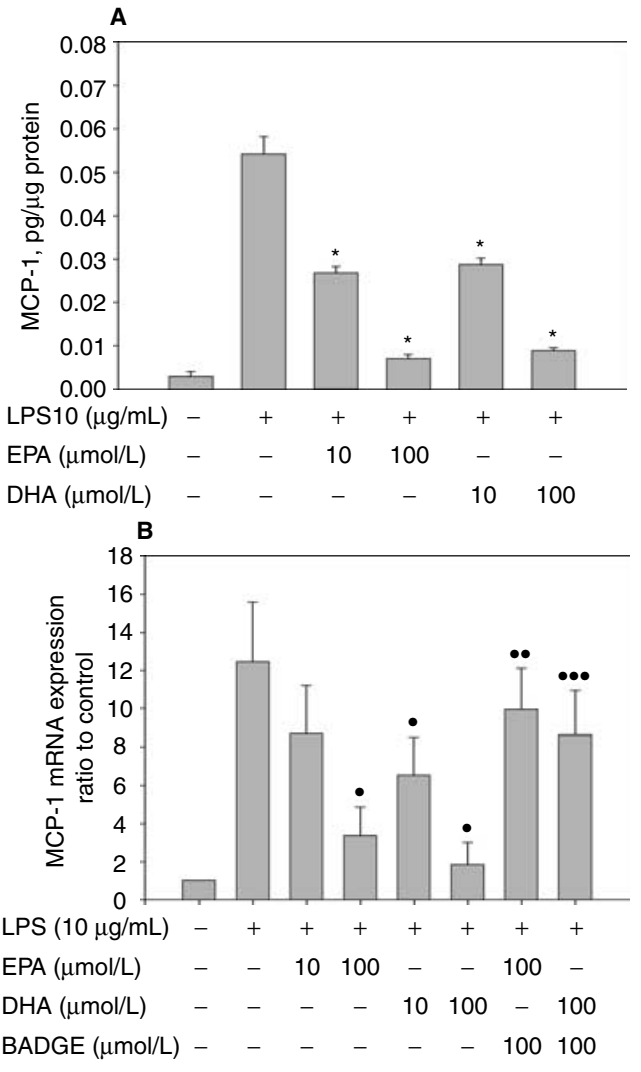

Fig. 2. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on monocyte chemoattractant protein-1 (MCP-1) protein levels and mRNA expression in lipopolysaccharide (LPS)-stimulated human kidney-2 (HK-2) cells. (*A*) HK-2 were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of EPA and DHA in the presence or absence of 10 μg/mL LPS for 24 hours. Supernatants were collected and assayed for MCP-1 as described in the **Methods** section. Results are expressed as means ± SD of four independent experiments. (*B*) HK-2 cells were cultured in K-SFM containing 10 μg/mL LPS with different concentrations of EPA and DHA in the absence or presence of 100 μmol/L bisphenol A diglycidyl ether (BADGE) for 24 hours. MCP-1 mRNA was determined following the ΔCt protocol for real-time reverse transcription-polymerase chain reaction (RT-PCR) as described in the **Methods** section. β actin served as the housekeeper gene. $^{*}P < 0.05$ vs. LPS induction control; $^{\bullet}P < 0.05$ vs. LPS induction group; $^{\bullet\bullet}P < 0.05$ vs EPA100 plus LPS; $^{\bullet\bullet\bullet}P < 0.05$ vs DHA 100 plus LPS.

removed the inhibitory effect of EPA and DHA on LPS-induced NF-κB activation in HK-2 cells (Fig. 4B). To further clarify the relationship between PPAR-γ and NF-κB, PPAR-γ was overexpressed in HK-2 cells by transient transfection. EPA and DHA increased PPAR-γ activation in a dose-dependent manner in the pSG5 plasmid control group (Fig. 5A). Overexpression of PPAR-γ further increased PPAR-γ activation compared to the plasmid control in the presence of EPA or DHA (Fig. 5A). It also decreased LPS-induced NF-κB activation compared to the plasmid control (Fig. 5B). This activation was further decreased in the presence of EPA or DHA (Fig. 5B).

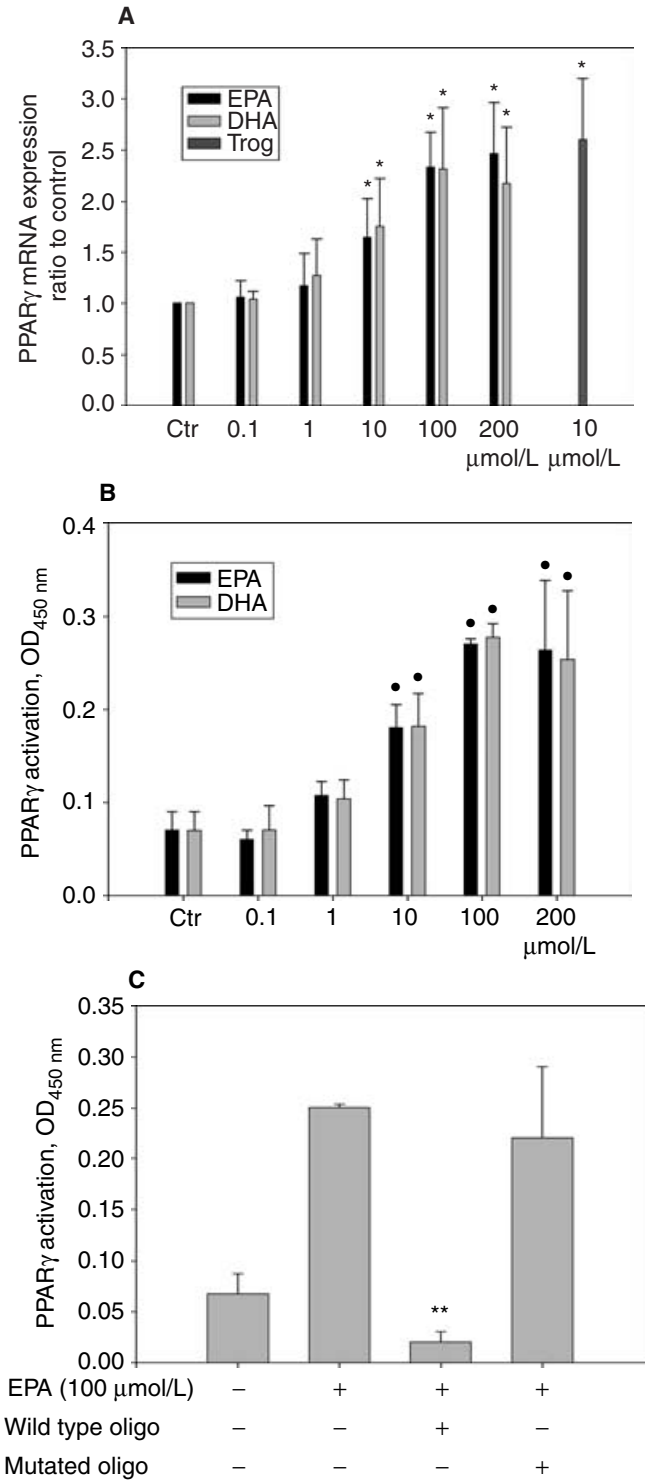

Fig. 3. Effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mRNA expression and activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) in human kidney-2 (HK-2) cells. (*A*) HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement (Ctr) but containing different concentrations of EPA and DHA for 24 hours, and troglitazone (Trog) (10 µmol/L) was used as a positive control. PPAR-γ mRNA was determined following the ΔCt protocol for real-time reverse transcription-polymerase chain reaction (RT-PCR) as described in the **Methods** section. β actin served as the housekeeper gene. (*B*) Nuclear extracts were prepared and assayed as described in the **Methods** section. (*C*) The wild-type and mutated oligonucleotide were provided as a competitor for PPAR-γ binding in order to monitor the specificity of the assay. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. vehicle control group; $^{\bullet}P < 0.05$ vs. vehicle control group; $^{**}P < 0.05$ vs. EPA alone. OD is optical density.

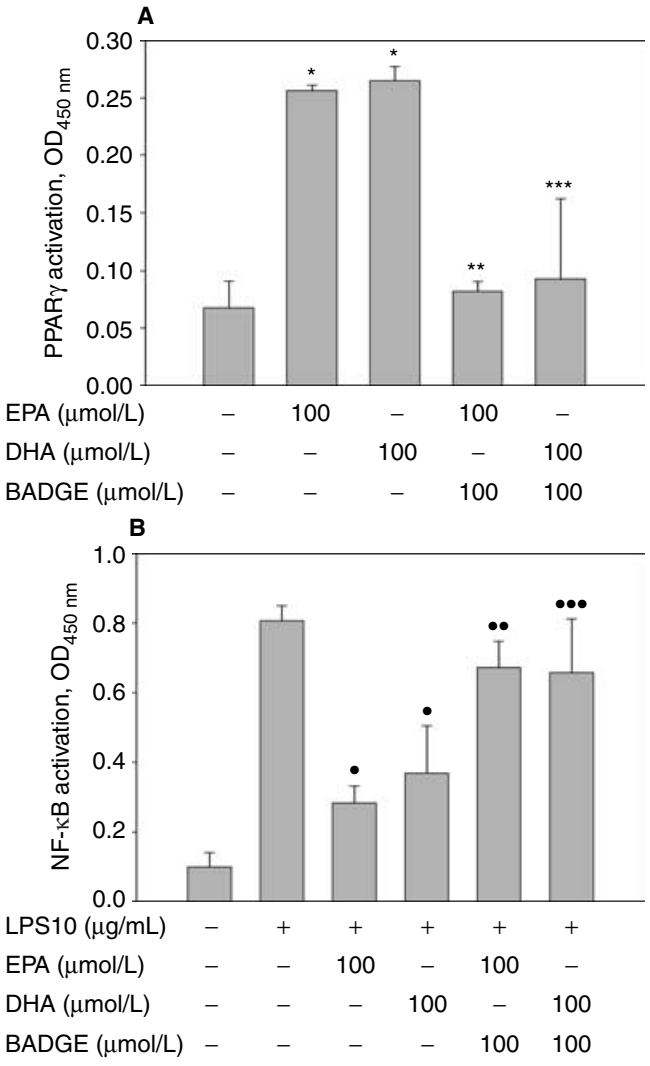

Fig. 4. Effects of the bisphenol A diglycidyl ether (BADGE) on the activity of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and nuclear factor-kappaB (NF-κB) in eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA)-treated human kidney-2 (HK-2) cells. HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing 100 µmol/L of EPA, DHA, and BADGE for 24 hours (*A*) or for 23 hours and then incubated for another 1 hours in the presence or absence of 10µg/mL lipopolysaccharide (LPS) (*B*). Nuclear extracts were prepared for measurements of PPAR-γ or NF-κB activation, respectively. Nuclear extracts were assayed as described in the **Methods** section. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. vehicle control; $^{**}P < 0.05$ vs. EPA alone; $^{***}P < 0.05$ vs. DHA alone; $^{\bullet}P < 0.05$ vs. LPS induction group; $^{\bullet\bullet}P < 0.05$ vs. EPA plus LPS; $^{\bullet\bullet\bullet}P < 0.05$ vs. DHA plus LPS. OD is optical density.

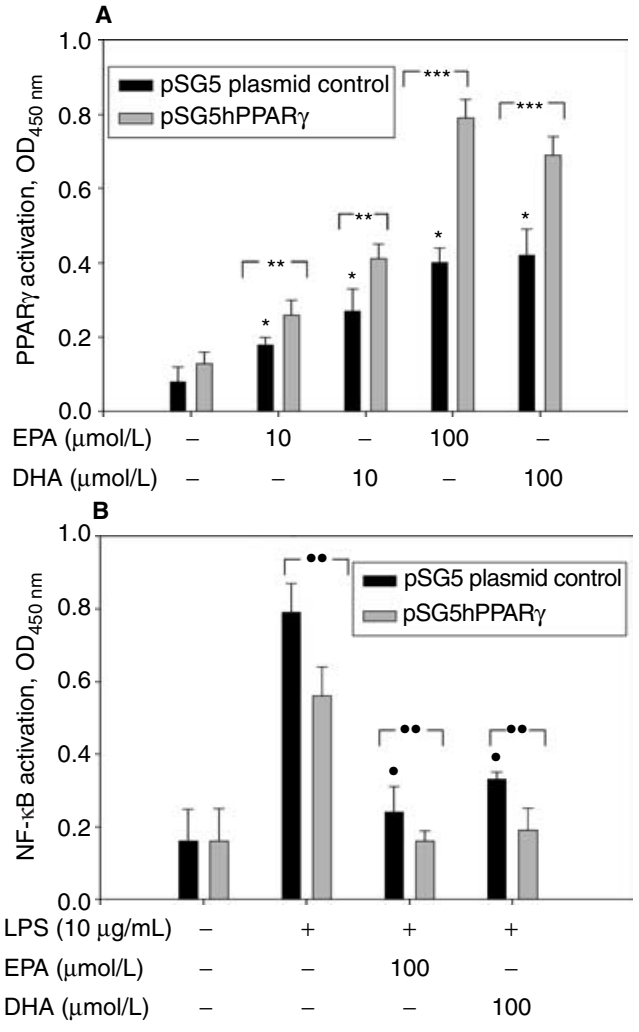

Fig. 5. Effects of over-expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) on activation of PPAR-γ and lipopolysaccharide (LPS)-induced nuclear factor-kappaB (NF-κB) activation in human kidney-2 (HK-2) cells. HK-2 cells were transiently transfected with pSG5 plasmid control (■) or pSG5hPPAR-γ (□) using electroporation as described in the **Methods** section. Both transiently transfected HK-2 cells were incubated in keratinocyte serum-free media (K-SFM) without supplement but containing different concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 24 hours. Nuclear extracts were prepared for measurement of PPAR-γ activation (*A*). Both transiently transfected HK-2 cells were pretreated in K-SFM without supplement but containing 100 μmol/L of EPA and DHA for 23 hours and then incubated for another 1 hour in the presence or absence of 10 μg/mL LPS. Nuclear extracts were prepared for measurement of NF-κB activation (*B*). Nuclear extracts were assayed using the TransAM enzyme-linked immunosorbent assay (ELISA) system as described in the **Methods** section. Data represent the means ± SD of four independent experiments. $^{*}P < 0.05$ vs. corresponding vehicle control; $^{**}P < 0.05$ vs. pSG5 plamid control; $^{***}P < 0.001$ vs. pSG5 plamid control; $^{\bullet}P < 0.05$ vs. corresponding LPS induction group; $^{\bullet\bullet}P < 0.05$ vs. pSG5 plasmid control. OD is optical density.

DISCUSSION

Despite increasing evidence of the beneficial effect of fish oil, little is known concerning the mechanisms of action of its fatty acid components. Pathogenic mechanisms that may be affected by fish oil therapy include proliferation of glomerular cells, infiltration of inflammatory cells, and production of inflammatory mediators. Recently, Grande et al [26] demonstrated that ω-3 PUFAs can be incorporated into renal tissue. They also demonstrated that the ω-3 PUFAs in fish oil significantly inhibits proliferation of cultured mesangial cells in vitro [26]. In vivo experiments have shown that fish oil reduces glomerular cell proliferation, α-smooth muscle actin expression, and proteinuria in antithymocyte serum (ATS) glomerulonephritis. This information is crucial for the rational use and refinement of pharmacotherapy with fish oil and/or its components for IgAN and other mesangial proliferative glomerulonephropathies.

Although mesangial cell proliferation is a central feature of IgAN, evidence suggests that the accumulation of extracellular matrix proteins in the cortical interstitium is a consistent feature of chronic glomerular diseases and a strong determinant of IgAN and other renal diseases [27]. Injury to PTCs and interstitial inflammation have been proposed as prerequisites for the development of matrix deposition and interstitial fibrosis [14, 28]. There are also reports that dietary enrichment with fish-oil could decrease urinary excretion of N-acetyl-beta-D-glucosaminidase (a marker of tubular injury) and protect renal function in proteinuric patients with primary glomerulopathies [29]. Increasing evidence suggests that MCP-1 may have an important role in the regulation of interstitial inflammation and possibly other processes related to matrix deposition [15, 30]. Our initial experiments demonstrated that both EPA and DHA inhibit LPS-induced MCP-1 production and mRNA expression in HK-2 cells. Both EPA and DHA also significantly inhibit LPS-induced NF-κB activation in a dose-dependent manner. This suggests that ω-3 PUFAs inhibit inflammatory responses and may have a protective effect on PTC.

We then investigated the molecular mechanism by which EPA and DHA inhibit NF-κB activation and MCP-1 expression. This could occur through several pathways. One potential mechanism may be through competition with arachidonic acid, a substrate for the lipoxygenase and cyclooxygenase pathways. The reduction of arachidonic acid derived products may reduce glomerular and interstitial inflammation, mesangial cell contractility, platelet aggregation, and vasoconstriction in response to renal injury [31]. ω-3 PUFAs may also directly inhibit production of the proinflammatory cytokines IL-1 and TNF-α by mononuclear cells although the mechanism remains unclear [32]. Another important mechanism by which ω-3 PUFAs may act as anti-inflammatory agents is by the activation of PPARs which have been demonstrated to inhibit production of proinflammatory cytokines, such as TNF and IL-6 [33].

Our results demonstrate that there was constitutive mRNA expression and activation of PPAR-γ in HK-2

cells, and that this was increased by both EPA and DHA. This suggests that EPA and DHA may inhibit inflammatory responses via activation of PPAR-γ. To provide further evidence for this mechanism, we investigated the effect of a PPAR-γ antagonist on the activation of PPAR-γ and NF-κB. BADGE is a high-affinity ligand for PPAR which has no apparent ability to activate the transcriptional activity of PPAR-γ activation; therefore, it can antagonize the ability of a ligand to activate PPAR-γ [34]. We demonstrated that BADGE (100 μmol/L) not only abolished the PPAR-γ activation by EPA and DHA, but also abolished the inhibitory effect of EPA and DHA on LPS-induced activation of NF-κB in HK-2 cells. However, we cannot exclude the possibility of an active metabolite or metabolites of EPA and DHA acting as a PPAR ligand in mediating the anti-inflammatory effect. To further clarify the relationship between PPAR-γ and NF-κB, PPAR-γ was overexpressed in HK-2 cell by transient transfection. Active NF-κB was decreased in transfected cells and both EPA and DHA effectively further decreased LPS-induced NF-κB activity compared to the untransfected cells. Taken together, these findings strongly suggest that anti-inflammatory activity of ω-3 PUFAs in HK-2 cells is, at least in part, mediated through PPAR-γ activation. Evidence for this also comes from studies demonstrating that ligand-activated PPAR-γ can inhibit NF-κB activity through direct interactions with the p65 subunit of NF-κB or increasing synthesis of the NF-κB inhibitor, IκB [35].

CONCLUSION

Both EPA and DHA are potent inhibitors of inflammatory responses in HK-2 cells. The effects of EPA and DHA are mediated by activation of PPAR-γ, which is responsible for the down-regulation of NF-κB activity. ω-3 PUFAs may represent a class of naturally occurring, low-toxicity, PPAR ligands with potent anti-inflammatory properties.

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Reprint requests to Dr. Xiong Z. Ruan, Centre for Nephrology, Royal Free and University College Medical School, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.
E-mail: xzruan@rfc.ucl.ac.uk

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