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1,4-Naphthoquinone Induces FcRn Protein Expression and Albumin Recycling in Human THP-1 Cells

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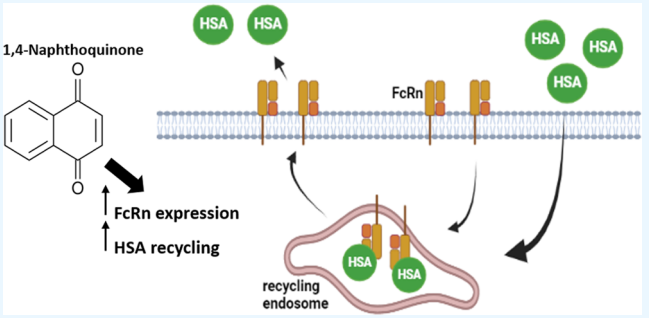
ABSTRACT: The neonatal Fc receptor (FcRn) has been established as a major factor in regulating the metabolism of albumin and IgG in humans by protecting them from intracellular degradation after they are endocytosed into cells. We assume that increasing the levels of endogenous FcRn proteins in cells would be beneficial to enhance the recycling of these molecules. In this study, we identify the compound 1,4-naphthoquinone as an efficient stimulator of FcRn protein expression in human THP-1 monocytic cells with potency at the submicromolar range. Also, the compound increased the subcellular localization of FcRn to the endocytic recycling compartment and enhanced human serum albumin recycling in the PMA-induced THP-1 cells. These results suggest that 1,4-naphthoquinone stimulates FcRn expression and activity in human monocytic cells *in vitro* and it could open a new avenue for designing cotreatment agents to enhance the efficacy of biological treatments such as albumin-conjugated drugs *in vivo*.



INTRODUCTION

The neonatal Fc receptor (FcRn) acts as a recycling receptor in cells that rescues endocytosed human serum albumin (HSA) and immunoglobulin (IgG) from lysosomal degradation and thereby extends the half-lives of these molecules in the blood.[1] Indeed, the FcRn-mediated protective pathway is responsible for prolonging the lifetime of albumin and IgG in the blood circulation by capturing these molecules in acidic intracellular compartments, preventing their degradation and recycling them to the cell surface where they are dissociated at neutral pH and released.[2]

The antibody-mediated immune response represents targeted and long-lived protection against many invading pathogens. Among the five existing antibody isotypes IgG, IgM, IgA, IgE, and IgD, IgG is the most abundant circulating antibody isotype in humans with the longest serum half-life. Also, it plays a key role in mediating immunity and protecting against infectious agents.[3] Additionally, albumin is a key plasma protein involved in many important physiological roles including the maintenance of appropriate osmotic pressure and the binding and transport of various molecules in the blood such as hormones, fatty acids, and drugs.[4]

Albumin- and antibody-based drugs are emerging class of therapeutics that have shown remarkable promise in the treatment of several diseases, particularly cancer. Albumin-based drug delivery systems offer natural transport proteins with multiple ligand-binding sites.[5] One of the most successful examples is Abraxane, an exogenous human albumin-bound paclitaxel formulation approved for the treatment of locally advanced or metastatic tumors.[6] Similarly, dozens of therapeutic antibodies have been dramatically developed over the last few years and have become the predominant treatment for various diseases.[7]

1,4-Naphthoquinone is a quinone-derived compound that has been shown to exhibit anti-inflammatory activity both *in vitro* and *in vivo*.[8,9] We have recently reported the ability of 1,4-naphthoquinone to target the IRAK1 enzyme, a master regulator of the IL1R/TLR immune signaling pathway in cells.[10] Notably, IL1R/TLR regulates key downstream signaling proteins including NF-κB.[11] The latter signaling molecule is a transcriptional inducer of FcRn expression in various cells.[12−14] In this study, we sought to define whether 1,4-naphthoquinone can affect FcRn expression in THP-1 monocytic cells and, thereby, increase the FcRn-mediated recycling of HSA in human cells.

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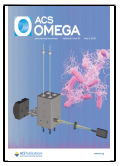

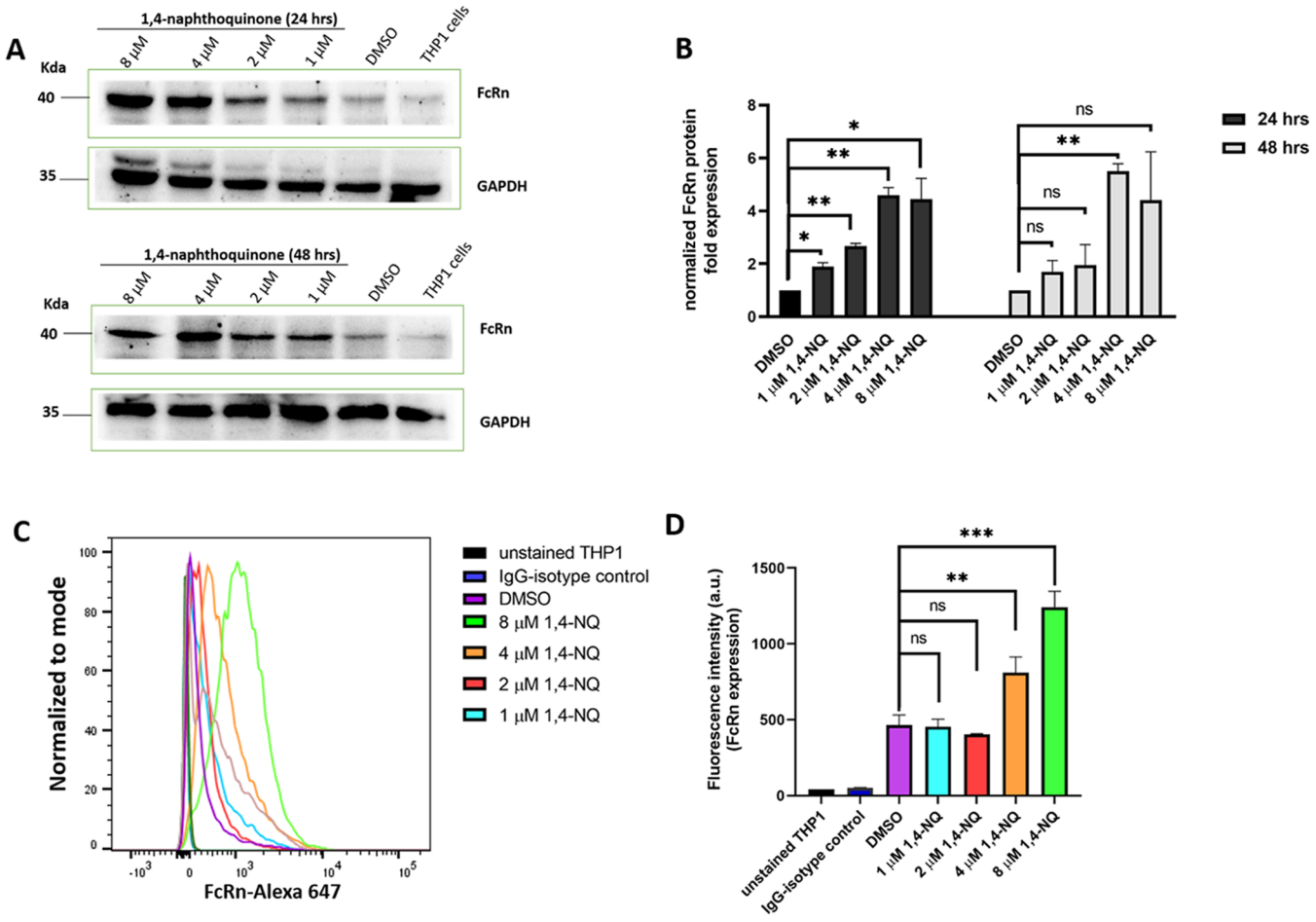

Figure 1. 1,4-Naphthoquinone induces FcRn protein expression in THP-1 cells. (A) Immunoblotting analysis of FcRn in THP-1 cells after treatment with different concentrations (1, 2, 4, 8 μM) of 1,4-naphthoquinone (1,4-NQ) and incubated for 24 and 48 h. Total protein lysate (40 μg) was subjected to 10% SDS-PAGE, transferred to a nitrocellulose membrane of 0.45 μm pore size, and probed with polyclonal rabbit antihuman FcRn antibodies and mouse antihuman GAPDH. Images were captured using a ChemiDoc MP Scanner (Bio-Rad). (B) Quantitation of the gel band density using ImageJ software. The immunoblotting results represent the mean ± SD of two independent experiments. (C) FACS analysis of FcRn protein expression in THP-1 cells after treatment with different concentrations (1, 2, 4, 8 μM) of 1,4-naphthoquinone and incubated for 24 h. The cells were fixed and stained with antihuman FcRn Alexa-Fluor 647-conjugated antibodies. A proper isotype-matching antibody (IgG isotype control) was used as a negative control. FACS results represent the mean ± SD of triplicate samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns, not significant.

RESULTS AND DISCUSSION

1,4-Naphthoquinone Treatment Induces FcRn Protein Expression in THP-1 Cells and Exhibits Low Cytotoxicity on Cells. To determine the effect of 1,4-naphthoquinone on FcRn protein expression, the monocytic THP-1 cells were treated with different concentrations of the 1,4-naphthoquinone compound and incubated at various time points. As demonstrated by immunoblotting, we could see an increase in FcRn protein expression in the cells treated with 4 μM 1,4-naphthoquinone compared with control cells treated with DMSO or untreated cells at both 24 and 48 h (Figure 1AB). Also, all concentrations of the compound (1, 2, 4, and 8 μM) significantly increased FcRn protein expression at the 24 h time point (Figure 1AB). Furthermore, FACS analysis confirmed the latter results and obtained a quantitative measurement of FcRn protein levels in live cells. Obviously, there was a statistically significant increase ($p < 0.05$) in FcRn protein expression, as can be inferred by the increase in the mean fluorescence intensity signal, in cells treated with 4 and 8 μM 1,4-naphthoquinone compared to control cells treated with DMSO or untreated cells (Figure 1CD). Notably, cells stained with Alexa-Fluor 647 mouse IgG2b (isotype control) showed very weak fluorescence signals, indicating very low cross-binding of the mouse IgG to the human Fcγ-receptors present on the surface of THP-1 cells.

To define the potential cytotoxicity of 1,4-naphthoquinone, differentiated THP-1 cells or HD fibroblasts were treated with different doses of 1,4-naphthoquinone and incubated at 37 °C for 24 and 48 h (Figure 2). In differentiated THP-1 cells, after 24 h of treatment, the drug exhibited very low cytotoxicity at low doses (<5 μM), with average cell death detected being 1, 3, and 7% at 1, 2, and 4 μM, respectively (Figure 2A). Similarly, after 48 h of drug treatment, very low toxicity was observed in the cells, with average cell death detected being 0, 0, and 13% at 1, 2, and 4 μM, respectively (Figure 2B). However, modest cytotoxicity of the drug at higher concentrations (8 and 16 μM) was detected at both 24 and 48 h time points, with average cell death ranging between 25 and 58% (Figure 2AB). Interestingly, 1,4-naphthoquinone at submicromolar doses (<5 μM) also demonstrated very low cytotoxicity on normal HD fibroblasts. At 24 h, the drug at concentrations of 1, 2, and 4 μM caused only 1, 2, and 7% cell

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ACS Omega 2023, 8, 16491−16499

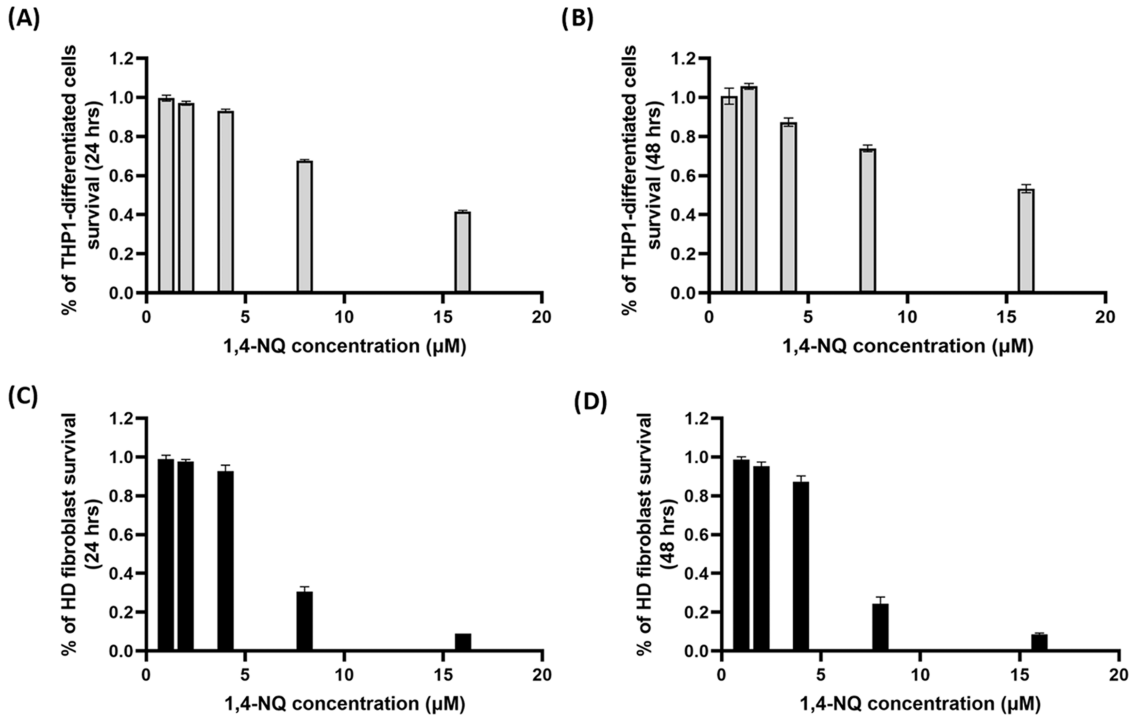

Figure 2. Cell cytotoxicity of 1,4-naphthoquinone on differentiated THP-1 cells and normal HD fibroblasts. MTT cytotoxicity assays show survival percentages of (A, B) differentiated THP-1 cells and (C, D) human normal HD fibroblasts. For differentiated THP-1 cells, the cells were resuspended in a medium supplemented with 150 nM PMA and seeded in 96-well plates and incubated at 37 °C for 24 h. The culture medium was removed, and the cells were treated with increasing concentrations (1, 2, 4, 8, 16 μM) of 1,4-naphthoquinone and incubated for 24 or 48 h at 37 °C. For HD fibroblasts, the cells were seeded in 96-well plates and incubated at 37 °C for 24 h. The cells were treated with 1, 2, 4, 8, and 16 μM 1,4-naphthoquinone and incubated for 24 or 48 h at 37 °C. The Colorimetric Cell Titer 96 nonradioactive cell proliferation assay was used to detect cell proliferation in each well. Data are expressed as mean ± SD.

death, respectively (Figure 2C). Also, very low cell death was noted at 48 h (1, 5, and 13% at 1, 2, and 4 μM, respectively) (Figure 2D). On the other side, high cytotoxicity of the drug (cell death 70−90%) was noticed at higher doses (8 and 16 μM) at both 24 and 48 h (Figure 2CD).

Importantly, these findings regarding the low cytotoxicity of small doses of 1,4-naphthoquinone on human fibroblasts nicely correlate with other reports that also have shown little toxicity of low doses of 1,4-napthoquinone or its derivatives on normal human cells, such as peripheral blood mononuclear cells and kidney, stomach, and liver cells,[15−17] indicating that 1,4-naphthoquinone at low doses may exert low toxicity on human normal cells.

1,4-Naphthoquinone-Induced FcRn Expression Increases Subcellular Localization of FcRn to the Recycling Compartment. To determine the effect of 1,4-naphthoquinone on the cellular expression and spatial subcellular localization of FcRn, PMA-induced THP-1 cells were treated with the compound followed by analysis by confocal laser scanning microscopy. In cells treated with 1,4-naphthoquinone, FcRn was predominantly localized to the perinuclear region, likely resembling the endocytic recycling compartment (Figure 3A)[18] and similar to the typical cellular FcRn localization that has previously been reported in the literature.[19] Additionally, some FcRn staining was observed in the cytoplasm as endosomal-like structures and very little FcRn was distributed on the cell surface (Figure 3A). Notably, the pattern of FcRn localization in cells treated with 1,4-naphthoquinone was very much similar to the DMSO-treated control cells (Figure 3A), which indicates that the compound probably maintains the cellular localization and function of FcRn. However, a significant increase in FcRn localization to the endocytic recycling compartment was observed with increasing dose of the compound. The latter observation was inferred by the increase in colocalization between FcRn proteins and the recycling compartment marker transferrin at 2 and 4 μM 1,4-naphthoquinone compared to DMSO (Pearson's correlation coefficient = 0.79 ± 0.06 and 0.86 ± 0.04, Manders coefficient = 0.57 ± 0.16 and 0.67 ± 0.12, respectively) (Figure 3B). This suggests that 1,4-naphthoquinone induces the expression of the FcRn protein and promotes the vesicular transport of FcRn *via* the recycling pathway.

It is worth mentioning that utilizing FcRn to increase the recycling of albumin- and IgG-based ligands has previously been reported. However, the majority of these studies were based on the enhanced affinity between IgG or albumin and FcRn proteins using mutagenesis strategies. For example, Sockolosky et al. have described the production of engineered proteins fused with the FcRn-binding peptide (FcBP) that showed high levels of internalization and recycling in cells.[20] Also, Lee et al. have recently demonstrated the generation of an Fc variant containing the L309D/Q311H/N434S (DHS) substitutions that displays improved persistence and pharmacokinetics characteristics *in vivo* compared to the native IgG1.[21] On the same side, Andersen et al. have reported the development of engineered human albumin by introducing single point mutations at the c-terminal of the protein, which increased the affinity to FcRn proteins and resulted in an extended serum half-life of the engineered human albumin in research animal models.[22]

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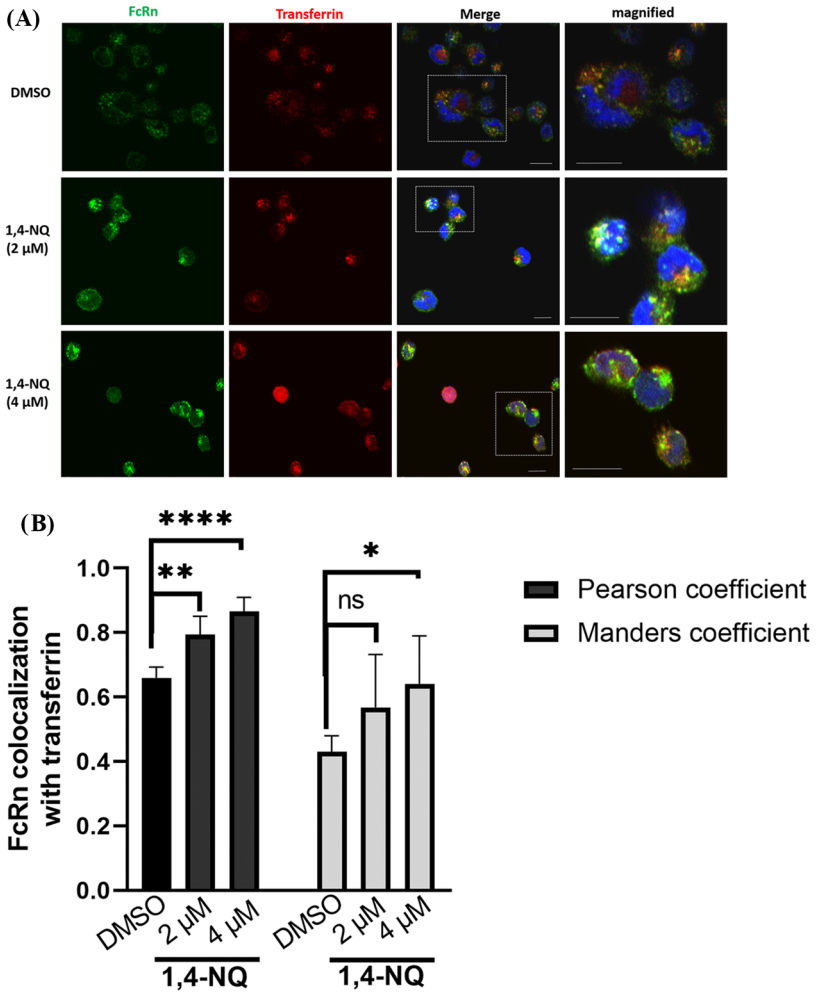

Figure 3. 1,4-Naphthoquinone increases subcellular localization of FcRn to the recycling compartment in THP-1-differentited cells. (A) Steady-state subcellular localization of FcRn (green) and colocalization with the internalized Alexa-488-trasnferrin (red). PMA-induced THP-1 cells were treated with different concentrations (2, 4 μM) of 1,4-naphthoquinone (1,4-NQ) for 24 h. The cells were incubated with Alexa-488-trasnferrin (25 μg/mL) at 37 °C for 20 min, then washed three times with PBS to remove excess transferrin, and fixed with 4% PFA. FcRn was stained with rabbit anti-FcRn antibodies followed by antirabbit Alexa647. Nuclei were stained with DAPI (blue). The confocal images were acquired using an LSM 780 (Zeiss, Germany). (B) Colocalization parameters: Pearson's correlation coefficient, which measures the linear correlation between the two channels, and Manders coefficient, which calculates the percentage of total signal from one channel that overlaps the signal from the other, were measured using the JACoP plugin of ImageJ software. Data are expressed as mean ± SD (15 cells for each point). Scale bars represent 10 μm. *p < 0.05, **p < 0.01, ****p < 0.0001.

1,4-Naphthoquinone Treatment Increases HSA Recycling in PMA-Induced THP-1 Cells. To define the effect of 1,4-naphthoquinone on HSA recycling, a pulse-chase experiment of native HSA-rhodamine was conducted in PMA-induced THP-1 cells, followed by confocal microscopy analysis to dissect the trafficking routes of the HSA in cells.[23] The cells pretreated with 1,4-naphthoquinone were allowed to take up HSA-rhodamine in cell culture for 10 min and then chased for another 10 min (Figure 4A). Remarkably, there was a large amount of HSA-rhodamine in the recycling compartment, as indicated by the colocalization between HSA-rhodamine and FcRn at pulse time points in cells treated with 1,4-naphthoquinone and DMSO (Manders coefficient = 0.38 ± 0.13, 0.33 ± 0.05, respectively), without a statistical difference (Figure 4B,C), indicating that the treatment does not alter the trafficking of HSA-rhodamine to the recycling pathway. Interestingly, a marked decrease in the fluorescence intensity of HSA-rhodamine at 10 min chase was clearly observed in the 1,4-naphthoquinone-treated cells compared to that in control cells (Figure 4C). This was confirmed by measuring the amount of FcRn that is colocalized with HSA-rhodamine. Indeed, a significant decrease in the amount of HSA-rhodamine was detected at the 10 min chase time point in cells treated with the compound compared to that in the DMSO-treated cells (Manders coefficient = 0.06 ± 0.03, 0.24 ± 0.07, respectively) (Figure 4C). This decrease probably indicated that a large amount of HSA-rhodamine was recycled to the culture medium.

To further assess the effect of 1,4-naphthoquinone on HSA recycling, the native unconjugated HSA was pulsed-chased in PMA-induced THP-1 cells and then the culture medium above the cells was collected at different time points and analyzed by a highly sensitive sandwich ELISA to measure the amount of recycled HSA in the culture medium.[24,25] A standard curve was prepared by making serial dilutions of HSA in a serum-free culture medium (Figure 5B). Remarkably, a significant increase of HSA levels in the conditioned medium was measured in the cells treated with 1,4-naphthoquinone, particularly at 4 and 8

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ACS Omega 2023, 8, 16491−16499

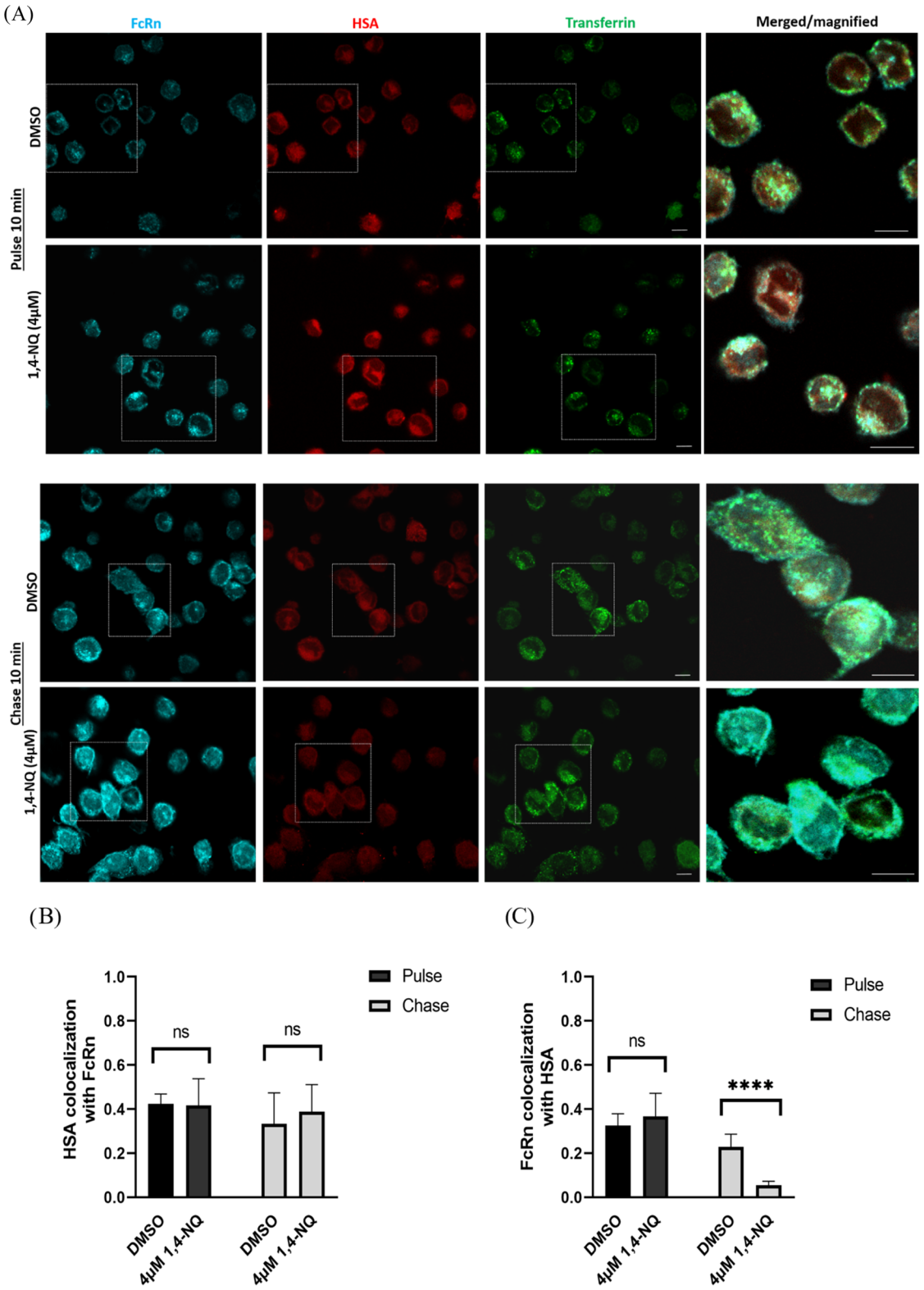

Figure 4. 1,4-Naphthoquinone enhances the intracellular recycling of the HSA in THP-1-differentiated cells. (A) PMA-induced THP-1-differentiated cells were treated with 4 μM 1,4-naphthoquinone (1,4-NQ) for 24 h. The cells were pulsed with HSA-rhodamine (200 μg/ml) (red) and Alexa-488-transferrin (50 μg/mL) (green) for 10 min at 37 °C. The cells were washed three times with PBS and chased for another 10 min at 37 °C. The cells at each time point were fixed with 4% PFA. FcRn was stained with rabbit anti-FcRn antibodies followed by antirabbit Alexa647 (cyan). The confocal images were acquired using an LSM 780 (Zeiss, Germany). (B, C) Quantification of the colocalization of internalized HSA-rhodamine and endogenous FcRn. The Manders coefficient colocalization parameter was measured using the JACoP plugin of ImageJ software. Data are expressed as the mean ± SD (at least 20 cells for each time point). Scale bars represent 10 μm. ****$p < 0.0001$; ns, not significant.

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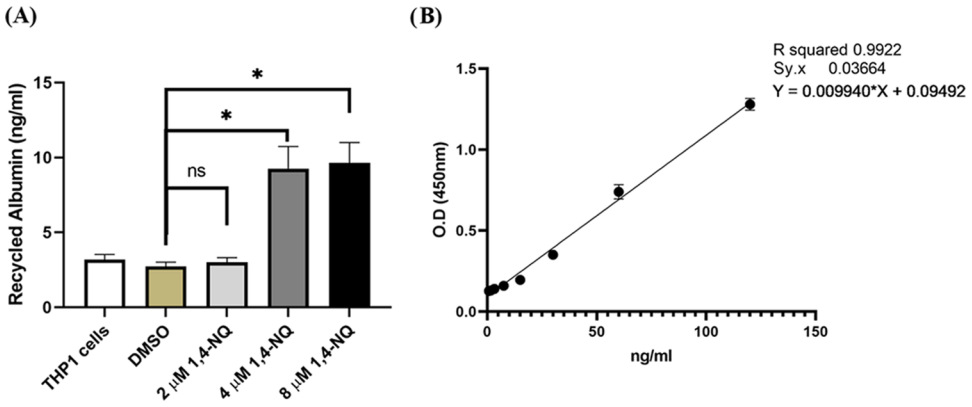

Figure 5. 1,4-Naphthoquinone increases the HSA recycling back to the culture medium in THP-1-differentited cells. (A) PMA-induced human THP-1 cells were treated with different concentrations (2, 4, 8 μM) of 1,4-naphthoquinone (1,4-NQ) for 15 h. For the recycling assay, the cells were incubated with the native HSA (100 ng/mL) dissolved in serum-free culture medium for 20 min at 37 °C. After incubation, the supernatant was removed and cells were washed four times with PBS to remove excess HSA. Fresh serum-free culture medium was added, and cells were incubated at 37 °C for 30 min. The culture medium above the cells was collected, and the HSA was measured by sandwich ELISA to determine the amount of recycled HSA. (B) Standard curve of HSA prepared in serum-free medium. *p < 0.05; ns, not significant.

μM doses, compared to that in DMSO-treated cells (Figure 5A). Hence, it can be concluded that 1,4-naphthoquinone potentiation of HSA recycling resulted in an increase in the amount of HSA that is released out of the cell.

CONCLUSIONS

There is considerable interest in modulating the FcRn receptor given its role in rescuing endocytosed IgG and albumin from lysosomal degradation. Importantly, preserving FcRn-mediated recycling may be an important task when attempting to generate albumin/antibody-based therapeutics with clinically favorable persistence. Here, we demonstrate that 1,4-naphthoquinone can be potentially used to induce the expression of FcRn proteins and the recycling of albumin in human monocytic cells, which presumably can be exploited to further extend the half-lives of therapeutic antibodies and/or albumin-conjugated drugs in blood circulation and, hence, promotes the efficiency of these treatments.

The molecular mechanism of 1,4-naphthoquinone-induction of FcRn protein expression still remains to be investigated. However, it can be surmised by the ability of 1,4-naphthoquinone to modulate the IRAK1-mediated signaling pathway. Indeed, it has been recently shown that 1,4-naphthoquinone effectively inhibits IRAK1 enzymes *in vitro* and potently suppresses the production and secretion of key proinflammatory cytokine proteins in THP-1 cells.[10] Several downstream signaling cascades such as NF-κB, JNK, and MAPKs can be activated by the IRAK1-mediated pathway.[13,26] Therefore, it would be interesting to define whether these pathways are involved in the 1,4-naphthoquinone-mediated FcRn induction mechanism in the future.

To our knowledge, this is the first small chemical stimulator of FcRn reported in the literature. Further preclinical and *in vivo* analyses of the compound are needed to clarify its potential use in clinical therapeutics and to unravel the molecular mechanisms of action of 1,4-naphthoquinone.

EXPERIMENTAL SECTION

Chemicals, Reagents, and Cell Lines. 1,4-Naphthoquinone (97%) (Sigma), phorbol 12-myristate 13-acetate (PMA) (Biotechne), colorimetric Cell Titer 96 nonradioactive cell proliferation assay kit (Promega), rabbit polyclonal antihuman FcRn antibodies—extracellular domain (Abcam, U.K.), goat antirabbit IgG (H + L)-Alexa-Fluor 647 (Thermo Fisher), mouse monoclonal (IgG2b) antihuman FcRn Alexa-Fluor 647-conjugated antibody (RD Systems, USA), Alexa-Fluor 647 mouse IgG2b, k isotype control (BD Biosciences, USA), mouse antihuman GAPDH antibody (Abcam, U.K.), horseradish peroxidase-conjugated goat antimouse and goat antirabbit antibodies (Abcam, UK), transferrin from human serum, Alexa-Fluor 488 conjugate (Invitrogen), human monocytic cell line (THP-1) and human dental (HD) fibroblasts (ATCC), native human serum albumin and human albumin sandwich ELISA kit (Abcam, U.K.), and native human serum albumin-conjugated-rhodamine (HSA-rhodamine) (Abcam, U.K.) were used.

Immunoblotting. Cells were lysed using RIPA lysis buffer. Protein concentration was determined using the colorimetric, detergent-compatible DC Protein Assay kit (Bio-Rad). Total protein lysate (40 μg) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane of 0.45 μm pore size (Thermo Scientific). The following antibodies were used for immunoblotting: polyclonal antihuman FcRn antibodies—extracellular domain (1:2000), mouse antihuman GAPDH (1:5000), and horseradish peroxidase-conjugated goat anti-mouse and goat antirabbit secondary antibodies. Images were captured using a ChemiDoc MP Scanner (Bio-Rad).

Fluorescence-Activated Cell Sorting (FACS). THP-1 cells (1×10^6) were plated into six-well plates and treated with the following concentrations of 1,4-naphthoquinone: 1, 2, 4, and 8 μM for 24 h. Untreated cells and cells treated with the vehicle (DMSO) were used as negative controls. After 24 h, the cells were collected and centrifuged at 250g for 5 min. Then, the cells were fixed with 4% paraformaldehyde (PFA) for 10 min at RT in the dark. The fixed cells were centrifuged at 250g for 5 min, suspended in 1 mL of ice-cold 100% methanol, and incubated for 30 min for permeabilization. The cells were then washed with FACS buffer (BD Biosciences, USA) and centrifuged at 400g for 5 min. Next, cells were stained with the antihuman FcRn Alexa-Fluor 647-conjugated antibody (RD Systems, USA) diluted in 100 μL of FACS buffer at 0.5 μg/1 $\times 10^6$ cells at room temperature in the dark for 40 min with shaking. A proper isotype-matching antibody

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ACS Omega 2023, 8, 16491−16499

was used as a negative control. The stained cells were washed with 1 mL of phosphate-buffered saline (PBS) and centrifuged at 250*g* for 5 min. Finally, cells were resuspended in 200 μL of FACS buffer and acquired by FACS Canto II, FACS DIVA software version 8 (BD Biosciences). Data was analyzed by FlowJo software version 10.

Immunofluorescence and Confocal Laser Scanning Microscopy. THP-1 cells were maintained in RPMI 1640 cell culture growth medium (Euroclone) supplemented with 2 mM L-glutamine, 100 mg/mL penicillin/streptomycin (Gibco), 4.5 g/L D-glucose (Sigma), and 20% fetal bovine serum (Euroclone). For the induction of cell differentiation into macrophages, THP-1 cells were resuspended to a concentration of 2×10^5 cells per mL in a fresh RPMI medium supplemented with 150 nM PMA and then seeded into 24-well culture plates containing glass coverslips for 24 h. The culture medium was removed, and the cells were treated with indicated concentrations of 1,4-naphthoquinone (diluted in complete culture medium) for 24 h at 37 °C. The cells were then washed twice with PBS and fixed with 4% PFA for 15 min at room temperature in the dark. The fixed cells were then quenched in 50 mM ammonium chloride (NH_4Cl) for 5 min to decrease any possible autofluorescence. FcRn in cells was stained using rabbit polyclonal antihuman FcRn antibodies followed by goat antirabbit IgG (H + L)-Alexa-Fluor 647. DAPI was used to stain the nucleus in cells. The confocal images were acquired using an LSM 780 (Zeiss, Germany). The objective lens used for acquiring the images was Plan-Apochromat 63X/1.4 Oil DIC M27. Lasers with wavelengths of 405, 488, 561, and 633 nm were activated for excitation of the nuclear stain DAPI, Alexa-Fluor 488, rhodamine, and Alexa-Fluor 647, respectively. The detection ranges for fluorescence emission signals were 420−520 nm for DAPI, 490−550 nm for Alexa-Fluor 488, 568−640 nm for Alexa-Fluor 568, and 645−755 nm for Alexa-Fluor 647.

Transferrin Uptake Assay. PMA-induced THP-1 cells were incubated with Alexa-488-conjugated transferrin (25 μg/mL), dissolved in serum-free culture medium, for 20 min at 37 °C. After incubation, the cells were washed three times with PBS to remove excess transferrin and then fixed with 4% PFA as aforementioned.

Pulse-Chase Assay. PMA-induced THP-1 cells were pulsed with HSA-rhodamine (200 μg/mL) and Alexa-488-conjuated transferrin (50 μg/mL), dissolved in serum-free culture medium, for 10 min at 37 °C. The cells were washed three times with PBS and chased for another 10 min at 37 °C. The cells were then fixed with 4% PFA.

Quantification of Cellular Colocalization. Quantification of the colocalization of internalized transferrin or HSA and endogenous FcRn was determined using the JACoP plugin on ImageJ software as previously described (Bolte and Cordelières, 2006). The colocalization parameters, Pearson's correlation coefficient, which measures the linear correlation between the two channels, and Manders coefficient, which calculates the percentage of the total signal from one channel that overlaps the signal from the other, were identified at each drug concentration.

Human Serum Albumin (HSA) Recycling Assay. PMA-induced THP-1 cells were treated with different doses of 1,4-naphthoquinone for 15 h. Cells treated with DMSO only were used as a control. For the recycling assay, the cells were incubated with native HSA (100 ng/ml), dissolved in serum-free culture medium, for 20 min at 37 °C. After incubation, the supernatant was removed and the cells were washed four times with PBS to remove excess HSA. Fresh serum-free culture medium was added, and the cells were incubated at 37 °C for 30 min. The culture medium above the cells was collected, and HSA was measured by sandwich ELISA (Abcam, U.K.). The amount of recycled HSA (ng/mL) was then calculated based on the standard curve after normalization of the absorbance readings to the number of live cells.

MTT Cell Cytotoxicity Assay. Human cell lines HD fibroblasts and THP-1 cells were subjected to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. For THP-1 cells, briefly, the cells were resuspended in fresh RPMI medium supplemented with 150 nM PMA, seeded at a density of 6×10^4 per well in 96-well plates, and incubated at 37 °C for 24 h for induction of differentiation (as described above). The culture medium was removed, and the cells were treated with increasing concentrations (1, 2, 4, 8, 16 μM) of 1,4-naphthoquinone and incubated for 24 h or 48 h at 37 °C. HD fibroblasts were seeded at 8×10^3 or 13×10^3 per well in 96-well plates and incubated at 37 °C for 24 h for attachment. The cells were treated with increasing concentrations (1, 2, 4, 8, 16 μM) of 1,4-naphthoquinone and incubated for 24 h or 48 h at 37 °C. The Colorimetric Cell Titer 96 nonradioactive cell proliferation assay (Promega, Madison) was used to detect cell proliferation in each well according to the manufacturer's instructions.

Statistical Analysis. Statistical analysis was performed using GraphPad Prism 9.0. An unpaired, parametric, two-tailed *t*-test was used to measure significant differences. *p*-values < 0.05 were considered statistically significant. The following symbols represent these *p*-values: ****p < 0.0001, ***p < 0.001, **p < 0.01, and *p < 0.05, and ns denotes nonsignificant.

■ ASSOCIATED CONTENT

Accession Codes

Human FcRn protein Uniprot accession ID: Q8N166_HUMAN.

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Author Contributions

E.I.E. performed most of the experiments. I.S.M. contributed to the conception, design, supervision, experiments, data analysis, and writing of the manuscript. M.A.A. performed immunoblotting experiments. D.A.A. was involved in FACS experiments. M.A.A. revised the manuscript. W.M. A. was involved in the supervision and revision of the manuscript. All authors have read and accepted the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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■ ABBREVIATIONS USED

FcRn:neonatal Fc receptor; IgG:immunoglobulin G; IRAK:interleukin-1 receptor-associated kinase; PMA:phorbol 12-myristate 13-acetate; HSA:human serum albumin; IL1R/TLR:interleukin (IL)-1 receptor/Toll-like receptor; HD fibroblast:human dermal fibroblast; FcBP:FcRn-binding peptide; ELIZA:enzyme-linked immunosorbent assay; MAPK:mitogen-activated protein kinase; JNK:Jun N-terminal kinase; NF-κB:nuclear factor kappa B; IL:interleukin; FACS:fluorescence-activated cell sorting; DMSO:dimethyl sulfoxide

■ REFERENCES

(1) Pyzik, M.; Rath, T.; Lencer, W. I.; Baker, K.; Blumberg, R. S. FcRn: The Architect Behind the Immune and Nonimmune Functions of IgG and Albumin. *J. Immunol.* **2015**, *194*, 4595−4603.

(2) Roopenian, D. C.; Christianson, G. J.; Sproule, T. J.; Brown, A. C.; Akilesh, S.; Jung, N.; Petkova, S.; Avanessian, L.; Choi, E. Y.; Shaffer, D. J.; et al. The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. *J. Immunol.* **2003**, *170*, 3528−3533.

(3) Patel, D. D.; Bussel, J. B. Neonatal Fc receptor in human immunity: Function and role in therapeutic intervention. *J. Allergy Clin. Immunol.* **2020**, *146*, 467−478.

(4) Miller, A.; Jedrzejczak, W. W. Albumina–funkcje biologiczne i znaczenie kliniczne [Albumin–biological functions and clinical significance]. *Postepy Hig Med Dosw.* **2001**, *55*, 17−36.

(5) Larsen, M. T.; Kuhlmann, M.; Hvam, M. L.; Howard, K. A. Albumin-based drug delivery: harnessing nature to cure disease. *Mol Cell Ther.* **2016**, *4*, No. 3.

(6) Cho, H.; Jeon, S. I.; Ahn, C. H.; Shim, M. K.; Kim, K. Emerging Albumin-Binding Anticancer Drugs for Tumor-Targeted Drug Delivery: Current Understandings and Clinical Translation. *Pharmaceutics.* **2022**, *14*, No. 728.

(7) Lu, R. M.; Hwang, Y. C.; Liu, I. J.; Lee, C. C.; Tsai, H. Z.; Li, H. J.; Wu, H. C. Development of therapeutic antibodies for the treatment of diseases. *J. Biomed. Sci.* **2020**, *27*, No. 1.

(8) Aminin, D.; Polonik, S. 1,4-Naphthoquinones: Some Biological Properties and Application. *Chem. Pharm. Bull.* **2020**, *68*, 46−57.

(9) Liu, H.; Yan, C.; Li, C.; You, T.; She, Z. Naphthoquinone Derivatives with Anti-Inflammatory Activity from Mangrove-Derived Endophytic Fungus *Talaromyces* sp. SK-S009. *Molecules* **2020**, *25*, No. 576.

(10) Mahmoud, I. S.; Hatmal, M. M.; Abuarqoub, D.; Esawi, E.; Zalloum, H.; Wehaibi, S.; Nsairat, H.; Alshaer, W. 1,4-Naphthoquinone Is a Potent Inhibitor of IRAK1 Kinases and the Production of Inflammatory Cytokines in THP-1 Differentiated Macrophages. *ACS Omega* **2021**, *6*, 25299−25310.

(11) Loiarro, M.; Ruggiero, V.; Sette, C. Targeting TLR/IL-1R signalling in human diseases. *Mediators Inflamm.* **2010**, *2010*, No. 674363.

(12) Liu, X.; Ye, L.; Christianson, G. J.; Yang, J. Q.; Roopenian, D. C.; Zhu, X. NF-kappaB signaling regulates functional expression of the MHC class I-related neonatal Fc receptor for IgG via intronic binding sequences. *J. Immunol.* **2007**, *179*, 2999−3011.

(13) Cervenak, J.; Doleschall, M.; Bender, B.; Mayer, B.; Schneider, Z.; Doleschall, Z.; Zhao, Y.; Bősze, Z.; Hammarström, L.; Oster, W.; et al. NFκB induces overexpression of bovine FcRn: a novel mechanism that further contributes to the enhanced immune response in genetically modified animals carrying extra copies of FcRn. *MAbs* **2013**, *5*, 860−871.

(14) Guo, J.; Li, F.; Qian, S.; Bi, D.; He, Q.; Jin, H.; Luo, R.; Li, S.; Meng, X.; Li, Z. TGEV infection up-regulates FcRn expression via activation of NF-κB signaling. *Sci. Rep.* **2016**, *6*, No. 32154.

(15) de Sousa Portilho, A. J.; da Silva, E. L.; Bezerra, E. C. A.; de Souza Moraes Rego Gomes, C. B.; Ferreira, V.; de Moraes, M. E. A.; da Rocha, D. R.; Burbano, R. M. R.; Moreira-Nunes, C. A.; Montenegro, R. C. 1,4-Naphthoquinone (CNN1) Induces Apoptosis through DNA Damage and Promotes Upregulation of *H2AFX* in Leukemia Multidrug Resistant Cell Line. *Int. J. Mol. Sci.* **2022**, *23*, No. 8105.

(16) Ravichandiran, P.; Subramaniyan, S. A.; Kim, S. Y.; Kim, J. S.; Park, B. H.; Shim, K. S.; Yoo, D. J. Synthesis and Anticancer Evaluation of 1,4-Naphthoquinone Derivatives Containing a Phenylaminosulfanyl Moiety. *ChemMedChem* **2019**, *14*, 532−544.

(17) Wang, H.; Luo, Y. H.; Shen, G. N.; Piao, X. J.; Xu, W. T.; Zhang, Y.; Wang, J. R.; Feng, Y. C.; Li, J. Q.; Zhang, Y.; et al. Two novel 1,4-naphthoquinone derivatives induce human gastric cancer cell apoptosis and cell cycle arrest by regulating reactive oxygen species-mediated MAPK/Akt/STAT3 signaling pathways. *Mol. Med. Rep.* **2019**, *20*, 2571−2582.

(18) Yamashiro, D. J.; Tycko, B.; Fluss, S. R.; Maxfield, F. R. Segregation of transferrin to a mildly acidic (pH 6.5) para-Golgi compartment in the recycling pathway. *Cell* **1984**, *37*, 789−800.

(19) D'Hooghe, L.; Chalmers, A. D.; Heywood, S.; Whitley, P. Cell surface dynamics and cellular distribution of endogenous FcRn. *PLoS One* **2017**, *12*, No. e0182695.

(20) Sockolosky, J. T.; Tiffany, M. R.; Szoka, F. C. Engineering neonatal Fc receptor-mediated recycling and transcytosis in recombinant proteins by short terminal peptide extensions. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 16095−16100.

(21) Lee, C. H.; Kang, T. H.; Godon, O.; Watanabe, M.; Delidakis, G.; Gillis, C. M.; Sterlin, D.; Hardy, D.; Cogné, M.; Macdonald, L. E.; et al. An engineered human Fc domain that behaves like a pH-toggle switch for ultra-long circulation persistence. *Nat. Commun.* **2019**, *26*, No. 5461.

(22) Andersen, J. T.; Dalhus, B.; Viuff, D.; Ravn, B. T.; Gunnarsen, K. S.; Plumridge, A.; Bunting, K.; Antunes, F.; Williamson, R.; Athwal, S.; et al. Extending serum half-life of albumin by engineering neonatal Fc receptor (FcRn) binding. *J. Biol. Chem.* **2014**, *289*, 13492−13502.

(23) Toh, W. H.; Louber, J.; Mahmoud, I. S.; Chia, J.; Bass, G. T.; Dower, S. K.; Verhagen, A. M.; Gleeson, P. A. FcRn mediates fast recycling of endocytosed albumin and IgG from early macropinosomes in primary macrophages. *J. Cell Sci.* **2019**, *133*, No. jcs235416.

(24) Nilsen, J.; Trabjerg, E.; Grevys, A.; Azevedo, C.; Brennan, S. O.; Stensland, M.; Wilson, J.; Sand, K. M. K.; Bern, M.; Dalhus, B.; et al. An intact C-terminal end of albumin is required for its long half-life in humans. *Commun. Biol.* **2020**, *3*, No. 181.
(25) Schmidt, E. G.; Hvam, M. L.; Antunes, F.; Cameron, J.; Viuff, D.; Andersen, B.; Kristensen, N. N.; Howard, K. A. Direct demonstration of a neonatal Fc receptor (FcRn)-driven endosomal sorting pathway for cellular recycling of albumin. *J. Biol. Chem.* **2017**, *292*, 13312−13322.
(26) Qian, S.; Li, C.; Liu, X.; Jia, X.; Xiao, Y.; Li, Z. Activation of the JNK/MAPK Signaling Pathway by TGF-β1 Enhances Neonatal Fc Receptor Expression and IgG Transcytosis. *Microorganisms* **2021**, *9*, No. 879.

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ACS Omega 2023, 8, 16491−16499