preliminarily verify the potential of hsa_circ_0000077 as a therapeutic target for the precision treatment of OA.
Methods: Gene expression changes of hsa_circ_0000077 in cartilage samples were confirmed by qPCR. Luciferase assays were used to detect any interaction between the target miRNA (miR-494-3p) and hsa_circ_0000077. CCK-8, western blotting and qPCR were used to investigate the effects of miR-494-3p on the proliferation and maintenance of cartilage extracellular matrix (ECM).
Results: The gene expression level of hsa_circ_0000077 was significantly higher in healthy cartilage than in OA cartilage. Luciferase assays confirmed the interaction between miR-494-3p and hsa_circ_0000077. CCK-8 assay confirmed that overexpression of miR-494-3p inhibited the proliferation of chondrocytes but this was rescued by overexpression of hsa_circ_0000077. Western blotting confirmed that overexpression of miR-494-3p inhibited the expression of SOX9 but again this was rescued by overexpression of hsa_circ_0000077. Similarly, qPCR assays confirmed that overexpression of miR-494-3p inhibited the expression of ECM-related genes but expression was rescued by overexpression of hsa_circ_0000077.
Conclusions: Our results have revealed the role of hsa_circ_0000077 in the regulation of chondrocyte proliferation and maintenance of ECM, improved understanding of the mechanism of OA disease progression, and provided a therapeutic target for the precision treatment of OA.

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ESTROGEN-RELATED RECEPTOR γ (ERRγ) IS A CATABOLIC REGULATOR OF OSTEOARTHRITIS PATHOGENESIS

Y.-O. Son, S.-K. Park, J.-S. Kwak, Y. Won, W.-S. Choi, J.-S. Chun. *GIST, Buk-gu, Republic of Korea*

Purpose: The estrogen-related receptor (ERR) family of orphan nuclear receptor is composed of ERRα, ERRβ and ERRγ, which are known to regulate various isoform-specific functions under normal and pathophysiological conditions. However, the functions of ERRs in regulating joint cartilage homeostasis and/or OA pathogenesis have not yet been studied. Here, we investigate the involvement of ERRs in the pathogenesis of osteoarthritis (OA) in mice.
Methods: Human OA cartilage was sourced from individuals undergoing arthroplasty. C57BL/6J mice were used for the experimental OA studies. *Esrrg*$^{+/-}$ mice (B6.129P2-*Esrrg*^{tm1Dgen}/Mmnc) were purchased from the Mutant Mouse Regional Resource Center (MMRRC). *Col2a1-Esrrg* Tg mice were generated using the *Col2a1* promoter and enhancer. Human OA cartilage was frozen, sectioned at a thickness of 5 μm, and fixed in paraformaldehyde. Sulfate proteoglycans were detected by alcian blue staining, and cartilage destruction in mice was examined using safranin-O staining. Femoral condyles and tibial plateaus were obtained from mice, and chondrocytes were isolated from cartilage tissue by digestion with 0.2% collagenase.
Results: Among the ERR family members, ERRγ was specifically upregulated in cartilage from human OA patients and various mouse models of OA. Gain-of-function (adenovirus-mediated overexpression in joint tissues or *Col2a1-Esrrg* Tg mice) and loss-of-function (*Esrrg*$^{+/-}$ mice or shRNA-mediated knockdown) approaches clearly indicated that ERRγ acts as a novel catabolic regulator of OA pathogenesis, at least in part, by the upregulating matrix-degrading enzymes, MMP3 and MMP13, in articular chondrocytes.
Conclusions: Our results are the first to demonstrate that ERRγ acts as a catabolic regulator of cartilage degeneration and OA pathogenesis, and collectively support the idea that ERRγ could be a therapeutic target for OA

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ZFP36L1 REGULATES OSTEOARTHRITIS BY MODULATING HSPA1A

Y.-O. Son, H.-E. Kim, W.-S. Choi, J.-S. Chun. *GIST, Buk-gu, Republic of Korea*

Purpose: Osteoarthritis (OA) is a whole-joint disease characterized by cartilage destruction and other whole-joint pathological changes. There is currently no effective disease-modifying therapy. Here, we investigated the post-transcriptional mRNA regulation of OA-modulating proteins in chondrocytes by focusing on RNA-binding proteins.
Methods: Human OA cartilage was sourced from individuals undergoing arthroplasty. C57BL/6J mice were used for the experimental OA studies. *ZFP36l1*$^{+/-}$ mice (8-bp insertion/42-bp deletion in exon 2) were generated by ToolGen, Inc. For mRNA decay assay, primary-culture chondrocytes were infected with 800 MOI of Ad-HSPA1A to overexpress HSPA1A. The cells were co-infected with 800 MOI of Ad-C or Ad-ZFP36L1 for 12 hours, and exposed to actinomycin D (1 μg ml^{-1}) for the indicated time periods. The RNA-binding protein immunoprecipitation assay was performed using a Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacturer's instructions.
Results: Microarray analysis revealed that the ZFP36 family member, ZFP36L1, is specifically upregulated in OA chondrocytes and OA cartilage of humans and mice. Adenovirus-mediated overexpression of ZFP36L1 alone in mouse knee-joint tissue did not modulate OA pathogenesis. However, genetic ablation or silencing of *Zfp36l1* significantly abrogated experimental OA in mice. Knockdown of *Zfp36l1* increased the mRNA expression of two heat shock protein 70 (HSP70) family members, which act as its direct targets. Furthermore, overexpression of HSPA1A in joint tissues protected mice against experimental OA by inhibiting chondrocyte apoptosis.
Conclusions: Our results collectively indicate that the RNA-binding protein, ZFP36L1, regulates HSP70 family members that appear to protect against OA pathogenesis by inhibiting chondrocyte apoptosis.

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APPA, A POTENTIAL NEW THERAPY FOR OSTEOARTHRITIS, INHIBITS NEUTROPHIL PRO-INFLAMMATORY FUNCTIONS WITHOUT IMPAIRING HOST DEFENCE

A. Cross [1], J. Hawkes [1], H. Wright [1], N. Larkins [2], S. Edwards [1], R. Moots [1]. [1] *LIVERPOOL Univ., LIVERPOOL, United Kingdom;* [2] *AKL RESEARCH & DEVELOPMENT, STEVENAGE, United Kingdom*

Purpose: APPA - a proprietary combination of two synthetic molecules, apocynin (AP) and paeonol (PA) - has already demonstrated significant pain relief from osteoarthritis (OA) in canine models, by improving functionality and slowing cartilage destruction. Trials are now underway for APPA that comprise the first in human pharmacokinetics study and first in patients Phase II study (scheduled to start early 2019) to examine safety and effectiveness of APPA as a drug to treat osteoarthritis of the knee. The efficacy of APPA is due, at least in part, to the effects of the constituent components, AP and PA, on various levels of regulation of the transcription factor, NF-κB. This transcription factor plays a key role in both constitutive and inducible gene expression, particularly of immune cells, and as such is an attractive therapeutic target not only for osteoarthritis but for other inflammatory conditions. Neutrophils are the most abundant white blood cell in the circulation and are an essential part of the innate immune system, migrating to inflammatory sites where they generate/release their armoury of cytotoxic enzymes and reactive oxygen species (ROS) to fight infection, but which may also induce bystander tissue damage. Neutrophils also contribute to the cytokine and chemokine cascades that accompany inflammation, and regulate immune responses via cell to cell interactions. Evidence also suggests that neutrophils have a role in autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (lupus) and psoriasis/psoriatic arthritis. These cells are potent producers of ROS (activators of NF-κB) via an NADPH oxidase and many inflammatory functions are also regulated by NF-κB activation. The purpose of this study was to investigate how APPA affected these and other key neutrophil functions.
Methods: Neutrophils were isolated from peripheral blood of healthy subjects. Cell functional tests performed included measurements of apoptosis, levels of cell surface markers, ROS production, chemotaxis, formation of neutrophil extracellular traps (NETs), measurements of phagocytosis and bacterial killing assays. All measurements were performed before and after treatment with APPA and following cytokine stimulation. RNA and proteins were also isolated pre- and post-treatment: western blotting was used to measure levels of proteins involved in signal transduction pathways in addition to those known to regulate neutrophil apoptosis and their capability to protect against infection; real time PCR was used to measure mRNA transcripts.
Results: APPA did not significantly affect the rate of constitutive apoptosis. Chemotactic migration towards IL-8 and fMLP were only affected at the highest concentrations of APPA used (>3 mM). Neutrophil phagocytosis, bacterial killing and expression of surface receptors that facilitate these processes (e.g. integrins and Fc receptors) did not change significantly. APPA did decrease neutrophil degranulation and ROS production, although the latter was due to the scavenging of

ROS rather than inhibition of the NAPDH oxidase. APPA significantly decreased the formation of NETs ($p<0.05$), which was probably via inhibition of NADPH oxidase and decreased degranulation. TNFα-induced NF-κB signalling was inhibited by APPA (600 μM), as was GM-CSF and IL-6 signalling via ERK1/2 and STAT3, respectively. APPA decreased TNFα-activated expression of IL-8 and TNFα mRNA but upregulated NRF2, an anti-inflammatory regulator of antioxidant proteins. APPA was also an effective inhibitor of IL-6 and chemokine expression (CCL3, CCL4) induced by the TLR8 agonist and chromatin remodelling agent, R848 ($p<0.05$). This agonist triggers the expression of these genes via endogenous TNFα secretion and activation of neutrophils; APPA was as effective as the biologic therapy Infliximab in this inhibition.

Conclusions: APPA does not significantly impair host defence neutrophil functions and may have significant anti-inflammatory potential in diseases characterised by dysregulation of cytokine expression or oxidative stress. APPA is a potent ROS scavenger, in addition to affecting neutrophil degranulation. These data also describe the novel finding that APPA is as effective as biologic drugs in inhibiting the effects of endogenous TNFα on immune cell activation. We believe APPA may have therapeutic potential in ROS- and/or TNFα-driven inflammatory conditions.

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INDUCIBLE DICKKOPF 1-MEDIATED INHIBITION OF CANONICAL WNT SIGNALING AMELIORATES CARTILAGE DESTRUCTION DURING EXPERIMENTAL OSTEOARTHRITIS

M.H. van den Bosch, A.B. Blom, F.A. van de Loo, M.I. Koenders, W.B. van den Berg, P.L. van Lent, P.M. van der Kraan. *Radboud Univ. Med. center, Oss, Netherlands*

Purpose: Balanced canonical Wnt signaling has been shown to be crucial for the development and homeostasis of joint tissues. Previously, we described increased expression of various members of the Wnt signaling in the synovium in two experimental osteoarthritis (OA) mouse models. In addition, we found increased expression of the canonical Wnt signaling key downstream target WISP1 in both the synovium and cartilage during experimental and human OA. Together, this suggests increased activation of the canonical Wnt signaling pathway in the OA joint. We and others described that increased canonical Wnt signaling associates with more joint pathology. Here, we determined the potency of Dickkopf 1 (*Dkk1*) overexpression-mediated inhibition of canonical Wnt signaling to reduce pathology in the complex environment during experimental OA. Because a constitutively enhanced expression has profound effects on the development of target tissues such as the articular cartilage and bone which affects the development of OA, we here used inducible *Dkk1*-transgenic mice.

Methods: Inducible *Dkk1*-transgenic mice were generated by backcrossing *rtTA* and *tetO-Dkk1* transgenic mice. The mice used in this study were homozygous for *rtTA* and heterozygous for *Dkk1*, since a complete inhibition of β-catenin as key intracellular factor in canonical Wnt signaling, similar to the constitutive activation of β-catenin mimicking increased canonical Wnt signaling, has been shown to be detrimental for the articular cartilage. *Dkk1*-overexpression was induced by feeding mice food supplemented with doxycycline. Collagenase-induced osteoarthritis (CIOA) or destabilization of the medial meniscus (DMM) experimental animal models of OA were induced in mice with (on dox) or without (off dox) doxycycline supplementation. Gene expression was determined with qRT-PCR. Joint pathology was assessed using histology after hematoxylin & eosin or Safranin O & Fast Green staining. Protease activity was determined by immunohistochemical staining of the aggrecan neoepitope NITEGE.

Results: First, we ensured that the $rtTA^{+/+}/Dkk1^{+/-}$ had no basal phenotype in their cartilage and subchondral bone when compared with $rtTA^{+/+}/Dkk1^{-/-}$ mice at the age of induction of the OA models. Mice off dox showed comparable articular cartilage and a comparable ratio between the surface of Fast Green stained collagenous and total area in the subchondral bone. Moreover, we observed that addition of dox to the food resulted in a strongly increased *Dkk1* expression in both the cartilage and synovium of $rtTA^{+/+}/Dkk1^{+/-}$ as compared to off dox controls. Next, we tested whether doxycycline supplementation itself affected the severity of the experimental OA models as studied in $rtTA^{+/+}/Dkk1^{-/-}$ mice. However, we observed comparable cartilage degeneration between mice that were on dox and off dox after induction of CIOA and DMM, therewith excluding dox effects. Interestingly, $rtTA^{+/+}/Dkk1^{+/-}$ mice on dox, and thus overexpressing *Dkk1*, developed significantly less cartilage damage both after induction of CIOA and DMM, which was associated with decreased levels of the aggrecan neoepitope NITEGE, but showed comparable levels of synovial inflammation.

Conclusions: Our study shows that inhibition of canonical Wnt signaling by DKK1 might prove an efficient way to limit OA-related cartilage degeneration and therefore might be an interesting option for clinical therapy.

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CHANGES IN SOX9 AND RUNX2 PROTEIN ACTIVITY CORRELATE TO THE HEALTH STATE OF HUMAN PRIMARY CHONDROCYTES

K. Govindaraj, M. Karperien, J. Post. *Univ. of Twente, Enschede, Netherlands*

Purpose: SOX9 and RUNX2 are the master transcription factors involved in cartilage and bone development respectively. Altered SOX9 and RUNX2 activity is implicated in osteoarthritis (OA) pathophysiology and hypertrophic differentiation of chondrocytes. We have previously shown that SOX9 and RUNX2 protein activity can be measured by Transcription Factor - Fluorescence Recovery After Photobleaching (TF-FRAP). To decipher the underlying signaling mechanism in OA pathophysiology, we aim to use TF-FRAP to correlate SOX9 and RUNX2 mobility to the health state of the chondrocytes. We hypothesize that the mobility of SOX9 increases with OA progression, while the mobility of RUNX2 decreases in chondrocyte hypertrophy. We measured the mobility and correlated protein activity of these transcription factors at the single cell level by TF-FRAP in healthy, preserved and OA chondrocytes.

Methods: Healthy human primary chondrocytes (hPCs) were purchased from Articular Engineering, USA. Human preserved and OA hPCs were isolated from patients undergoing total knee replacement therapy. hPCs were seeded on glass coverslips and transfected with either SOX9-mGFP or eGFP-RUNX2. TF-FRAP was performed in at least 40 cells per condition in two healthy donors and three donors for each preserved and OA chondrocytes. TF-FRAP measurements were done using a Nikon A1 confocal microscope (Japan). In TF-FRAP, a small circular region (Ø 25 pixels) of the nucleus was bleached using a high intensity laser and the recovery of the fluorescence in the bleach spot is recorded. For every TF-FRAP measurement, 25 pre-bleach images and 260 post-bleach images were acquired at 4 frames per second for 60 seconds, with a frame size of 256x256 pixels. Higher immobile fraction (i.e., the fraction of transcription factors bound to DNA) and higher half-time recovery (longer DNA interaction times) were associated with a higher transcriptional activity. For all donors, early passage ($\leq$3) cells were used for TF-FRAP and qPCR measurements. To correlate protein mobility and DNA binding with the protein activity, we quantified the expression levels of SOX9 target genes (*ACAN* and *COL2A*) and RUNX2 target genes (*MMP13* and *COL10*) by qPCR.

Results: In the healthy hPCs, the SOX9 immobile fraction and half-time recovery were 60% (SD ±6%) and 14.2 sec (SD ±3.2 sec) respectively. In preserved hPCs, the SOX9 immobile fraction and half-time to recover was significantly lower, ranging from 50% - 44% (SD ±7% - ±9.7%), and 14.2 - 13.3 sec (SD ±3.9 - ±4.5 sec) respectively. In OA hPCs, the SOX9 immobile fraction and half-time recovery was significantly lower than in the healthy and preserved hPCs, ranging from 48.6% - 42.8% (SD ±7.3% - ±8.4%), and half-time to recover ranged from 13.3 - 12.2 sec (SD ±3.9 - ±5.3 sec). RUNX2 immobile fraction and half-time to recover data spread was large in healthy, preserved and OA hPCs. In healthy hPCs, immobile fraction and half-time to recover were ranging from 33.6% - 47.9% (SD 19.4% - 20.7%) and 13.97 sec - 14.46 sec (SD 7.9 - 10.1 sec) respectively. In the preserved hPCs, the immobile fraction and half-time to recover were ranging from 38.8% - 47.0 % (SD 19.7% - 22.2%) and 11.7 sec - 14.8 sec (SD 6.8 - 8.2 sec) respectively. In OA hPCs, the immobile fraction and half-time to recover ranged from 33.0% - 41.02% (SD 20.3% - 25.7%) and 11.2 sec - 12.9 sec (SD 7.4 - 9.1 sec) respectively. We have an initial non-quantitative indication for a distinct cell population that, irrespective of the health state, shows decreased RUNX2 mobility, which could be indicative of a hypertrophic cell state. We will show the quantification and segregation. RT-qPCR measurements show decreased SOX9 and increased RUNX2 target gene expression during OA progression.

Conclusions: Our data show that the SOX9 and RUNX2 transcriptional activity is differentially regulated depending on the health state of hPCs. If we assume a correlation between mobility and transcription factor

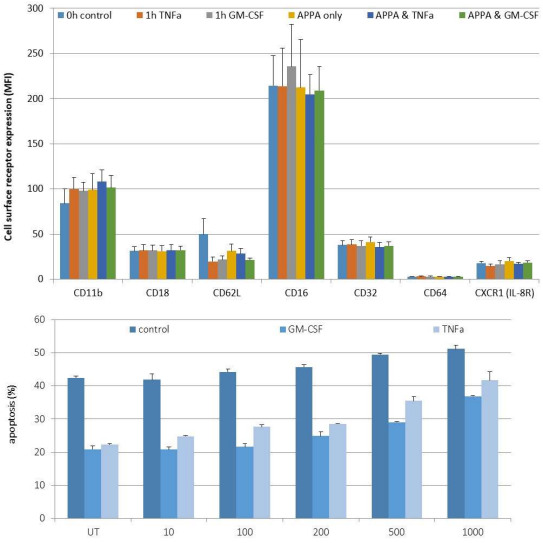
APPA, A POTENTIAL NEW THERAPY FOR OSTEOARTHRITIS, INHIBITS NEUTROPHIL PRO-INFLAMMATORY FUNCTIONS WITHOUT IMPAIRING HOST DEFENCE

Andrew Cross[1,2], Jennifer Hawkes[1,2], Helen Wright[2], Nicholas Larkins[3], Steven Edwards[2] and Robert Moots[1,2]

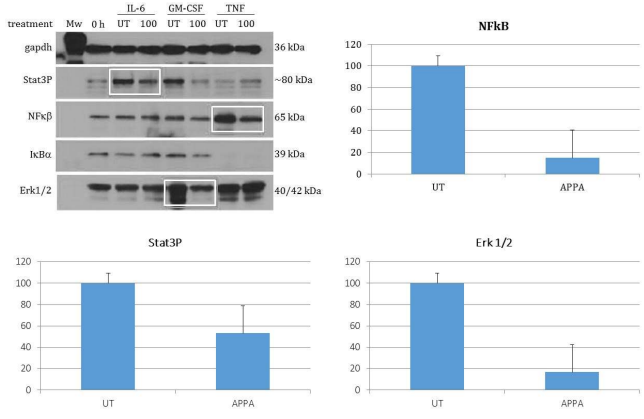
[1]Aintree University Hospital, [2]Liverpool University, [3]AKL Research & Development

Background: APPA - a proprietary combination of two synthetic molecules, apocynin (AP) and paeonol (PA) - has already demonstrated significant pain relief from osteoarthritis (OA) in canine models, by improving functionality and slowing cartilage destruction. Trials are now underway for APPA that comprise the first in human pharmacokinetics study and first in patients Phase II to examine safety and effectiveness of APPA as a drug to treat osteoarthritis of the knee. The efficacy of APPA is due, at least in part, to the effects of the constituent components, AP and PA, on various levels of regulation of the transcription factor, NF-κB. This transcription factor plays a key role in both constitutive and inducible gene expression, particularly of immune cells, and as such is an attractive therapeutic target not only for osteoarthritis but for other inflammatory conditions. Neutrophils are the most abundant white blood cell in the circulation and are an essential part of the innate immune system, migrating to inflammatory sites where they generate/release their armoury of cytotoxic enzymes and reactive oxygen species (ROS) to fight infection, but which may also induce bystander tissue damage. Neutrophils also contribute to the cytokine and chemokine cascades that accompany inflammation, and regulate immune responses via cell to cell interactions. Evidence also suggests that neutrophils have a role in autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (lupus) and psoriasis/psoriatic arthritis. These cells are potent producers of ROS (activators of NF-κB) via an NADPH oxidase and many inflammatory functions are also regulated by NF-κB activation. The purpose of this study was to investigate how APPA affected these and other key neutrophil functions.

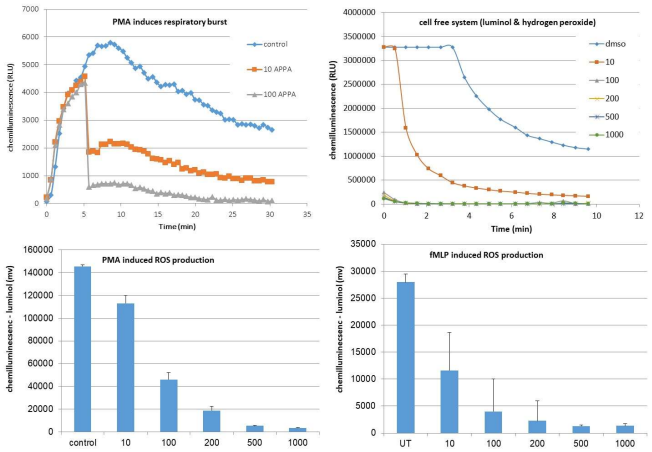
METHODS: Neutrophils were isolated from peripheral blood of healthy subjects. Cell functional tests performed included measurements of apoptosis, levels of cell surface markers, ROS production, chemotaxis, formation of neutrophil extracellular traps (NETs), measurements of phagocytosis and bacterial killing assays. All measurements were performed before and after treatment with APPA and following cytokine stimulation. RNA and proteins were also isolated pre- and post-treatment: western blotting was used to measure levels of proteins involved in signal transduction pathways in addition to those known to regulate neutrophil apoptosis and their capability to protect against infection; real-time PCR was used to measure mRNA transcripts.



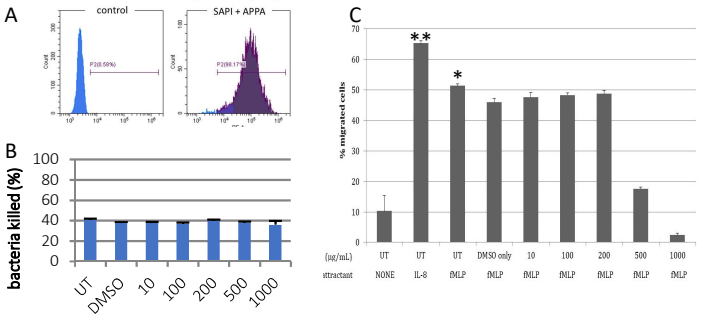
There was no significant difference in surface marker expression (A) following 10 min pre-incubation with APPA and subsequent stimulation with cytokines GM-CSF and TNFa. APPA does not affect the constitutive rate of neutrophil apoptosis or the cytokine-delayed levels of apoptosis (B), neutrophils (10^6/mL) from healthy controls were incubated for 20 h in the absence (UT) or presence of APPA (10 - 1000 μg/mL) in the absence (control) or presence of cytokines known to regulate neutrophil apoptosis.



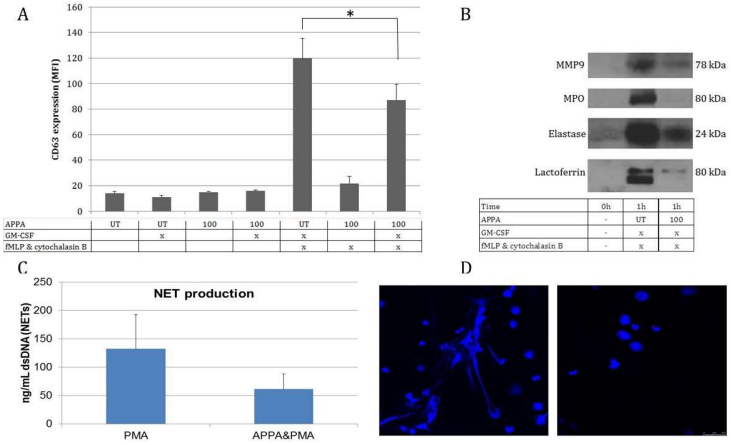
Effects of APPA on activation of cytokine-regulated cell signalling. Neutrophils (5 x 10^6) from healthy controls were incubated in the absence (UT) or presence of APPA (100 μg/mL) for 10 min. APPA-treated neutrophils were then stimulated for 15 min with either IL-6, GM-CSF or TNFα. Cell lysates were collected, proteins separated by SDS-PAGE before western blotting and probing with antibodies recognising activated (phosphorylated) signalling molecules. Typical result of 3 separate experiments, n = 3, p = 0.03, 0.03 & 0.008 for STAT3P, Erk1/2 & NFκB, respectively after normalisation to GAPDH protein levels.



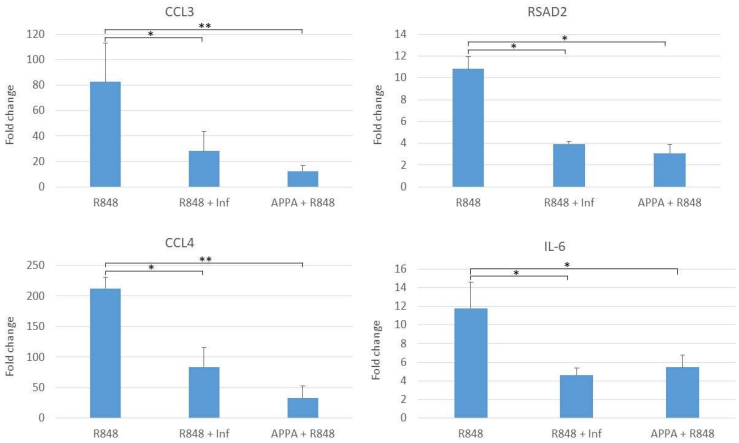
APPA scavenger's the cheiluminescence signal in both a cell and cell-free system. In (A) PMA was used to stimulate ROS production by neutrophils before either APPA (10 & 100 μg/mL) were injected during the experiment, while in (B) APPA (10 - 1000 μg/mL) was added to a cell-free system utilizing hydrogen peroxide. APPA is a strong scavenger/quencher at all concentrations. C & D summarize receptor-independent and receptor-dependent respiratory burst and the effects of APPA upon it.



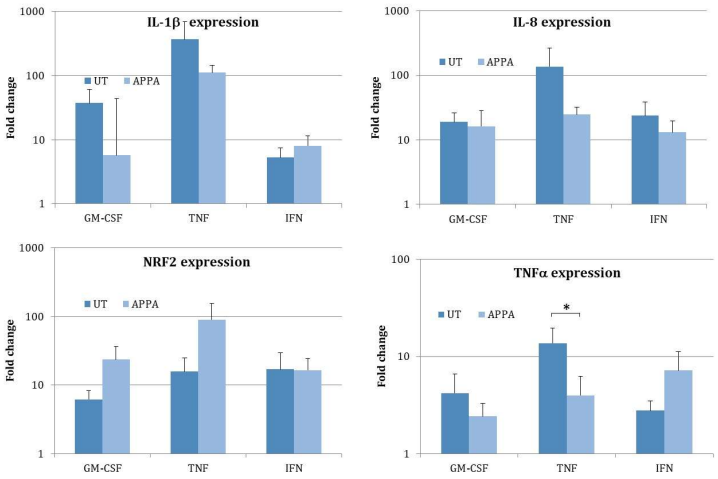
APPA does not inhibit neutrophil phagocytosis or killing of *S. aureus* (SA). Neutrophils were incubated in the absence (UT) or presence of APPA (10 – 1000 μg/mL) for 10 min. (A) APPA treated neutrophils were then incubated for 30 min with SAPI (heat-inactivated *S. aureus* (SA), labelled with propidium iodide (PI) and opsonized with human AB serum) before analysing uptake by flow cytometry. In (B) APPA pre-treated neutrophils were incubated for 60 min with live, opsonized *S. aureus*, lysed and plated out on LB agar plates after suitable dilutions in water. Results are expressed as a percentage of colony forming units (CFUs), compared to bacteria counts after incubation under identical conditions in the absence of neutrophils. DMSO was used as an APPA solvent control. High concentrations of APPA inhibit neutrophil chemotaxis (C). Migration of APPA pre-treated neutrophils towards fMLP (10^{-8}M) was measured after a 90 min incubation period. Untreated neutrophils migrating towards fMLP (10^{-8}M) and IL-8 (100 ng/mL) are shown as positive controls (** p<0.01, * p<0.05). Values shown are means (± SE) of n = 4.



APPA decreases neutrophil degranulation and NET formation. In (A) and (B), neutrophils (5 x 10^6) from healthy controls were incubated in the absence (UT) or presence of APPA (100 µg/mL) for 10 min. APPA treated neutrophils were then primed for 30 min with GM-CSF before stimulating degranulation with fMLP (1 µM) plus cytochalasin B (5 µg/mL). in (A) neutrophils were analysed for expression of CD63, a marker of degranulation using flow cytometry (* p<0.05, n = 7). In (B) supernatants were collected, from neutrophils stimulated to degranulate and proteins separated using SDS-PAGE before western blotting and probing for expression of the labelled granule proteins. In (C), neutrophils were treated with PMA for 4 h in the absence and presence of 100 µg/mL APPA. Neutrophil extracellular trap (NET) formation was measured by DNA release n = 4, p = 0.04 (D) and by microscopy utilizing dual DAPI and neutrophil elastase staining.



APPA is as effective as infliximab in down-regulating chemokine and cytokine expression. Neutrophils were incubated with 5 µM R848 for 7h in the absence (R848) or presence of 200 µg/mL infliximab (IFX) or 100 µg/mL APPA. Expression levels of mRNA for CCL3, CCL4, RSAD2 and IL-6 (normalised to GAPDH mRNA levels) were then measured by qPCR. * p < 0.05, ** p = 0.01 (n = 3).



APPA down-regulates TNFα-stimulated gene expression but up-regulates expression of NRF2. Nrf2 is a factor that regulates gene transcription of anti-oxidants, thereby reducing damage by the reactive oxidant species (ROS) generated during inflammation. Neutrophils (1 x 10^7) from healthy controls were incubated in the absence (UT) or presence of APPA (100 µg/mL) for 10 min. APPA-treated neutrophils were then stimulated with GM-CSF, IFNα or TNFα for 1 h. RNA was isolated, processed and cDNA synthesized before being used to measure transcript levels of IL-1β, IL-8, NRF2 and TNFα. Values shown are mean (± SE), n = 6, * p = 0.012.

RESULTS: APPA did not significantly affect the rate of constitutive apoptosis. Chemotactic migration towards IL-8 and fMLP were only affected at the highest concentrations of APPA used (>3 mM). Neutrophil phagocytosis, bacterial killing and expression of surface receptors that facilitate these processes (e.g. integrins and Fc receptors) did not change significantly. APPA did decrease neutrophil degranulation and ROS production, although the latter was due to the scavenging of ROS rather than inhibition of the NAPDH oxidase. APPA significantly decreased the formation of NETs (p<0.05), which was probably via inhibition of NADPH oxidase and decreased degranulation. TNFa-induced NF-κB signalling was inhibited by APPA (600 mM), as was GM-CSF and IL-6 signalling via ERK1/2 and STAT3, respectively. APPA decreased TNFα-activated expression of IL-8 and TNFα mRNA but upregulated NRF2, an anti-inflammatory regulator of antioxidant proteins. APPA was also an effective inhibitor of IL-6 and chemokine expression (CCL3, CCL4) induced by the TLR8 agonist and chromatin re-modelling agent, R848 (p<0.05). This agonist triggers the expression of these genes via endogenous TNFα secretion and activation of neutrophils; APPA was as effective as the biologic therapy Infliximab in this inhibition.

CONCLUSIONS: APPA does not significantly impair host defence neutrophil functions and may have significant anti-inflammatory potential in diseases characterised by dysregulation of cytokine expression or oxidative stress. APPA is a potent ROS scavenger, in addition to affecting neutrophil degranulation. These data also describe the novel finding that APPA is as effective as biologic drugs in inhibiting the effects of endogenous TNFα on immune cell activation. We believe APPA may have therapeutic potential in ROS- and/or TNFα-driven inflammatory conditions, by resolving inflammation via the harmonization of the cross-talk between Nrf2 and NF-κB.

AKL Research & Development Ltd
Stevenage Bioscience Catalyst
Stevenage, Herts SG1 2FX, UK
www.aklrd.com