Dexamethasone induces ω3-derived immunoresolvents driving resolution of allergic airway inflammation

Katerina Pyrillou, PhD,[a,b] Aikaterini-Dimitra Chairakaki, PhD,[a] Constantin Tamvakopoulos, PhD,[b,†] and Evangelos Andreakos, PhD[a,†]

[a]Laboratory of Immunobiology, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece

[b]Division of Pharmacology-Pharmacotechnology, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece

[†]Evangelos Andreakos and Constantin Tamvakopoulos contributed equally to this work

Reprints and correspondence:

Evangelos Andreakos

Biomedical Research Foundation of the Academy of Athens

Soranou Efesiou 4, Athens, Greece

Tel: +30 210 6596 338

vandreakos@bioacademy.gr

Or

Constantin Tamvakopoulos

Soranou Efessiou Street, 4

11527 Athens, Greece

Tel. +30 210 6597475

ctamvakop@bioacademy.gr

Funding Support: This work was supported by core funding from the Hellenic Ministry of Education, Research and Religious Affairs and the Hellenic Ministry of Health, and by the research grants PREDICTA (Contract No 260895) from the European Commission, MIDAS (Contract No MIS 377047) and RESOLVE-ASTHMA (Contract No 2601) from the Hellenic Ministry of Education, Research and Religious Affairs.

Capsule Summary

Dexamethasone induces the production of DHA-derived specialized proresolving lipids and the resolution of allergic airway inflammation. This adds glucocorticosteroids to the short list of drugs that are known to possess direct pro-resolving functions.

To the Editor

Resolution of inflammation has long been considered to be a passive process that ensues in an inflammatory response following the dilution of pro-inflammatory cues. However, this paradigm is shifting as it is becoming increasingly evident that resolution is an active reaction that employs a complex molecular machinery that orderly terminates inflammation. Among the multiple mechanisms involved, specialized proresolving lipid mediators (SPMs) such as resolvins, protectins and lipoxins have taken center stage[1]. These are derived from ω3 and ω6 polyunsaturated fatty acids through complex intra- and trans-cellular biosynthetic pathways, and promote resolution of inflammation by their manifold and concerted functions inhibiting leukocyte trafficking, dampening pro-inflammatory signaling, inducing apoptosis, and promoting anti-inflammatory M2-like macrophage phenotypes efferocytosis and tissue restitution[1].

Impaired induction of SPMs has been associated with defective resolution of inflammation and chronic diseases including asthma. Thus, reduced Lipoxin A4 (LXA4) levels have been detected in blood, bronchoalveolar lavage fluid (BAL), sputum and exhaled breath condensates of severe asthma patients, correlating with disease severity[2-4]. Moreover, impaired protectin D1 (PD1) production has been described in asthma patients compared to healthy individuals[5,6]. These observations are functionally relevant since administration of synthetic forms of SPMs in animal models of asthma have been notably effective in resolving inflammation[7]. Still, little is known about the effect of known drugs in the production of proresolving lipids, hampering the exploitation of this emerging new knowledge for therapy.

Here, we have investigated the role of dexamethasone (DXM), a ~~prototype~~ prototypical glucocorticosteroid, in the generation of SPMs and the resolution of inflammation. We employed an established model of allergic airway disease based on ovalbumin (OVA) sensitization and challenge (**Fig E1, A**) which triggers a Th2-driven inflammatory response in the airways peaking at day 1 post-challenge and gradually resolving thereafter (**Fig E1, B-D**)[7]. This is accompanied by the production of DHA-derived protectins such as PD1 and PDX, and their intermediate metabolite 17-HDHA, in the lung which starts at day 1 and peaks at day 4 post-challenge (**Fig E1, E**), in line with their proresolving activity. By contrast, vehicle control (PBS) challenge does not trigger any SPMs (**Fig E1, E**). Prostaglandins PGD2 and PGE2 which are required for the activation of SPM pathways[8] are also induced, while the EPA-derived metabolite RvE1 is not detectable (data not shown). The induction of SPMs is topical as none of these mediators is up-regulated in serum (**Fig E2**).

Using this setting, we administered DXM therapeutically, at days 1, 2 and 3 post-challenge, and assessed its effects (**Fig 1, A**). Surprisingly, we observed rapid and robust activation of the DHA•17-HDHA pathway within 6h of DXM administration (day 1 post-challenge), resulting in over ~~10~~3-fold higher concentrations of PD1 and PDX compared to naïve animals whereas vehicle (PBS)-treated controls exhibited no difference (**Fig 1, B**). This may even be an underestimation as in naïve and most vehicle-control treated animals PD1 and PDX are completely undetectable but an arbitrary value of half the limit of quantification was still used for fold calculations. RvE1 and LXA4 were not detected while PGD2 and PGE2, although present, were not differentially altered (data not shown). This early boost in SPM production was not anymore detectable 24h or 72h after DXM administration (day 2 or 4 post-challenge),

as levels in vehicle-control treated animals were also increased, while at 144h (day 7 post-challenge) higher, yet not significant, levels of PD1 and PDX were observed in the control group (**Fig 1, B**). These findings demonstrate that DXM acutely induces the mobilization of the DHA•17-HDHA•PD1/PDX pathway during allergic airway disease in mice.

We then examined whether the ability of DXM to up-regulate PD1 and PDX could also modulate resolution of allergic airway disease. DXM was unable to generate a notable anti-inflammatory or proresolving effect within 6h of treatment, a very short time interval (**Fig 1, C-D**). However, at 24h (day 2 post-challenge) and onwards DXM reduced total leukocyte and eosinophil numbers in the BAL more rapidly than vehicle control, and decreased OVA-specific IL-5 and IL-13 levels in mediastinal lymph nodes (MLNs). Accordingly, DXM shortened the resolution interval (R*i*), the time interval from the peak of inflammation at day 1 post-challenge to the 50% reduction point, by 2 days, a very significant effect for this model (**Fig 1, E-F**). To confirm that DXM accelerates the resolution of inflammation through the induction of DHA-derived SPMs, rather than other effects on the immune system, we employed 12,15-lipoxygenase deficient (*Alox15*$^{-/-}$) mice which cannot metabolize DHA to downstream bioactive mediators such as protectins (**Fig 2, A-B**). Abrogation of PD1 and PDX generation completely reversed DXM-mediated resolution of allergic airway inflammation including total leukocyte and eosinophil infiltration in the BAL, and Th2 cytokine production in MLNs (**Fig 2, C-D and E3, A-B**). Although this does not exclude the involvement of other bioactive lipids requiring 12,15-lipoxygenase for their generation, the potent proresolving activity of syntheric PD1 administration in similar

settings[5, 7] indicates that at least PD1 and its functionally similar isomer PDX are principally involved.

In conclusion, our study describes a novel function of DXM, a ~~prototype~~ prototypical glucocorticosteroid, in the activation of SPM networks. It reveals that DXM enhances the ~~catabolism~~ metabolism of DHA to its bioactive derivatives PD1 and PDX. This effect is rapid, inducing at ~~10~~3-fold higher levels than ~~background~~ vehicle within 6h of treatment, providing an early boost to the process of resolution, markedly reducing both the duration and magnitude of inflammation. This effect is also transient as no differences between DXM and vehicle-treated animals were observed later on, possibly reflecting the induction of counter-regulatory pathways suppressing SPM networks, and lack of need for additional SPMs in an effectively resolving situation. Notably, the effect of DXM is specific for the D-series SPMs since there was no observed mobilization of other proresolving pathways such as lipoxin A4 or E-series resolvins nor PGD2 and PGE2.

At present, very few pharmaceutical substances have a documented ability to activate SPM networks. Aspirin and statins provoke conformational changes on cyclooxygenases altering their enzymatic activity and leading to the production of epimeric forms of lipoxins, resolvins and protectins, depending on the substrate[9]. Resiquimod, a TLR7/8 agonist, induces in turn DHA-derived SPMs in human macrophages and experimental settings of airway disease[7]. Synthetic forms of SPMs such as RvE1 and LXA4 are also being explored although their short half-life and chemical stability constitute major hurdles towards their clinical application. Our study therefore adds DXM as a new agonist of resolution, expanding the short list of clinically used drugs that are able to actively induce SPM pathways. It also provides

novel insight into the mechanism of action of DXM, a well-known and widely-used drug, with important implications for glucocorticosteroid use in the clinic.

We acknowledge support of Ms M. I. Saridaki for technical assistance and Prof. Nikos Papadopoulos and Charles Serhan for advice.

Katerina Pyrillou, PhD,[a,b] Aikaterini-Dimitra Chairakaki, PhD,[a] Constantin Tamvakopoulos, PhD,[b,†] and Evangelos Andreakos, PhD[a,†]

From [a]Laboratory of Immunobiology, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece; [b]Division of Pharmacology-Pharmacotechnology, Center for Clinical, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece. E.mail: vandreakos@bioacademy.gr

[†]Evangelos Andreakos and Constantin Tamvakopoulos contributed equally to this work

Author contributions: KP, CT and EA conceived and designed the experiments; KP and ADC performed the experiments; KP, ADC, CT and EA analyzed and interpreted the data; KP and EA wrote the paper.

Funding Support: This work was supported by core funding from the Hellenic Ministry of Education, Research and Religious Affairs and the Hellenic Ministry of Health, and by the research grants PREDICTA (Contract No 260895) from the European Commission, MIDAS (Contract No MIS 377047) and RESOLVE-ASTHMA (Contract No 2601) from the Hellenic Ministry of Education, Research and Religious Affairs.

Disclosure of potential conflict of interest: The authors declare no conflict of interest.

References

1. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014; 510:92-101.
2. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E, et al. Diminished lipoxin biosynthesis in severe asthma. Am J Respir Crit Care Med 2005; 172:824-30.
3. Kazani S, Planaguma A, Ono E, Bonini M, Zahid M, Marigowda G, et al. Exhaled breath condensate eicosanoid levels associate with asthma and its severity. J Allergy Clin Immunol 2013; 132:547-53.
4. Vachier I, Bonnans C, Chavis C, Farce M, Godard P, Bousquet J, et al. Severe asthma is associated with a loss of LXA4, an endogenous anti-inflammatory compound. J Allergy Clin Immunol 2005; 115:55-60.

5. Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, et al. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. J Immunol 2007; 178:496-502.
6. Miyata J, Fukunaga K, Iwamoto R, Isobe Y, Niimi K, Takamiya R, et al. Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma. J Allergy Clin Immunol 2013; 131:353-60 e1-2.
7. Koltsida O, Karamnov S, Pyrillou K, Vickery T, Chairakaki AD, Tamvakopoulos C, et al. Toll-like receptor 7 stimulates production of specialized pro-resolving lipid mediators and promotes resolution of airway inflammation. EMBO Mol Med 2013; 5:762-75.
8. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol 2001; 2:612-9.
9. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res 2010; 107:1170-84.

Keywords

Allergic asthma, allergic airway disease, anti-inflammation, resolution of inflammation, glucocorticosteroids, dexamethasone, protectins, Alox15

Abbreviations

SPMs = specialized pro-resolving lipid mediators, DHA = docosahexaenoic acid, PUFAs = polyunsaturated fatty acids, PD1 = protectin D1, PDX = protectin DX, LM = lipid mediators, LXA4 = lipoxin A4, LTB4 = leukotriene B4, BAL = bronchoalveolar lavage fluid, LOX = lipoxygenases, COX = cyclooxygenases, NSAIDs = non-steroidal anti-inflammatory drugs, PGs = prostaglandins, LTs = leukotrienes, GCs = glucocorticosteroids, GR = glucocorticoid receptor, DXM = dexamethasone, OVA = ovalbumin, EXC4 = eoxin C4, RvE1 = resolvin E1, RvD1 = resolvin D1, 17-HDHA = 17-hydroxy-docosahexaenoic acid, 7-HDHA = 7-hydroxy-docosahexaenoic acid, EPA = eicosapentaenoic acid, LC-MS/MS = liquid chromatography tandem mass spectrometry, R*i* = resolution interval, IL = interleukin

Figure Legends

Figure 1. Effect of DXM on the biosynthesis of DHA-derived proresolving lipid mediators and the resolution of allergic airway inflammation. (**A**) Schematic of the experimental protocol. DXM treatment is depicted in red. (**B**) Tissue lipidomic profiling over a time-course of 7 days post-challenge showing early activation of the DHA-17-HDHA-PD1/PDX pathway triggered by DXM. Data are expressed as fold induction relative to naïve non-inflamed mice. (**C**) Total leukocyte, eosinophil and lymphocyte numbers in BAL. (**D**) T_H2 cytokine responses in mediastinal lymph nodes. (**E**) Resolution curves for total leukocyte and eosinophil numbers in BAL. (**F**) Calculated resolution intervals (R*i*) based on total leukocyte and eosinophil resolution curves. Data represent mean±SEM of n=9-23 mice/group pooled from at least three independent experiments. In fold calculations half the limit of quantification (LOQ) was used for samples beyond detection. ***P<0.001, **P<0.01 and *P<0.05 compared to PBS-treated controls.

Figure 2. Dependence of DXM pro-resolving capacity on the activation of the DHA-17-HDHA-PD1/PDX pathway. (**A**) Schematic of the experimental protocol employing wild type (WT) and Alox15-deficient (*Alox15*$^{-/-}$) mice depicted in red. Data are expressed as fold induction relative to naïve WT mice. (**B**) Tissue lipidomics analysis ~~in~~ of non-treated *Alox15*$^{-/-}$ mice compared to WT mice. (**C**) Effect of DXM administration on total leukocyte, eosinophil and lymphocyte numbers in BAL at day 4 post-challenge. (**D**) Effect of DXM administration on T_H2 cell responses at day 4 post-challenge. Data represent mean±SEM of n=6-23 mice/group pooled from at least three independent

experiments. ~~BLQ: Below the Limit of Quantification.~~ ***P<0.001, **P<0.01 and *P<0.05 compared to WT mice.