

Results Sample 1

Hultqvist M, Olofsson P, Gelderman KA, Holmberg J, Holmdahl R (2006) A New Arthritis Therapy with Oxidative Burst Inducers. PLoS Med 3(9): e348

Problem: Rheumatoid arthritis

Purpose of study: In this study we used rats as an experimental model of RA to identify compounds that increase oxidative burst capacity in vivo and investigate whether these substances thereby could have a therapeutic effect on arthritis.

Results

Oxidative Burst-Inducing Agents Prevent Arthritis

Since low oxidative burst capacity increased arthritis susceptibility [7], we proposed that substances elevating ROS production could have therapeutic effects on arthritis. We had already seen that certain oils with an alkane structure, such as phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and pristane (2,6,10,14-tetramethylpentadecane), had an oxidative burst-inducing capacity in vitro. Additionally, despite structural similarity, pristane induced arthritis, whereas phytol protected against it [7]. **To examine the structure/function relationship**, we screened a number of short saturated alkanes (C8–C17) using an isoluminol-based oxidative burst assay [14] on human promyelocytes (HL-60) differentiated to granulocytes in the presence of DMSO. Alkanes 11–13 carbons long were the most potent in activating the NADPH oxidase complex (Figure 1A). **To investigate their arthritogenic potential**, we injected the alkanes into arthritis-susceptible DA rats carrying the DA *Ncf1* allele (*Ncf1*^{DA}). Alkanes with 15 carbons or more, such as pristane, induced arthritis, whereas shorter alkanes did not (Figure 1B). We therefore concluded that the arthritis-inducing effect of these oils is independent of their oxidative burst-inducing capacity.

Next we wanted to see **if oxidative burst-inducing effect correlated with arthritis prevention** using PIA [20] as a model of human RA. C11, shown to induce oxidative burst in vitro, was potent in reducing arthritis severity when

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Comment: Background justifying the question. Includes references.

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Comment: Purpose of the experiment (question)

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Comment: Reference to a protocol (same as in methods)

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Comment: Summary of experimental approach/method

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Comment: Observations. Note the reference to figures, and the summary of the data.

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Comment: Concluding statement.

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Comment: Linking one experiment to another. Chronological link.

administered 5 d before arthritis induction (Figure 1C). Similar results were also seen with C12 and C13 (unpublished data). In contrast, C16, which was arthritogenic and did not increase oxidative burst, did not protect against arthritis (Figure 1C). These results clearly demonstrate that small changes in structure determine the effect of the molecule. Since phytol was superior in inducing oxidative burst in vitro as well as in preventing arthritis when injected before induction of disease (Figure 1A and 1D), it was chosen for further studies of efficacy.

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Comment: Link between this result and the following studies. Logical link.

We then investigated the dependency of arthritis-preventing effects on route of administration. When phytol was administered SC, IP, or intranasally 5 d before and after PIA induction it was evident that SC administration resulted in the best preventive effect (Figure 1E).

Phytol Increases Oxidative Burst Capacity In Vivo

We also wanted to know whether phytol has the same oxidative burst-inducing effect in vivo. In particular, we asked whether phytol would correct the low oxidative burst capacity seen in rats carrying the *Ncf1*^{DA} allele. We analyzed spleen granulocytes 5 d after phytol injection for intracellular oxidative burst response to PMA stimulation in vitro. Phytol clearly increased the oxidative burst capacity in DA.*Ncf1*^{DA} rats to the level of the DA.*Ncf1*^{E3} congenic rats carrying the arthritis-protective E3 *Ncf1* allele (Figure 2A). Serum levels of malondialdehyde, the main product of lipid peroxidation, were also increased after phytol injection (Figure 2B), reflecting a higher level of oxidation in vivo.

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Comment: Refinement of question by describing methodology.

Since a single phytol injection mediated a surprisingly long suppression of arthritis (Figure 1D) we investigated whether this protective effect correlated with a sustained increase in oxidative burst capacity. Thus, we followed the oxidative burst response to PMA in blood granulocytes for 7 wk after injection of either pristane or phytol. In order to avoid intraexperiment variations, results were normalized to a naïve standard group. An increase in oxidative

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burst capacity was evident the day after phytol (but not after pristane injection) and persisted for several weeks (Figure 2C). During development of arthritis the PMA response of pristane-injected rats increased compared to naïve rats, possibly reflecting a systemic response to the local inflammation; this finding agrees with reports of oxygen radicals in synovial neutrophils in human RA [21].

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One possible explanation for the long-lasting effect of phytol could be that the phenotype of cell populations with longer lifespans than those of neutrophilic granulocytes, such as stem cells in BM, is altered. To address this hypothesis we analyzed BM cells from DA.Ncf1^{DA} rats 3 and 24 h after phytol. No effect of injection was seen after 3 h, but 24 h after administration of phytol the oxidative burst capacity was elevated up to the level of the protected DA.Ncf1^{E3} rats (Figure 2D).

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Comment: Speculation justifying the next experiment.

Increased Oxidative Burst Alters Cell Distribution

Next we investigated the effect of phytol in vivo in blood, spleen, and draining LNs. ...

Phytol Suppresses CIA and CII Autoimmunity

Since PIA is a model with a unique pathogenesis involving $\alpha\beta$ T cells, operating in the effector phase as well [20,22], we analyzed whether phytol operates also in CIA [23], a model in which antibodies are important in the joint inflammatory attack [24]. A significant decrease in arthritis severity comparable to the effect on PIA was observed after preventive administration of phytol also in CIA (Figure 4A). ...

Phytol Inhibits Arthritis Induced without Adjuvant Oil

So far, arthritis was induced with an oil component such as pristane or IFA in the CII immunization. To exclude a role of oil in the induction of disease we

developed a new model, NOCIA. CII was emulsified in a mixture of nonoil adjuvants, such as alum, lipopolysaccharide, and a bacterial DNA sequence (CpG) and injected SC. Phytol injection before immunization completely blocked the development of disease (Figure 4D). In addition, when the immune system was challenged at the end of experiment (day 67 after NOCIA induction) by a CII injection in the ear, we found that phytol totally inhibited the DTH response (Figure 4E).