Fullerene Nanomaterials Inhibit Phorbol Myristate Acetate-induced Inflammation

By: Anthony Dellinger, Zhiguo Zhou, Robert Lenk, Darren MacFarland, Christopher L. Kepley

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Abstract:

Inflammation is a natural biological response that occurs when vascular tissues are subjected to harmful stimuli. This process may be beneficial to the host during wound healing and infections but can be detrimental if left unchecked. Oxidative stress, the generation of reactive oxygen species, is thought to be one component of this response. Fullerenes can counteract reactive oxygen species due to their potent antioxidant capabilities. Thus, we hypothesized that these molecules may inhibit inflammation. To test this hypothesis we used an *in vivo* model of phorbol 12-myristate 13-acetate (PMA)-induced inflammation and examined the effects fullerenes have on mitigating this response. We show that PMA-induced inflammation and oedema is dramatically inhibited when fullerenes are given prior to challenge. Thus, fullerene derivatives may be a novel way to blunt certain inflammatory conditions and facilitate faster recovery of damaged tissue.

Keywords: fullerene | inflammation | oxidative stress

Article:

Background

The inflammatory process is essential for survival, playing an important role in both health and disease. However, if left unchecked this inflammatory processes may contribute to a wide variety of human disease processes including the more traditionally defined chronic inflammatory diseases, such as allergy, arthritis and inflammatory bowel disease as well as systemic cardiovascular diseases (atherosclerosis) and blood vessel disorders (circulatory shock). The mechanisms leading from the initial injury involves an intricate series of events including oedema, oxidative stress, immune cell recruitment, proliferation, migration and differentiation. Inappropriate inflammation is usually treated with certain steroids such as glucocorticoids or with non-steroidal anti-inflammatory drugs. Both classes of compounds can have undesirable side effects (1,2). Discovering new ways to treat inflammation is of clinical importance.

Questions addressed

Fullerenes have unique biological capabilities and are being investigated as possible therapies for a wide range of diseases (3–7). We recently demonstrated that derivatized fullerenes can inhibit allergic inflammation *in vitro* and *in vivo* in part through the reduction in reactive oxygen species (ROS) (8). These studies strongly suggest that fullerenes are capable of targeting mitochondria, as others have shown in various cell lines (9–12), where they can affect cellular functions. However, the lack of suitable pharmacological formulations of fullerenes has largely prevented their development into therapies. Given their wide ranging potential as a new therapeutic platform we hypothesized that derivatized fullerene compounds could inhibit the inflammatory cascade associated with phorbol 12-myristate 13-acetate (PMA)-induced inflammation. A well-established mouse model of inflammation was used to show that fullerenes can significantly reduce the inflammatory events that lead to cutaneous inflammation.

Experimental design

All mice (8-week-old males) were purchased from Charles River Labs, Wilmington, MA, USA and humanely treated under standard conditions and with IRB approval. Fullerene derivatives were synthesized by adding various molecular entities to the carbon cage. A representative fullerene used herein is shown in Fig. 1. Each derivative was purified and characterized using matrix-assisted laser desorption ionization mass spectrometry, nuclear magnetic resonance and high performance liquid chromatography. A description of the synthesis and characterization is described elsewhere (manuscript in preparation).

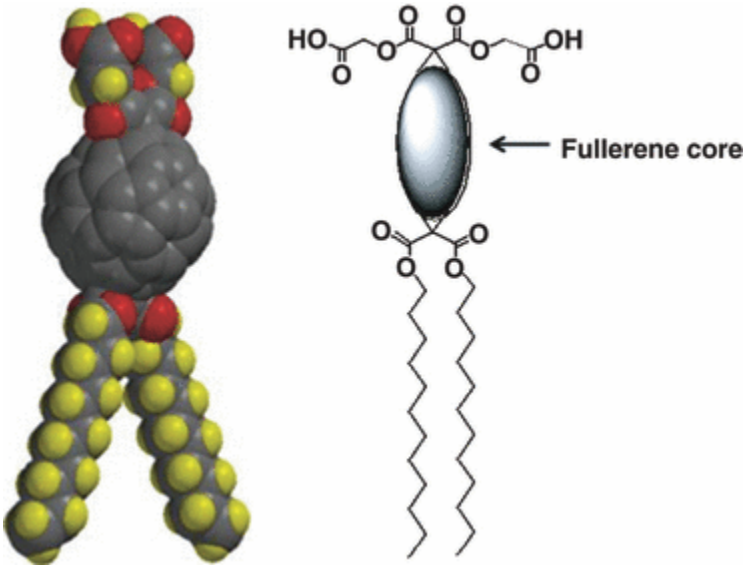

Figure 1. Representative fullerene derivative.

PMA-induced mouse ear oedema model of inflammation

Phorbol 12-myristate 13-acetate (Sigma) is a lipophilic, long-acting stimulant of protein kinase C (PKC) and elicits a well-characterized delayed-type hypersensitivity reaction (13,14). To induce an inflammatory response we used a mouse model of PMA-induced inflammation (15–18). For control mice, phosphate-buffered saline (PBS, 20 μl/ear) was injected subcutaneously into the left ear approximately 5 min prior to injection with PMA (20 μl of 0.01% w/v per ear in acetone). For treated mice, fullerenes in PBS (2 μg/20 μl; 667 μM) were injected subcutaneously into the left ear approximately 5 min prior to injection with PMA. The right ear of each mouse was not challenged and served as a control to measure differences in the weights of biopsies. After 5.5 h, the extent of inflammation was photo-documented and the swelling quantified by weighing two separate, 3-mm biopsies obtained at different parts of the left ear (fullerene treated ± PMA or non-treated ± PMA) and right ear (non-treated) and calculating the differences in weights. Thus, the more the mouse ear weighed the greater the inflammation and oedema.

Results

We tested fullerene derivatives for their ability to prevent the acute irritation in a mouse model of irritant dermatitis. As seen in Fig. 2a and b, the vehicle-treated ears that received only PBS before PMA exhibited considerable redness and swelling as indicated by the arrows. However, when fullerenes were injected into the ear prior to PMA challenge there was a noticeable inhibition of this response (Fig. 2c and d). Quantification of the ear swelling is shown in Fig. 2e as the average change in weight in the left ear of treated and non-treated mice compared with the right ear of each. In the PBS ± PMA mice the average increase in ear weight was 0.015 g (± SD: 0.01) while the fullerene ± PMA mice had an average increase of 0.002 g (±SD: 0.004) indicating that the inflammatory cascade leading to oedema was inhibited when fullerenes were used prior to PMA challenge.

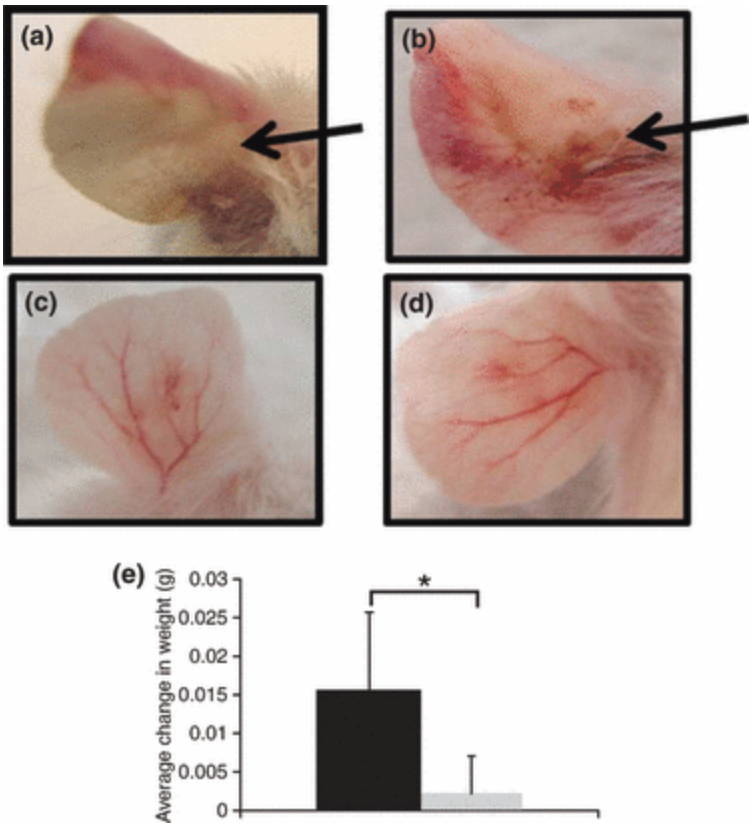

Figure 2. Fullerenes inhibit phorbol 12-myristate 13-acetate (PMA)-induced inflammation. The left ears of control mice treated with phosphate-buffered saline (PBS) ± PMA (a,b) or mice treated with fullerene ± PMA (c,d) were photo-documented approximately 5.5 h after PMA challenge. In the embedded graph (e) mouse biopsies (two per ear) obtained from the left ear of fullerene treated (■) and non-treated (▪) mice (±PMA) were weighed and the average compared with the weights of biopsies (two per ear) of non-treated right ears (no PBS, fullerene, or PMA). The difference in the average weights (±SD) in grams is shown (n = 4/group). *Indicates statistical significance (P = 0.025 using t-test).

Discussion

Inflammation occurs naturally within the body and serves a useful purpose in the defense against microbes and damaged tissues. Though inflammation is protective in some situations if left unchecked or during disease processes this inflammation can lead to serious complications. The inflammatory response in general occurs in three distinct phases. Initially, increased vascular permeability results in oedema followed by the second phase of leucocytes infiltration. Lastly, granuloma formation and tissue repair occur. In this study we demonstrated that fullerenes blocked the acute inflammatory mechanisms that led to oedema and leucocyte infiltration.

It is not clear how the fullerene derivatives inhibited this response. However, we and others have demonstrated certain mechanisms of fullerenes which may be relevant here. First, the release of

histamine and prestored tumor necrosis factor-α from cutaneous mast cells was one well-characterized component of inflammation (19). We demonstrated that preincubation of mast cells with fullerenes significantly inhibited the release of inflammatory mediators *in vitro* and *in vivo* (8). Second, oxidative stress through the generation of ROS plays a key role in inflammatory responses. It has been well documented that PMA-induced PKC activation leads to elevated ROS levels in disease-related inflammation (20–22). Given that fullerenes have previously been shown to be very potent ROS-inhibiting antioxidants (23) the fullerene derivatives described here may inhibit inflammation through blunting ROS and subsequent ROS-dependent pathways. We are currently focusing on these two theories to determine how fullerenes inhibit PMA-induced inflammation *in vivo*.

In conclusion, we showed that PMA-induced activation of the inflammatory cascade(s) leading to cutaneous inflammation was blocked by certain fullerene derivatives. Such a therapeutic intervention that targets these inflammation precursors and blocking their effects may open new avenues for controlling inflammation and block diseases associated with unchecked inflammation.

Acknowledgements

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