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D-pinitol inhibits Th1 polarization *via* the suppression of dendritic cells

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Abstract

D-pinitol has been demonstrated to exert insulin-like and anti-inflammatory effects. However, the effects of the maturation and immunostimulatory functions of dendritic cells (DC) remain to be clearly elucidated. In this study, we have attempted to determine whether D-pinitol regulates surface molecule expression, cytokine production, endocytosis capacity, and underlying signaling pathways in murine bone marrow-derived DC. We also attempted to ascertain whether D-pinitol could influence Th1/Th2 immune response *in vivo*. The DC used in this study were derived from murine bone marrow cells, and were used as immature or LPS-stimulated mature DC. The DC were then assessed with regard to surface molecule expression, dextran-FITC uptake, cytokine production, capacity to induce T-cell differentiation, and underlying signaling pathways. D-pinitol was shown to significantly inhibit CD80, CD86, MHC class I, and MHC class II expression in the LPS-stimulated mature DC. The DC also evidenced impaired IL-12 expression and IFN-γ production. The D-pinitol-treated DC were found to be highly efficient in regards to Ag capture *via* mannose receptor-mediated endocytosis. D-pinitol was also demonstrated to inhibit LPS-induced MAPKs activation and NF-κB nuclear translocation. Moreover, the D-pinitol-treated DC manifested impaired induction of Th1 responses, and normal cell-mediated immune responses. These novel findings provide new insight into the immunopharmacological role of D-pinitol in terms of its effects on DC. These findings also broaden current perspectives concerning our understanding of the immunopharmacological functions of D-pinitol, and have ramifications for the development of therapeutic adjuvants for the treatment of DC-related acute and chronic diseases.

Keywords: D-pinitol; Dendritic cell; Th1 polarization; IFN-γ; NF-κB; IL-12

Abbreviations: DC, dendritic cells; MAPKs, mitogen-activated protein kinases; BM, bone marrow; APC, antigen-presenting cells; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; TNCB, 2,4,6-trinitrochlorobenzene; CHS, contact hypersensitivity.

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1. Introduction

A substantial body of research is currently focused on the biology of dendritic cells (DC), specifically with regard to their possible clinical use as cellular adjuvants in the treatment of chronic infectious diseases and tumors. DC are professional antigen-presenting cells (APC) that have been determined to possess immune sentinel properties, initiating T-cell responses against microbial pathogens and tumors [1]. Immature DC capture and process exogenous agents within peripheral tissues, in which they begin to mature. Maturing DC migrate into the lymphoid organs, where they stimulate naive T cells *via* the signaling of both major antigen-peptide-presenting histocompatibility complex (MHC) molecules and co-stimulatory molecules [2,3]. Maturation and differentiation are generally understood to require the activation phosphorylation of mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), p38 MAPK, and the transcription nuclear factor kappa B (NF-κB) [4,5]. DC have also been demonstrated to be highly responsive to both inflammatory cytokines and bacterial products, such as TNF-α and lipopolysaccharides (LPS). When encountered in the peripheral organs, these products are known to induce a series of phenotypic and functional alterations in the DC. Similar changes, indicative of maturation, have also been reported after the infection of cells with mycoplasma, viruses, intracellular bacteria, and parasites [7]. DC located within the peripheral tissues generally tend to be both phenotypically and functionally immature. Immature DC do not induce primary immune responses, as they do not express the requisite costimulatory molecules. In addition, they don't express antigenic peptides as stable complexes with MHC molecules. While in an immature state, DC have been shown to effectively capture and process exogenous antigens (Ag) within the peripheral tissues, where the DC then begin to mature. DC maturation has previously been associated with decreased or absent Ag uptake, high levels of MHC class II and accessory molecule expression, as well as IL-12 generation upon stimulation [6].

The flavonoids comprise a family of common phenolic plant pigments, which have been identified as dietary anticarcinogens and antioxidants [7]. We reported previously that a variety of phytochemicals exhibit profound immunoregulatory activity, particularly in DC [8–11]. D-pinitol (3-*O*-methyl-chiroinositol), an active principle of the traditional antidiabetic plant, *Bougainvillea spectabilis*, reportedly exerts insulin-like effects [12]. Pinitol is also known to exert insulin-like effects, *via* the driving of creatine and other nutrients into muscle cells [12]. D-pinitol has been suggested to possess multifunctional properties, including anti-inflammatory properties [13]. In addition, it has also been implicated in the prevention of cardiovascular diseases [14]. Until now, the cellular targets of D-pinitol in the immune system have remained enigmatic, thereby leaving the issue of the global function of D-pinitol in relation to DC maturation and immuno-regulatory activities open to discussion in terms of future research.

In this study, we have attempted to characterize the effects of a noncytotoxic concentration of D-pinitol on the maturational and functional properties of murine bone marrow (BM)-derived DC. Our findings demonstrated, for the first time, that D-pinitol treatment inhibited phenotypic and functional maturation in the DC. D-pinitol treatment also suppressed the LPS-induced activation of ERK1/2, JNK, and p38 MAPK, as well as NF-κB nuclear translocation, in the DC. The *in vivo* data indicate that, although the D-pinitol-treated DC were observed to migrate to the T-cell areas of secondary lymphoid tissue, they did not induce normal cell-mediated contact hypersensitivity (CHS). Moreover, this readily available drug may constitute a simple, inexpensive, and highly effective means for the manipulation of the immunostimulatory capacity of DC. Considering, then, the critical functions of these professional APC in the initiation and regulation of immune responses, coupled with the ready availability of D-pinitol, our findings appear to bear important implications with regard to the manipulation of the functions of DC in potential therapeutic applications.

2. Materials and methods

2.1. Animals and chemicals

Male 8–12-week-old C57BL/6 (H-2K^b and I-A^b) and BALB/c (H-2K^d and I-A^d) mice were purchased from the Korean Institute of Chemistry Technology (Daejeon, Korea). They were housed in a specific pathogen-free environment within our animal facility for at least 1 week before use. D-pinitol was purchased from Sigma.

2.2. Reagents and Abs

Recombinant mouse (rm)GM-CSF and rmIL-4 were purchased from R&D Systems. D-pinitol, dextran-FITC (molecular mass, 40,000), and LPS (from *Escherichia coli* 055:B5) were obtained from Sigma-Aldrich. An endotoxin filter (END-X) and an endotoxin removal resin (END-X B15) were acquired from Associates of Cape Cod. Cytokine ELISA kits for murine IL-12 p70, IL-4, and IFN-γ were purchased from BD Pharmingen. FITC-conjugated or PE-conjugated mAbs used to detect the