Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/etap

Systemic immunomodulatory effects of topical dinitrochlorobenzene (DNCB) in rats. Activity of peripheral blood polymorphonuclear cells

Sandra Belij[a,1], *Aleksandra Popov*[a,1], *Lidija Zolotarevski*[b], *Ivana Mirkov*[a], *Jelena Djokic*[a], *Dragan Kataranovski*[a,c], *Milena Kataranovski*[a,d,*]

[a] *Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia*

[b] *Institute for Pathology, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia*

[c] *Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

[d] *Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form
7 November 2011
Accepted 23 November 2011
Available online 2 December 2011

Keywords:
Rats
DNCB
Skin sensitization
Peripheral blood
ploymorphonuclears

ABSTRACT

Topical application of dinitrochlorobenzene (DNCB) is employed in the immunotherapy of skin diseases. Activation of T-cell mediated immune responses (Th1/type1) is the supposed mechanism of the clinical effect of DNCB, but there are no data concerning innate/inflammatory mechanisms. In this study, the effect of repeated topical DNCB application on peripheral blood polymorphonuclear (PMN) leukocytes has been examined in two rat strains which differ in the propensity to mount Th1/type1 or Th2/type2 responses. The dynamics of changes in PMN numbers and effector activities (respiratory burst, nitric oxide production and myeloperoxidase content), as well as in adhesion and TNF-α production following the rat skin sensitization with low (0.4%) and high (4%) DNCB doses were measured. Both priming and activation of PMNs were observed following skin sensitization with DNCB, with dose-dependent as well as time-dependent differences in some PMN activities. Obtained data might be relevant for understanding the immune mechanisms of topical DNCB therapy.

1. Introduction

Skin sensitizers are chemicals with an intrinsic ability to induce contact allergy. Contact allergens are often low molecular weight substances that can act as haptens, as they can induce an immune response only after binding to tissue macromolecules. The majority of haptens are chemicals whose lipophilicity facilitates their passage through the corneal layer and their electrophilic protein-reactivity leads to skin protein binding (Divkovic et al., 2005). Once the proteins are haptenated, a skin and skin-associated lymphoid tissue immune response results. Previous experimental contact hypersensitivity (CHS) reactions in animal models using strong haptens ("experimental haptens") such are dinitrohalobenzenes, have demonstrated that two discrete phases are necessary to achieve an optimal CHS reaction. The sensitization phase starts with the topical application of hapten

* *Corresponding author at*: Department of Ecology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia. Tel.: +381 11 2078375; fax: +381 11 2761433.
E-mail address: milena@ibiss.bg.ac.rs (M. Kataranovski).

[1] Both authors contributed equally to the study.

1382-6689/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.etap.2011.11.006

to the animal's abdominal or dorsal area. Antigen-presenting cells in the skin take up haptenated proteins and migrate to skin-draining lymph nodes, where presentation of the haptenated peptides to hapten-specific $CD4^+$ and $CD8^+$ T cells occurs. This stimulates their activation and proliferation (Bour et al., 1995). Primed specific T cells then leave lymph nodes and enter the circulation. The expression of contact hypersensitivity occurs following application of the hapten to the skin of sensitized animals several days later. It is characterized by infiltration of hapten-primed Th1/type1 T cells in the epidermis and dermis (Engeman et al., 2004). Effector activities of hapten-induced immune response contribute to the clinical expression of allergic contact dermatitis (ACD).

The immunostimulatory potential of some contact sensitizers was employed as topical immunotherapy for some skin dermatoses including alopecia areata and viral warts (Buckley and Du Vivier, 2001; Rokhsar et al., 1998). From 1965, when the first topical sensitizer tri-ethyleneiminobenzoquinone (TEIB or Trenimon) was used to treat cutaneous diseases (Helm et al., 1965) several contact sensitizers were employed in the treatment of these conditions including urushiol (from poison ivy), nickel, formalin and primin, although they were non-specific agents as they occur in the environment. Other substances not encountered in the environment such as dinitrochlorobenzene (DNCB), squaric acid dibutyl ester (SADBE) and diphenylcyclopropenone (DPCP) superseded the use of the early contact sensitizers. Among these latter agents, DNCB was used as topical immunotherapeutic for more than thirty years in the treatment of benign dermatoses and in malignant cutaneous disorders (reviewed in Buckley and Du Vivier, 2001). However, the mutagenicity *in vitro* (Ames *Salmonella tiphymurium* test) of DNCB suggested that this agent was potentially too hazardous for topically usage as an immunomodulator (Happle, 1985) and its use declined, though no carcinogenicity was noted in treated patients. Despite such reservations, topical DNCB therapy is still employed in the treatment of alopecia areata (Mohan et al., 2008) and some cutaneous malignancies (Herrmann et al., 2004; von Nida and Quirk, 2003). Because it is efficacious and inexpensive, it is recommended as a rational and effective option in the treatment of chronic resistant alopecia areata (Mohan et al., 2008).

Beside local effects, topical DNCB therapy has been proposed as a systemic immunotherapy for immunodeficiency (HIV) disease (Stricker et al., 1993) and lupus erythematosus (Stricker et al., 1995). The efficacy of repeated topical DNCB application was shown in HIV patients (Stricker et al., 1997; Traub et al., 1997), chronic prurigo (Yoshizawa et al., 1999) and refractory atopic dermatitis (Yoshizawa et al., 2000). Increase in peripheral blood T cell counts and stimulation of proinflammatory T helper type 1 (Th1) response are supposed underlying mechanisms of beneficial effects of topical DNCB therapy in these patients. Data from animal contact hypersensitivity studies revealed systemic Th1 response as judged on serum hapten-specific IgG2a (Dearman and Kimber, 1991) and IgG antibodies (Kuper et al., 2008) following DNCB sensitization of mice and rats, respectively. Data obtained with other haptens showed increases in plasma levels of interleukin-6 (IL-6) and acute phase reactants (haptoglobin and serum amyloid A) in serum of hapten-challenged mice (Kimber et al., 1989, 1990) implying association of skin sensitization with systemic inflammation.

Our data showed the priming effect of repeated (for two consecutive days) skin sensitization with DNCB on peripheral blood PMNs respiratory burst and adhesion in rats one day post repeated application of high doses (2% and 4%) of DNCB (Kataranovski et al., 2001) which indicated involvement of a cellular component of systemic inflammation in contact sensitization with this hapten. Topical application of DNCB was associated with PMN migration to the lungs of sensitized rats (Belij et al., 2011). These data signified the relevance of the engagement of this peripheral blood cell compartment in contact sensitivity to DNCB. Various aspects of the activity of peripheral blood PMN cells during contact sensitivity to DNCB were measured in addition to respiratory burst and adhesion, including nitric oxide (NO) production and myeloperoxidase (MPO) cell content, as well as tumour necrosis factor (TNF)-α production, as activities relevant for proinflammatory function of these cells. The PMN activity of animals sensitized with low (0.4%) and high (4%) DNCB doses was evaluated and measurements were conducted one, three and five days following DNCB application, as sensitization phase of experimental contact sensitivity usually lasts 5 days in mice (Saint-Mezard et al., 2004) and rats (Popov et al., 2011). Having in mind that CHS is considered as Th1/type1 reaction (Saint-Mezard et al., 2004) PMN activity was measured in T_H1-prone Dark Agouti (DA) rats and compared to their activity in T_H2-prone Albino Oxford (AO) rats. Time-dependent priming and activating effect of skin sensitization with DNCB on peripheral blood PMNs were noted, with strain differences in some of the aspects of their activity. Presented data might be of relevance for understanding the mechanisms of topical DNCB immunotherapy.

2. Materials and methods

2.1. Chemicals

1-Chloro-2,4-dinitrobenzene (DNCB) was obtained from BDH Chemicals Ltd., UK and dissolved in 4:1 acetone:olive oil (vehicle). Phorbol-12-myristate-13-acetate (PMA), lipopolysaccharide (LPS) from *E. coli* 0111:B4, N-(1-naphthyl)ethylenediamine dihydrochloride, sulfanilamide (p-aminobenzenesulfonoamide), hexadecyltrimethylammonium bromide (HTAB), o-dianisidine dihydrochloride, myeloperoxidase (MPO) and aminoguanidine (bicarbonate salt) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), nitroblue tetrazolium (NBT) from ICN Pharmaceutical (Costa Mesa, CA, USA), sodium nitrite from Fluka Chemika (Switzerland) and hydrogen peroxide (H_2O_2) from Zorka Farma, Sabac (Serbia). mAb OX-42 (mouse anti-rat CD11b/CD11c, IgG2a) and FITC-conjugated $F(ab')_2$ goat anti-mouse IgG were purchased from Serotec Ltd., Bicester, UK.

PMA was dissolved in dimethylsulfoxide (DMSO) at 1 mg/ml and diluted before the use in cell culture medium. Myeloperoxidase and nitroblue tetrazolium were dissolved in water. All solutions for cell culture experiments were either prepared under sterile conditions or were sterile filtered (Flowpore, pore size 0.22 μm) before the use. Culture medium RPMI-1640 (PAA