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Exposure of mice to the nitroso metabolite of sulfamethoxazole stimulates interleukin 5 production by $CD4^+$ T-cells

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Abstract

Sulfamethoxazole hypersensitivity may be caused by production of the protein-reactive metabolite nitroso sulfamethoxazole (SMX-NO) and interaction of SMX-NO with T-cells. We have characterised the nature of the immune response induced by administration of sulfamethoxazole, sulfamethoxazole metabolites and nitrosobenzene to BALB/c mice. Drugs were administered over a 13-day period to induce polarised cytokine secretion profiles. Proliferation was measured by [^{3}H] thymidine incorporation. Cytokine secretion was monitored by ELISA. Results were compared with those provoked by exposure to type 1 and type 2 chemical allergens, 2,4-dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA). $CD4^+$ or $CD8^+$ T-cells were depleted ex vivo to identify the primary source of cytokines. Lymph node activation was observed following treatment with DNCB, TMA, nitrosobenzene and SMX-NO, but not with sulfamethoxazole or sulfamethoxazole hydroxylamine (SMX-NHOH). DNCB and TMA induced type 1 and type 2 cytokine profiles, respectively. SMX-NO treatment stimulated the production of high levels of IL-5, variable amounts of IFN-γ, and relatively low levels of IL-10 and IL-4. Nitrosobenzene-activated lymph node cells secreted only low levels of IFN-γ and IL-5. Depletion of $CD4^+$ or $CD8^+$ T-cells from SMX-NO stimulated lymph node cells revealed that $CD4^+$ T-cells were the major source of IL-5. In conclusion, the data presented indicates that subcutaneous administration to mice of SMX-NO, but not the parent drug, stimulated the secretion of high levels of IL-5 from activated $CD4^+$ T-cells, which is consistent with the clinical profile of the drug.

Keywords: Drug allergy; Sulfamethoxazole; Drug metabolism; Cytokine

Abbreviations: AOO, 4:1 acetone:olive oil; DNCB, 2,4-dinitrochlorobenzene; ELISA, enzyme-linked immunosorbant assay; LNC, lymph node cell; NOB, nitrosobenzene; PBS, phosphate buffered saline; SI, stimulation index; SMX, sulfamethoxazole; SMX-NHOH, sulfamethoxazole hydroxylamine metabolite; SMX-NO, nitroso sulfamethoxazole metabolite; Tc, cytotoxic T-cell; Th, helper T-cell; TMA, trimellitic anhydride

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1. Introduction

Drug hypersensitivity reactions can be serious and account for many drug-induced deaths (Park et al., 1998). Administration of sulfamethoxazole (SMX), a sulfonamide, is associated with a high incidence (30% of treated patients with HIV infection) of hypersensitivity (Pirmohamed and Park, 2001). $CD4^+$ T lymphocytes isolated from the peripheral blood of hypersensitive patients proliferate in the presence of both SMX and nitroso SMX (SMX-NO) (Schnyder et al., 2000; Burkhart et al., 2001). SMX-NO is formed by oxidation of the metabolite SMX hydroxylamine (SMX-NHOH; Fig. 1) (Cribb and Spielberg, 1992; Rieder et al., 1988; Gill et al., 1997). All patients treated with SMX are, therefore, exposed to SMX-NHOH, which readily oxidises to SMX-NO (Cribb et al., 1991). SMX-NO is protein-reactive and has been shown to haptenate the surface of viable lymphocytes and keratinocytes (Naisbitt et al., 1999, 2001a,b; Reilly et al., 2000). Human T lymphocytes and clones specific for SMX secrete high levels of interleukin 5 (IL-5) and relatively low levels of interferon gamma (IFN-γ) (Pichler et al., 1997; Mauri-Hellweg et al., 1995), and can be stimulated to kill autologous keratinocytes, providing indications that the immune system is involved in the pathogenesis of SMX-mediated hypersensitivity (Schnyder et al., 1998, 1997). Secretion of IL-5 is thought to play a fundamental role in the differentiation of eosinophils and their accumulation at sites of inflammation (Clutterbuck et al., 1987).

We have investigated the nature of the primary drug signal presented to the immune system. Administration of SMX-NO to rats resulted in antigen formation and a specific T lymphocyte response to SMX-NO (Naisbitt et al., 2001b). T-cells from primed animals proliferated in the presence of a MHC-restricted peptide derived from both viable and dead cells haptenated with low and high levels of SMX-NO, respectively (Naisbitt et al., 2002). Treatment of rats with the parent compound SMX did not stimulate an immune response.

It is now clear that the major classes of T lymphocytes, $CD4^+$ T helper (Th) cells and $CD8^+$ T cytotoxic (Tc) cells, display functional heterogeneity. In both instances, two main phenotypes with distinct cytokine secretion profiles have been identified, and defined as type 1 (Th1, Tc1) or as type 2 (Th2, Tc2) cells (Coffman and Mosmann, 1988; O'Garra, 1998). Chemical contact allergens such as 2,4-dinitrochlorobenzene (DNCB) induce in mice a polarised type 1 cytokine secretion profile, with high levels of both interferon-γ and interleukin 12 (IL-12), but only comparatively low levels of type 2 cytokines (Dearman et al., 2002; Dearman and Kimber, 2001). In contrast, chemical respiratory allergens, such as trimellitic anhydride (TMA), provoke in mice IgE antibody responses and a preferential type 2 cytokine secretion profile, with high levels of IL-4, IL-5, IL-10 and IL-13, but only relatively low levels

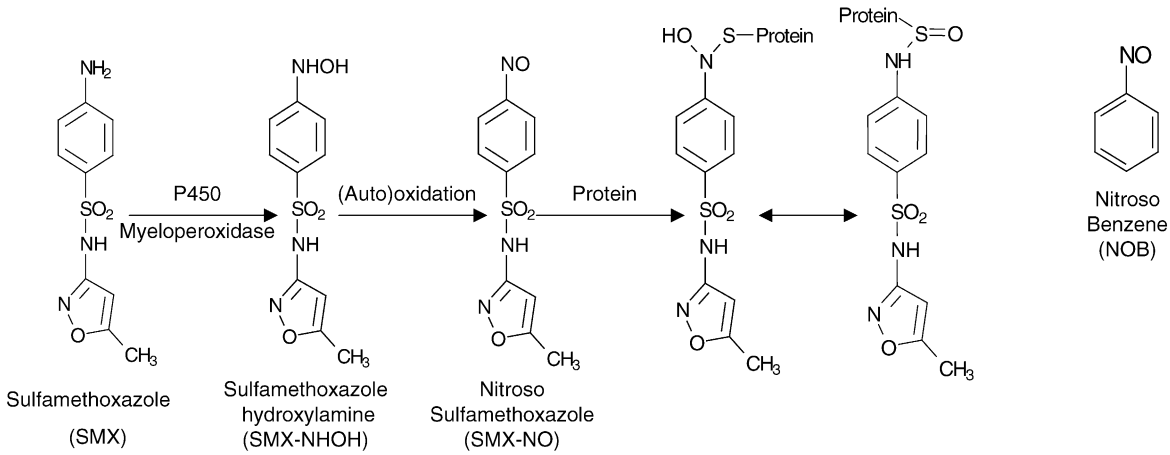

Fig. 1. Schema depicting the metabolism and covalent binding of SMX and its metabolites. SMX is metabolised by CYP2C9 to SMX-NHOH. SMX-NHOH is oxidised in aqueous solution to SMX-NO. SMX-NO binds covalently to thiol rich proteins. The protein-reactive substructure, nitrosobenzene (NOB), is also shown.