BIOCHEMICAL PHARMACOLOGY 72 (2006) 1432–1438

available at www.sciencedirect.com

ScienceDirect

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

Alkylating drugs applied in non-cytotoxic doses as a novel compounds targeting inflammatory signal pathway

A. Pukhalsky [a,*], G. Shmarina [a], V. Alioshkin [b], A. Sabelnikov [c]

[a] Research Centre for Medical Genetics, Moscow, Russia
[b] Gabrichevsky Institute of Epidemiology and Microbiology, Moscow, Russia
[c] East Carolina University, Greenvill, NC, USA

ARTICLE INFO

Article history:
Received 1 February 2006
Accepted 9 March 2006

Keywords:
Alkylating drugs
Regulatory T cell
IL-2 receptor
TNF receptor
NFκB
Experimental colitis

ABSTRACT

Alkylating drugs (ADs) belonging to the nitrogen mustard family are commonly used as cytostatic and immunosuppressive agents. Our previous in vitro studies demonstrated that in the case of gradual dose decrease, the number of targets for alkylation in the cell is also reduced and the drug switches from brutal cytostatic to cell growth modifier. At doses of 0.3 μg/ml and lower, the effects of ADs are no longer associated with DNA damage or stress/MAPK pathways activation. Instead, the disruption of signal transduction by the IL-2β and/or TNFα cell surface receptors is observed. As a result, ADs in the doses 100-fold lower than cytostatic ones are capable to modify lymphocyte activity including the activity of regulatory T cells. We hypothesized that ADs may have a beneficial effect in the treatment of inflammatory diseases. Indeed, the application of non-cytotoxic doses of an AD melphalan reduces the severity of murine experimental colitis. Daily administration of melphalan (25 μg/kg body weight) markedly reduced the severity of DSS-colitis as determined by clinical and histological criteria. Moreover, the beneficial effect of melphalan was also shown in asthmatic patients. In 60% of these patients histological and ultrastructural signs of bronchial epithelium regeneration were also revealed. Thus, ADs at non-cytotoxic concentrations exert beneficial effect both in acute and chronic inflammatory diseases. Such anti-inflammatory activity is thought to be due to blocking of signal transduction through various cell surface receptor including IL-2R and TNFR. Consequently different steps of inflammatory cascade turn out to be inhibited.

1. Introduction

Alkylating drugs (ADs) are derived from sulfur mustards which were used as chemical warfare agents during World War I. The poisoned soldiers demonstrated leucopenia, bone marrow aplasia, dissolution of lymphoid tissue and ulceration of gastro-intestinal tract. The clinical course of bronchopneumonia in these subjects was characterized by the absence of leukocyte response [1]. Subsequent studies revealed that the susceptible tissues were those with rapid regenerative capacity. So bone marrow, lymphoid tissue and epithelium of gastro-intestinal tract turned out the principal targets for alkylating agents. These cytostatic effects prompted the creation of numerous antineoplastic drugs belonging to the nitrogen mustard family (cyclophosphamide, chlorambucil, melphalan). Subsequently, these drugs began to be used as immunosuppressive agents in the treatment of non-malignant diseases [2]. Thus, the efficacy of pulse cyclophosphamide treatment of severe connective tissue diseases, idiopatic pulmonary fibrosis, gastrointestinal vasculitis in

* Corresponding author.
E-mail address: osugariver@medgen.ru (A. Pukhalsky).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.03.008

systemic lupus erythematosus and acute steroid refractory bowel disease and nephrotic syndrome has been demonstrated [3–7]. The mechanism of such beneficial effect remains unclear, although the most of authors believe that it is associated with immunosuppressive activity of the drug. In the same time there are numerous works demonstrating that cyclophosphamide treatment is able to stimulate concomitant immunity due to regulatory T cell inactivation. In particular, a single dose of 150 mg/kg prevents a poorly immunogenic melanoma in mice [8]. It was also shown that a single injection of cyclophosphamide significantly accelerates the diabetes onset in non-obese diabetic mice [9,10]. Such diabetes acceleration is thought to occur through the selective depletion of regulatory T cells that otherwise inhibit the disease process in untreated mice [10]. These data are not in accordance with the common concept of mechanisms of cytostatic effects of alkylating agents, which are mainly associated with cross-linking of DNA double strands [11] and, at higher concentrations, with induction of DNA strand breaks [12]. Although DNA is not a unique target for alkylation in the cell the others (RNA and some proteins) do not play any role in the cytostatic effect realization if the drug is used at a DNA-altering dose. However, when the dose is gradually decreased, the number of targets for alkylating agents will also be reduced. Thus, after cell treatment with various concentrations of an AD, different scenarios will be realized. That may be demonstrated by the example of lymphocytes stimulated in vitro with a T cell mitogene (e.g. phytohaemagglutinin or concanavalin A; Fig. 1). If the concentration of ADs is high (more than 100 μg/ml or 300 μM), the cell dies within few hours due to irreversible DNA damage [13]. If the concentration of ADs is lower (30–100 μg/ml) numerous sites of DNA are also alkylated, but the damaged segments restored during DNA repair. However, the affected cells are anyway died due to apoptosis induction. It has been recently shown

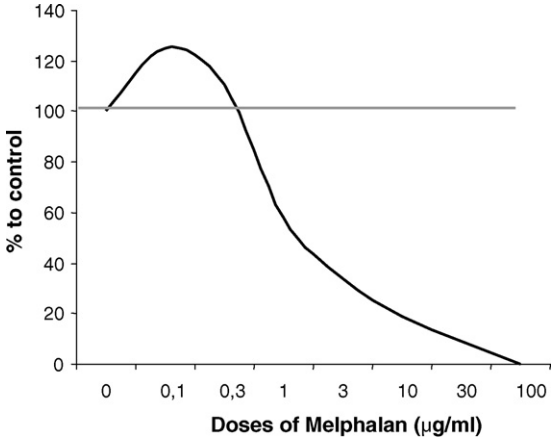

Fig. 1 – Effects of alkylating agents on proliferative response of murine spleen limphocytes stimulated with Con A. Freshly isolated spleen cells were exposed to mafosfamide or melphalan for 1 h at a concentration ranging from 0.01 to 100 μg/ml (or nearly 0.03–300 μM). Subsequently, the cells were washed, stimulated with optimal dose of Con A and cultured for 72 h. Cell proliferation was evaluated by [^{3}H]-thymidine incorporation.

that AD like other stress-induced agents, such as UV irradiation, heat shock, and protein synthesis inhibitors, activate both the JNK/SAPKs and another member of the MAPK family, the HOG1 homolog p38 MAPK [14]. Persistent activation of stress-induced kinases JNK/SAPK and p38 has been shown to trigger c-Jun-dependent CD95-L expression that is seemed to be rate-limiting step in the induction of apoptosis [15,16]. Moderate concentrations of ADs do not kill a cell but make it resistant to proliferative stimuli, possibly due to interference between mitogene signaling and stress/MAPK pathways that lead to the inhibition of IL-2 production in lymphocytes [17]. The activation of stress-induced kynases is believed to be independent of cytotoxic properties of ADs. Thus, JNK/SAPK activity is significantly induced even at relatively low concentrations (near 10 μM) that did not affect cell viability [16]. Nevertheless this dose range is much higher than those minimum concentrations, which can still modulate cultured lymphocyte proliferation [17,18]. Thus, ultra-low concentrations of ADs (0.3 μg/ml and lower) can augment the proliferative response of lymphocytes to phytohaemagglutinin (PHA) or concanavalin A (Con A) due to selective inhibition of suppressor cells [19] (see Fig. 2).

2. Immunomodulating effects of low concentrations of alkylating agents

Although skepticism developed regarding the existence of suppressor cells, studies in recent years have confirmed a central role of suppressor cell population in regulating immunity. Naturally occurring suppressor T cells (renamed regulatory T cells) constitutively express the transcription factor FoxP3 [20,21], CD25 [22], and glucocorticoid-induced TNF receptor (GITR) [23]. Regulatory T cells (Tregs) not only express IL-2Rα but also IL-2Rβ and the γc: that is, all of the subunits that are required to express a functional high-affinity IL-2R [24]. In the same time, Tregs do not secret IL-2 [25,26], so they depend on paracrine IL-2 for any responsiveness to this cytokine. In our previous experiments [19] it was shown that low concentrations of AD mafosfamide (a synthetic analogue of alkylating metabolite of cyclophosphamide) inhibit activity of suppressor cells induced by recombinant IL-2 (rIL-2). As seen in Fig. 2, addition of untreated suppressor lymphocytes to the culture of freshly isolated spleen cells significantly decreased their response to Con A. The pretreatment of suppressor cells with ultra-low doses of mafosfamide restored the level of lymphocyte response to mitogene. The effect of mafosfamide on suppressor lymphocyte activity can be mimicked by exposing of suppressor cells to anti-p75 mAb (antibody against β chain of IL-2R), but not to anti-p55 mAb (antibody against α chain of IL-2R). These data suggest that ADs are able to directly affect suppressor lymphocytes due to IL-2 signaling impairment. Similar results were obtained in our experiments with cytotoxic lymphocytes (CTL) [19]. As seen in Fig. 3, CTL were induced in a semi-allogeneic mixed lymphocyte culture. The cells were positive for IL-2Rβ but negative for IL-2Rα surface expression. Treatment with mafosfamide strongly suppressed the response of CTL to IL-2 stimulation. Thus, β chain of IL-2R and/or other components of IL-2 signal cascade seem to be critical molecular targets for