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The effect of azelastine on an acute non-specific inflammation in comparison to the effect of dexamethasone

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Introduction

The calcium pyrophosphate (CaPP)-induced pleurisy in mice – considered as an acute non-specific inflammation – is a suitable model to investigate the influence of compounds on different inflammatory mediators including the family of interleukin-1 (IL-1). Previous studies have revealed that an IL-1-like activity is increased in pleural exudates, in serum and in urine after CaPP injection into the pleural cavity of mice [1]. In the present paper the influence of azelastine was investigated on the inflammatory process in this model. Azelastine is a novel antiallergic agent with an inhibitory effect on the early and late phase asthmatic reaction [2]. Its antiallergic properties may stem not only from antagonizing the histamine-induced effects but also from a capacity to inhibit the release of histamine and other allergic mediators from mast cells [for instance 3].

To analyze whether azelastine might also exert a suppression of cytokine release, we examined its effect on the chemotactic activity and the expression of charge-changing lymphokines (CCLKs) in pleural exudates, which were partly characterized as IL-1 like activity. Simultaneously, the influence of dexamethasone was tested, because glucocorticoids are known as inhibitors of IL-1 production and secretion [4].

Material and methods

Induction of the pleurisy: Male AB mice were injected into the right pleura (ether anesthesia) with 0.7 ml of a sterile 1% suspension of CaPP microcrystals in pyrogen free saline.

Evaluation of the inflammatory reactions: At the time intervals indicated in the results, mice were anesthetized, killed and the exudates harvested and recorded. The cell count, -type, protein concentration, chemotactic activity for lymph node cells and CCLKs were estimated. Chemotaxis assay (Boyden-technique; 5): The lymphocytes were derived from lymph nodes of Wistar rats. After washing and passage through nylon wool columns, cells were placed into the top of modified Boyden chambers separated by 8 μm Sartorius filters. After an incubation time of 5 hours an average locomotion index was quantitated. Random motility of leukocytes derived from pleural exudates and the chemotaxis of polymorphonuclear leucocytes (PMML) *in vitro* were also determined using the Boyden-technique. 3 μm pore size Sartorius membrane filters were used.

Tanned electrophoretic mobility (TEEM)-test [6]: The test is based on the determination of charge changing lymphokines (CCLK) as products of activated leukocytes using target cells (stabilized tanned sheep red bloond cells) in a cytopherometer. The lymphokines induced in the pleural cavity or after stimulation in spleen cell cultures *in vitro* were assayed directly as CCLK or after gelfiltration on ultrogel AcA54. Preparation of lymphokine-containing supernatants *in vitro*: A total of 1.5×10^6 mouse spleen cells were incubated with the given concentration of azelastine or dexamethasone in Eagle's MEM at 37 °C for 4.5 hours.

Simultaneously cells were stimulated with concanavalin A in the presence of various concentrations of azelastine or dexamethasone. The supernatants were separated by centrifugation and assayed in the TEEM-test.

Treatment of animals: Azelastine (5×10^{-6} *M*/kg), dexamethasone (5×10^{-6} *M*/kg) or 0.9% NaCl were given intraperitoneally 30 min or orally 60 min prior to initiating the CaPP-pleurisy.

Agents used: Azelastine (ASTA Pharma AG, Frankfurt/M.); dexamethasone (VEB Kombinat GERMED, GDR); Concanavalin A (Sigma, St. Louis, USA); Il-1 and anti-IL-1 were a kind gift from Dr. Ch. A. Dinarello, Tufts University School of Medicine Boston, USA.

Results and discussion

The intrapleural injection of CaPP in mice induced an acute inflammation which was characterized by exudates containing $3.3/8.2/7.2 \times 10^6$ leukocytes with 60 to 80% PMNL at 2, 4 and 6 hours after the injection. Azelastine as well as dexamethasone caused a pronounced inhibition of the exudate volume as absolute cell number in the exudates, whereas the percentages of PMNL and the protein concentration were not influenced. However, the chemotactic activity of the pleural exudates was reduced after azelastine and dexamethasone treatment. In the same manner both compounds were able to decrease the concentration of CCLKs in the exudates (Fig. 1).

The random migration of the leukocytes derived from the pleural exudates of azelastine-treated animals was significantly ($p<0.05$) decreased (2.1 cells $\pm$ 0.3 per high power field) in comparison to non-treated mice (14.3 ± 2.8 p.h.p.f.). This corresponded with data showing that, *in vitro*, azelastine is able to inhibit the chemotaxis of PMNL to zymosan-treated serum in a dose-dependent manner (in the range 10^{-6} to 10^{-4} *M*). Exudates derived from non-treated mice showed, after gelfiltration on ultrogel AcA54, pronounced activities at 65, 35 and 17 kda measured in the TEEM-test and in the chemotaxis assay. In the chemotaxis test an additional active peak at 14 to 12 kda was found. The activities of CCLKs and chemotactic factors at 35 and 17 kda corresponded well with patterns found after testing of a standard IL-1. Moreover, both activities were antagonized by a polyclonal anti-IL-1 anti-serum (1:1000). Taken together these results support the idea that interleukins from the IL-1 family and/or TNF were determined. In pleural exudates derived from animals treated with azelastine these activities disappeared or showed reduced peaks at 17 and 35 kda.

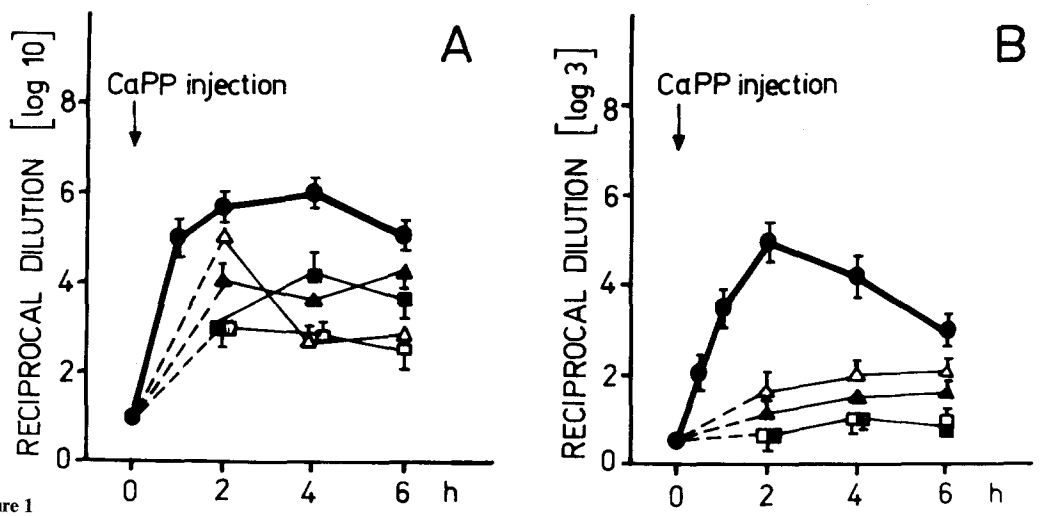

Figure 1

Influence of azelastine (5×10^{-6} *M*/kg) and dexamethasone (5×10^{-6} *M*/kg) (A) on the chemotactic activity of pleural exudates on T cells of rats, (B) on the IL-1 like activity measured as CCLK using the TEEM-test in pleural exudates (starting dilution 1:100). –●– 0.9% NaCl, i.p.; –▲– azelastine i.p.; –△– azelastine oral; –■– dexamethasone i.p.; –□– dexamethasone oral.

Therefore we propose that azelastine possible acts as an inhibitor of the cytokine (IL-1 – like activity) production and secretion.

To obtain more evidence about the mechanism of action of azelastine its influence on mice spleen cell cultures *in vitro* was investigated. Under these conditions azelastine and dexamethasone were able to induce the secretion of CCLKs with a peak in the dose-response curve at 10^{-10} to 10^{-9} *M*. However, already at a concentration of 10^{-13} to 10^{-12} *M* azelastine and dexamethasone inhibited the Con A-evoked secretion of CCLKs.

Taken together, these data support the idea that azelastine is not only an antihistaminic drug with the capacity to inhibit the release of histamine but also – as is dexamethasone – a compound capable of influencing the release of interleukins, probably interleukin-1. Moreover, azelastine can inhibit leukocyte mobility, probably by a direct mechanism.

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