Compound 48/80 induced mast cell activation in the lung

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Introduction:

Mast cells play a key and very versatile role in allergic inflammation ranging from acute IgE mediated reactions such as those seen in anaphylaxis, asthma and rhinitis to roles in the late phase allergic reaction and chronic inflammation (see 1 for a recent review). Detection of inflammation in the lungs by proton MRI following ovalbumin (2) or lipopolysaccharide challenge (3) has been previously demonstrated. Our aim was to investigate non-invasively in spontaneously breathing animals the pulmonary effects following mast cell activation induced by compound 48/80 (4) *in vivo* and in naïve Brown Norway (BN) rats.

Methods:

Treatments: Male BN rats were challenged intra-tracheally (i.t.) with compound 48/80 (Sigma, St. Louis; 1 mg/kg) and scanned prior to (baseline) and 1, 3, 6 and 24 h after challenge. To better characterize the effects of this compound, rats were pre-treated (1 h prior to 48/80) with: *(i)* the histamine (H_1) receptor antagonist, mepyramine (10 mg/kg i.t.); *(ii)* the histamine (H_2) receptor antagonist, tiotidine (6 mg/kg i.t.); *(iii)* a combination of both mepyramine and tiotidine; *(iv)* the 5-hydroxytriptamine (5-HT_2) receptor antagonist, methysergide (100 µg/kg i.t); *(v)* the mast cell stabilizer, di-sodium cromoglycate (DSCG, 10 mg/kg i.t. applied immediately before or 3 h after 48/80); and *(vi)* the steroid budesonide (3 mg/kg i.t. applied 1 h before or 3 h after 48/80). Histology was performed 1, 6, and 24 h after 48/80. Broncho-alveolar lavage (BAL) analysis was performed 24 h after 48/80. Compound 48/80 was from Sigma (St Louis), budesonide from Sicor (Milano), and tiotidine from Tocris (Norfolk, UK). Mepyramine, methysergide and DSCG were synthetized in house.

MRI: Rats were anaesthetized with forene (1.5-2.0%) in a mixture of O_2/N_2O (1:2), administered via a face mask. Measurements were carried out with a Bruker Biospec 47/40 system. A gradient-echo sequence was used throughout the study for detecting fluid signals (TR = 5.6 ms; TE = 2.7 ms; FOV = 6x6 cm^2; matrix = 256x128; slice = 1.5 mm; 45 image averages with an interval of 530 ms between each image acquisition) induced by PPE. Neither cardiac nor respiratory triggering was applied, and rats respired spontaneously. For details of image acquisition, see (4)

Results and Discussion:

An edematous signal was observed by MRI at 1, 3 and 6 h after treatment with 48/80 (1 mg/kg i.t.) reaching a peak 24 h after challenge (Fig. 1) The edematous response 24 h after 48/80 was accompanied by an influx of inflammatory cells (macrophages, neutrophils and eosinophils) and an increase in protein concentration in BAL fluid analysis and correlated with increased perivascular edema observed histologically.

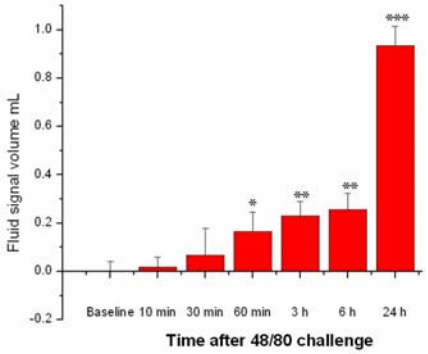

Fig. 1 – *Fluid signals detected by MRI following challenge with compound 48/80*

The edema observed 24 h after 48/80 treatment was fully blocked following pre-treatment with DSCG and moderately reduced when DSCG was applied 3 h after 48/80. Budesonide administerd 1 h before or 3 h after 48/80 fully inhibited the effects of 48/80 (fig. 2). The edema was not blocked by the histamine receptor antagonists mepyramine and tiotidine, either alone or in combination, nor by the 5-HT_2-receptor antagonist methysergide given 1 h prior to 48/80.

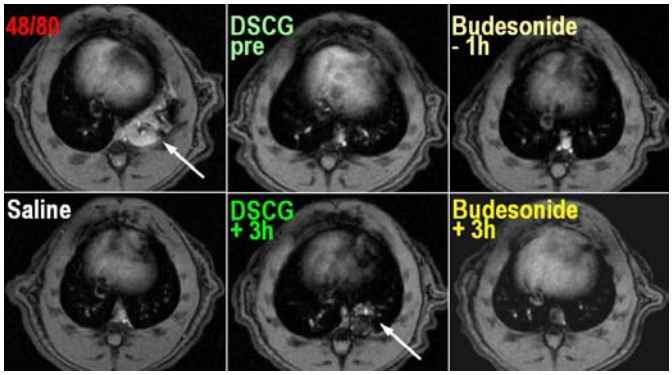

Fig. 2 – *Transversal sections through the chest of rats treated with 48/80 or saline, or pre-treated with DSCG (immediately before or 3 h after 48/80), or with Budesonide (1 h prior to or 3 h after 48/80). All images were taken 24 h after challenge of 48/80 or saline. The white arrows indicate the presence of edematous signals.*

These results suggest that mast cell degranulation by 48/80 is an important factor in the induction of edema at 24 h, and that the acute release of histamine and 5-HT is not responsible for the late phase (at 24 h) edema. Blockade by budesonide suggests that late phase inflammatory mediators may be responsible for this reaction.

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