# Suspected pholcodine allergy : the conundrum of clinically irrelevant immunoglobulin E and basophil responsiveness to morphine and codeine

# A patient with suspected pholcodine allergy: the conundrum of clinically irrelevant IgE and basophil responsiveness to morphine and/or codeine.

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Immunoglobulin E antibody (IgE)-mediated allergies to opioid analgesics such as morphine and codeine and related antitussives such as pholcodine remain rare. Since 2006/2007[1], pholcodine has become increasingly infamous because of its association with anaphylaxis to neuromuscular blocking agents (NMBAs). A reputation that, simultaneously with the publication of two "incriminating" case control studies, eventually led to the recommendation to stop the sales of all pholcodine-containing medicines throughout the EU[2, 3].

Correct diagnosis of IgE-mediated opioid allergy is fraught by difficulties that mostly relate to the unavailability of reliable specific IgE-assays and uncertainties associated with skin testing[4]. However, we reported three patients with a pholcodine allergy in whom the basophil activation test (BAT) appeared to be the only technique capable of correct diagnosis and, together with skin testing, to advance exploration of cross-reactivity with morphine, codeine and different NMBAs[5].

Here we present a 56-year-old woman with a blank history who experienced angioedema of tongue and lips with swallowing difficulties within 10 minutes after intake of a pholcomeripine syrup. She was treated at home with epinephrine 0.5 mg and methylprednisolone 125 mg, both intramuscularly. Upon arrival at the emergency department there was a residual angioedema of the upper lip and mild wheezes attributed to the respiratory infection. The patient was hemodynamically stable. An acute serum tryptase was not measured.

Total IgE and specific IgE was quantified by ImmunoCAP (Thermofisher Scientific) as in[5] and revealed a total IgE of 3429 $kU.L^{-1}$, pholcodine sIgE of 435 $KUA.L^{-1}$, morphine sIgE of 777 $kUAL^{-1}$, rocuronium sIgE of 57.9 $kUAL^{-1}$.

Analysis of BAT was performed 3 months after the index reaction and as described[5]. Briefly, heparinized whole blood was incubated with buffer as negative control, anti-IgE (Pharmingen, BD Bioscience) as positive control, or pholcodine (Fagron) (0.125–125 µM), morphine (StellorphineVR , Sterop) (17.5–1750 µM), codeine (Escapo C.V.) (16.7–1670 µM) or rocuronium (Esmeron; Organon) (8.2-82 µM). Reactions were stopped by placing the cells on ice, adding ice-cooled PBS-EDTA and removing the supernatant after centrifugation. To quantify basophil activation, cells were stained with anti-human IgE (clone GE-1, Sigma Aldrich GmBH) labelled with Alexa Fluor 405 (Molecular Probes, Invitrogen), anti-human CD63-FITC (clone H5C6, BD Bioscience) and anti-human CD203c-APC (clone NP4D6, Biologend). To stain the mas-related G protein-coupled receptor X2 (MRGPRX2), PE-labelled anti-human MRGPRX2 (clone K125H4, Biolegend) was added. Cells were lysed/fixed with Phosflow

Lyse/Fix buffer. Cells were washed and re-suspended in PBS with 0.1% sodium azide and measured.

The chemical structures of pholcodine, morphine, codeine and rocuronium are shown in figure 1A. Unlike our previous cases[5], as shown in figure 1B/1C, this patient demonstrates a positive BAT for morphine and codeine, indicative for some recognition at position C-6, since codeine and pholcodine differ at position C-3. Moreover, reactivity with all three compounds suggests that the recognition may be the same or similar to the antibody specificity described by Harle *et al*[6], i.e., the cyclohexenyl ring with a hydroxyl at position C-6 and, most important of all, a N-methyl at position C-17. However, IgE-inhibition with nalorphine, which has an N-propyl at position C-17 instead of the N-methyl, was negative (not shown).

To find an alternative explanation for basophil responsiveness to all three compounds, we stained the cells for MRGPRX2. This receptor, mainly expressed by mast cells (MCs), seems associated with immediate drug hypersensitivity reactions[7]. In contrast, it is barely expressed by resting basophils[8], explaining why the MRGPRX2-agonistic activity of opioids goes undetected in BAT[4, 5]. However, MRGPRX2-expression can be upregulated, and it cannot be excluded that some individuals have a spontaneous expression[8]. As shown in figure 1D, in our patient, 20% of the basophils spontaneously expressed MRGPRX2, suggesting that the cells could have reacted non-specifically to morphine and codeine at concentrations up to 3-log higher than required for pholcodine.

Finally, to explore the clinical significance of these unexpected basophil responses, the patient was challenged with codeine (31 mg) and morphine (11 mg) as detailed in[4]. Both challenges revealed negative. Likely, the explanation for the apparently false positive BAT has to be sought in the supratherapeutic stimulation concentrations. MRGPRX2-mediated reactions likely stem from a higher doses, and thus would require a sustained or prolonged period of high plasma or tissue concentrations[9]. The half-maximum concentration ($EC_{50}$) for morphine is 88 µg/mL for cultured primary human MCs[9]. According to the summary of product characteristics, Cmax for morphine is 23.5 $ng.mL^{-1}$ approximately 1.1 hour after intake of 20 mg orally and Cmax for codeine is 88.1 $ng.mL^{-1}$ approximately 1.2 hour after intake of a single dose of 60 mg orally. In other words, the morphine $EC_{50}$ for human MCs is approximately a 3.500-fold the Cmax.

Paralleling earlier observations[5], the patient showed IgE-reactivity to rocuronium. However, the clinical relevance of this finding remains obscure as skin testing and BAT) were negative. The latter observation, reflecting rocuronium not to be potent MRGPRX2 agonist in men.

As indicated, pholcodine has become increasingly infamous because of its the link with anaphylaxis from NMBAs. However, as debated elsewhere[10], the mechanism(s) for such a selective (temporarily) sensitization and the underlying "immunologic boostering effect" on IgE reactive to tertiary and quaternary substituted ammoniums as described earlier[1] through pholcodine remain(s) obscure. Pholcodine structurally differs only from morphine and codeine by its morpholinyl-ethyl-3 group at position C-3, and, as with these two analogues, has a freely accessible tertiary methyl group. Paralleling earlier observations[5], our patient was uneventfully challenged with both pholcodine analogues. Inversely, patients with rocuronium allergy and a positive morphine sIgE tolerate morphine and codeine[4]. As rocuronium is a mono-quaternary structure with a morpholinyl group (position C-2) and a propenyl pyrrolidinium quaternary ammonium group (position C-16), synthetically, these observations strengthen the model of different antibody recognition profiles as explained by Baldo *et al*[10]. Despite the common recognition of tertiary and quaternary substituted ammonium ions, individual populations of such antibodies would recognize different NMBAs via different N-alkyl groups and by recognition of neighbouring structures.

In conclusion, we present a patient with a suspected IgE-mediated pholcodine allergy with clinically irrelevant sIgE and basophil responses to morphine and/or codeine. The explanation for these false positive results is different. Although we cannot exclude nonspecific binding to the solid phase because of an elevated total IgE level, the reason for the irrelevant sIgE results has likely to be sought in the structural homology between these compounds. The false positive basophil responses could relate to a rare spontaneous basophilic MRGPRX2 expression.

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**Figure**

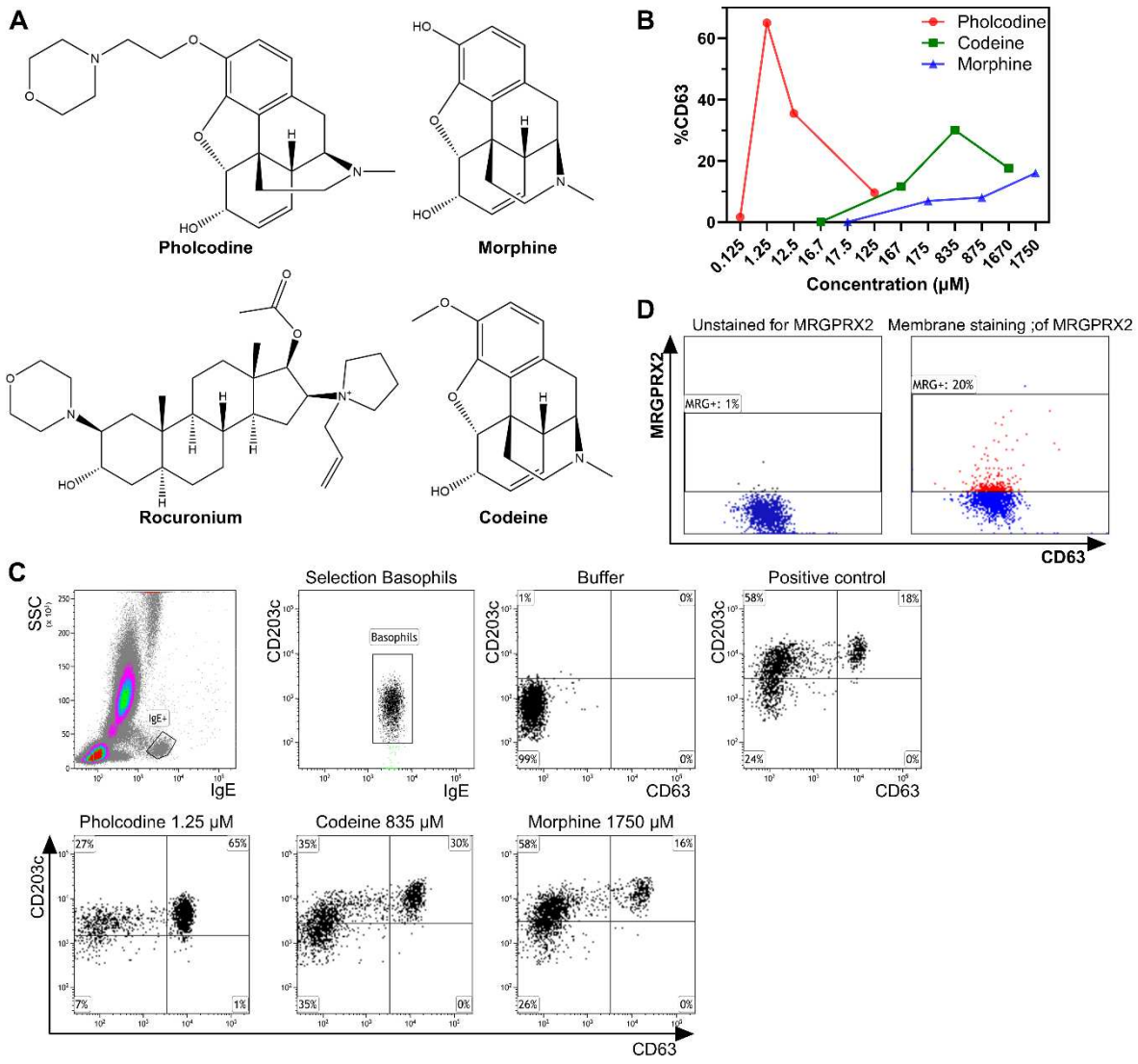


**Figure 1: Panel A:** Chemical structure of pholcodine, morphine, codeine and rocuronium. **Panel B**: CD63 up-regulation on basophils after stimulation with pholcodine, codeine and morphine. Activation of basophils is expressed as net percentage CD63 positive basophils. Upon stimulation with all three compounds, basophils of the patient show a dose-dependent up-regulation of CD63 expression from 0% up to 56% for pholcodine, 30% for codeine and 16% for morphine. Note that maximal responses for morphine and codeine were obtained at concentrations 2 to 3-log higher than that required for the antitussive. **Panel C**: Representative sample CD63 upregulation after activation of the basophils with buffer, anti-IgE as positive control, and the optimal stimulation concentration for pholcodine (1.25 µM), codeine (835 µM) and morphine (1750 µM). Basophils are characterized using side scatter (SSC), anti-IgE and CD203c positive cells. **Panel D:** membrane MRGPRX2 expression on resting basophils.