

# Meglumine gadoterate induces immunoglobulin-independent human mast cell activation and MRGPRX2 internalization

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To the Editor,

Gadolinium-based contrast agents (GBCA) are intravenous drugs used to enhance resolution in magnetic resonance imaging. They can induce immediate hypersensitivity reactions, yet their pathogenic mechanisms remain poorly characterized. This hampers the ability to predict which patients are at risk of developing them.<sup>1</sup> In fact, affected patients usually show negative skin-tests and can react upon the first known GBCA exposure, which implies that IgE-independent mechanisms might be driving this inflammatory response.

The Mas-related G protein-coupled receptor member X2 (MRGPRX2) has been recently associated with non-IgE mediated immediate hypersensitivity reactions.<sup>2</sup> Some drugs, such as fluoroquinolones, vancomycin, neuromuscular blockade agents, icatibant, morphine, leuprolide and iodinated contrast media, have been reported to activate MRGPRX2, which is highly expressed in mast cells (MCs).<sup>3</sup>

To assess the ability of GBCA to induce non-IgE-mediated hypersensitivity reactions, we stimulated the human MC line LAD2 with several commercial GBCA, namely, meglumine gadoterate, gadobutrol, gadoxetate disodium and gadoteridol. Then, we determined cell viability and degranulation by flow cytometry<sup>4</sup> (see a detailed material and methods section in this article's *online supplementary* ).

Of the GBCA tested, only meglumine gadoterate was able to induce significant MC activation (**Figure 1A**) without compromising cell viability (**Figure 1B**), as compared to unstimulated MCs. We further assessed MRGPRX2 expression on LAD2 cells by flow cytometry, as well as changes in its expression following stimulations with either meglumine gadoterate or vancomycin (a known agonist of MRGPRX2).<sup>5</sup> Under basal conditions, LAD2 cells expressed high levels of MRGPRX2 (**Figure 1C**). Following incubation with vancomycin, the level of MRGPRX2 expression was reduced, as compared to untreated LAD2 cells. Interestingly, we observed a similar decrease in MRGPRX2 expression levels upon meglumine gadoterate and vancomycin challenges, as compared to controls, suggesting both the signaling and the internalization of this receptor (**Figure 1D**).

Meglumine gadoterate is an ionic macrocyclic paramagnetic contrast media. It is composed by gadolinium, which together with the chelating agent tetraxetan (also known as DOTA), yields gadoteric acid. The base meglumine and gadoteric acid form the salt meglumine gadoterate (**Figure 2A**). Given that MRGPRX2 has affinity for cationic amphiphilic compounds,<sup>6</sup> we ascertained the ability of meglumine to induce MC activation. Meglumine itself induced MC degranulation without affecting cell viability, as compared to untreated cells (**Figure 2B**), although a reduction in MRGPX2 expression could not be confirmed (data not shown). Interestingly, meglumine caused MC activation at lower concentrations than meglumine gadoterate,

according to the half maximal effective concentration ( $EC_{50}$ ) of both substances (**Figure 2C**). The logarithmically transformed  $EC_{50}$  for meglumine gadoterate was 2.04 ( $R^2= 0.75$ ), and for meglumine was about one order of magnitude lower (1.06;  $R^2= 0.71$ ). Considering the  $EC_{50}$  for meglumine and its proportion in meglumine gadoterate (~26%), meglumine could be its main component responsible for MC degranulation.

In conclusion, our study demonstrates the ability of meglumine gadoterate to induce MC activation, by an immunoglobulin-independent mechanism that is likely mediated by MRGPRX2. Furthermore, we have delved into the meglumine gadoterate components that are involved in MC activation, and identified meglumine as a potential causative of non-IgE mediated hypersensitivity reactions. These data raise the possibility that immediate hypersensitivity reactions following intravascular administration of ionic iodinated contrast media may be at least partly mediated by meglumine. Further studies should be performed to define clinically relevant interactions between diverse radiological contrast media and MRGPRX2.

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**Figure 1. Meglumine gadoterate (MeGa) induces mast cell (MC) activation and MRGPRX2 internalization without affecting cell viability.** LAD2 cells were stimulated with different concentrations of GBCAs (MeGa, gadobutrol, gadoxetate disodium and gadoteridol) or vehicle control (-), and activation (A) and viability (B) were assessed by flow cytometry. Gating strategy and fluorescent minus one (FMO) control used to determine MRGPRX2 expression by flow cytometry (C). Geometric median fluorescence intensity (MFI) of MRGPRX2 following LAD2 cell stimulation with vancomycin (VAN, positive control) and MeGa, represented as the percentage of MRGPRX2 expressed by media-stimulated LAD2 cells (D). Pooled data from 3-4 independent experiments represented as mean  $\pm$  SEM, \*p[?]0.05; \*\*p[?]0.01; \*\*\*p[?]0.001.

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**Figure 2. Meglumine (ME) causes mast cell (MC) activation without affecting viability.** Circular diagram showing the composition of meglumine gadoterate (MeGa) (A). LAD2 cell viability and activation was assessed by flow cytometry following stimulation with ME or vehicle control (-) (B). LAD2 cells were stimulated with increasing concentrations of ME and MeGa to determine the half maximal effective concentration (EC<sub>50</sub>) of LAD2 cell activation (C). Pooled data from 10-12 (B) or 3-5 (D) independent experiments represented as mean  $\pm$  SEM, \*p[?]0.05; \*\*p[?]0.01; \*\*\*p[?]0.001.