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Brief Communication

Hydroxychloroquine as a novel therapeutic approach in mast cell activation diseases

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ABSTRACT

There is no therapeutic agent approved in cutaneous mastocytosis and mast cell activation syndrome. We report the efficacy of *hydroxychloroquine* in four patients with cutaneous mastocytosis ($n = 2$) and mast cell activation syndrome (n = 2).

We show that this molecule reduces the long-term survival of primary human mast cells, interferes with lysosome function and leads to the accumulation of non-functional tryptase in the mast cell granules. Furthermore, *hydroxychloroquine* decreases the production of pro-inflammatory mediators.

1. Introduction

Mast cell activation diseases (MCAD) include non-clonal and clonal pathologic mast cell states [1]. MCAD are classically divided in aberrant mast cell activation disorders classified as mast cell activation syndromes (MCAS) and in proliferation and/or accumulation of abnormal mast cells in various organs, classified as mastocytosis [2]. Mastocytosis can be divided between cutaneous mastocytosis (CM) if only skin involvement is present and in systemic mastocytosis if at least one internal organ is involved [3]. There are internationally approved diagnosis criteria for MCAS, CM and systemic mastocytosis [3–7]. No treatment is specifically approved for CM and MCAS. H1 anti-histamines are recommended by international guidelines for the treatment of clinical symptoms associated with MCAD [8].

Hydroxychloroquine (*HCQ*) is a lysosomatropic drug that accumulates in lysosomes where it perturbs some important functions by increasing the pH. *HCQ* has proved to be effective in some autoimmune diseases such as systemic lupus erythematosus [9]. We report here that *HCQ* improve clinical symptoms and signs present in MCAD patients. Moreover we show that *HCQ* alters some important biological function of human mast cell *in vitro*.

2. Material and methods

2.1. Clinical study

We included our patients with history of MCAD and unclassified inflammatory rheumatism, treated by *HCQ* for more of 12 month between January 2012 and December 2017.

The following data were collected: demographic, classification of MCAD, presented mast cell activation-related symptoms, previous treatment and achieved percentage of the improvement of mast cell mediator-related symptoms, during of treatment with *HCQ* and its dosage, presence/absence of *D816V KIT* mutation on the skin and marrow, initial serum tryptase level and during the last follow-up visit, achieved percentage of the improvement of mast cell mediator-related symptoms after six months of *HCQ* treatment and during the last follow-up visit. We also collected the clinical evolution of skin lesions and the number of mast cells in the skin biopsies (×40 magnification) before *HCQ* treatment and during the last follow-up visit if available. All diagnosis

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were performed and patients classified according WHO/international classification [3–7]. Systemic extension evaluation to search a SM according WHO criteria of our patients is showed in Table E1 [3–7]. The daily dosage of *HCQ* was adapted to reach a serum level of *HCQ* of 1000 μg/L [9].

The percentage of the improvement of mast cell mediator-related symptoms was evaluated by every patient on visual analog scale of 0 to 100 mm [10].

The mast cells were identified in skin biopsies by immunohistochemical analysis with anti-CD117 antibodies (polyclonal rabbit; Dako Glostrup, Denmark).

The analysis of the *D816V* mutation of *KIT* on the skin and smear marrow was performed by the method described by Lanternie F et al. [11].

2.2. Reagents

Anti-mast cell tryptase (clone G-12, Santa Cruz Biotechnology, Santa Cruz, CA, USA), Anti-CD117-APC (clone 104D2), anti-IL8-PE (clone AS14), anti-CD63-PE (clone H5C6), all from BD Biosciences, San Jose, *CA*. Anti-mast cell chymase (clone B7, Millipore, Billerica, MA, USA), Anti-FcεRI-FITC (clone AER-37, eBioscience, San Diego, CA, USA). All the secondary antibodies used (Alexa Fluor-conjugated) were from Molecular Probes, Inc., Eugene, OR. Mast cell granule matrix was stained using avidin-sulforhodamine 101 (Av. SRho, Sigma-Aldrich, Saint-Louis, Missouri, USA). Hydroxychloroquine-sulfate, Phorbol 12-myristate 13-acetate (PMA) and Ionomycin were from Sigma-Aldrich.).

2.3. Human mast cells (hMCs)

Peripheral blood mononuclear cells (PBMCs) were obtained from buffy coats (Etablissement Français du Sang). $CD34^+$ precursors cells were isolated from PBMCs (EasySep™ Human CD34 Positive Selection Kit, STEMCELL Technologies, Vancouver, Canada) and grown under serum-free conditions using StemSpanTM medium (STEMCELL Technologies) supplemented with recombinant human IL-6 (50 ng/mL; PeproTech, New Jersey, USA), human IL-3 (10 ng/mL; PeproTech) and 3% supernatant of CHO transfectants secreting murine SCF (a gift from Dr. P. Dubreuil, Marseille, France, 3% correspond to ~50 ng/mL SCF) for one week. Cells were next grown in IMDM Glutamax I, sodium pyruvate, 2-mercaptoethanol, 0.5% BSA, Insulin-transferrin selenium (all from Invitrogen, Carlsbad, CA, USA), ciprofloxacin (10 μg/mL; Sigma Aldrich), IL-6 (50 ng/mL) and 3% supernatant of CHO transfectants secreting murine SCF for 8 weeks then tested phenotypically ($Tryptase^+$, $CD117^+$, $FcεRI^+$) and functionally (β-hexosaminidase release in response to FcεRI crosslinking) before use for experiments. Only primary cell lines showing > 95% $CD117^+/FcεRI^+$ cells were used for experiments. Some hMCs were cultured with culture medium plus 2.5, 5 or 10 μM *HCQ* for 3 to 5 weeks. Culture medium containing *HCQ* was replaced weekly.

2.4. Confocal microscopy analysis

5 × 104 hMCs treated or not with *HCQ* were transferred to poly-L-lysine-coated slides then fixed, permeabilized and stained with anti-CD63 mAb followed by secondary antibodies and 2 μg/mL avidin-sulforhodamine 101 (highly cationic glycoprotein that selectively stains mast cell granules, Sigma-Aldrich).The samples were mounted and examined using a Zeiss LSM 710 confocal microscope and ZEN software with a 63× Plan-Apochromat objective (1.4 oil), electronic zoom 3. Scoring of the slides was performed in a blinded fashion by evaluating for each condition at least 50 cells in randomly selected fields from 2 independent experiments.

2.5. Quantitation of intracellular enzymes

To quantify cellular granule enzymes contents, 10×10^5 hMCs treated or not with *HCQ* were disrupted with 100 μL 1% Triton X-100 in PBS and the cell lysates were immediately processed. Enzymatically active tryptase was measured spectrophotometrically by adding 50 μL of 0.5 mmol/L Tosyl-Gly-Pro-Lys-p-nitroanilide (Sigma-Aldrich, T6140) 100 mmol/L Tris-HCl, pH 8, 100 mmol/L NaCI, to 50 μL cell lysate. Enzymatically active chymase was quantified by adding 50 μL of 1 mmol/L Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (Sigma-Aldrich, S7388) 300 mmol/L Tris, pH 8, 1.5 mol/L NaCl to 50 μL cell lysate. Changes in absorbances at 405 nm were immediately registered for 5 min at 30-s intervals at room temperature. Enzymatically active β-hexosaminidase was quantified by incubating 50 μL of 1.3 mg/mL para-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma-Aldrich, N9376), 0.1 mol/L sodium citrate, pH 4.5 plus 10 μL cell lysate for 30 min. at 37 °C. The enzymatic reaction was stopped by adding 150 μL of 0.2 mol/L glycine, pH 10.7. Absorbance was read at 405 nM.

2.6. Intracellular cytokine staining

5×10^4 hMCs were stimulated with PMA (100 ng/mL) and ionomycin (1 μg/mL) for 12 h in the presence of brefeldin A (10 μg/mL). Cells were washed, fixed (2% PFA), permeabilized (0.1% saponin in PBS containing 1% BSA, Sigma-Aldrich), and stained with anti-IL8-PE or anti-GMCSF-PE or anti-CCL4-FITC (BD pharmingen™, Becton, Dickinson and Company, San Diego, CA, USA) mAbs for 45 min. Flow cytometric data were acquired on a MACSQuant® Analyzer 10 (Miltenyi Biotec, Bergisch Gladbach, Deutschland) and were analyzed by using FlowJo software (Tree Star, Inc., Ashland, OR, USA).

2.7. Viability assays

MTT assay was used to measure *HCQ* toxicity. Briefly, cells were seeded into 96-well plates (3×10^4 cells in 100 μL culture medium) and treated for 16 h with *HCQ*. 20 μL of MTT solution (5 mg/mL in PBS, Sigma-Aldrich) were added to each well. After 3 h, supernatant was carefully discarded and 150 μL DMSO (Sigma-Aldrich) were added. Absorption was measured at 570 nm. The viability of hMCs cultured in presence of *HCQ* was regularly checked by flow cytometry by using a calcein-acetoxymethyl (AM) assay. Briefly, hMCs (5 × 104 cells) were incubated with 1 μmol/L of calcein-AM (Invitrogen) at 37 °C for 30 min. Cells were next washed with PBS and analyzed by flow cytometry. Apoptosis was measured by using 7ADD (7-aminoactinomycin D, BD Pharmingen) and annexin-V-FITC (Invitrogen). Briefly cells were washed in PBS and incubated with the two probes at the concentration recommended by the manufacturer for 15 min at room temperature and next analyzed by flow cytometry.

3. Results and discussion

We investigated the efficacy of *HCQ* in four patients with mast cell activation disease: two cases of cutaneous mastocytosis and two patients with mast cell activation syndrome. All four patients presented unclassified inflammatory rheumatism, which was legitimizing the treatment by *HCQ*. Our data from four patients are summarized in Table E2.

The first patient was a 57 year-old man with a four-year history of CM (Fig. 1) without *D816V* mutation of *KIT*. The diagnosis of CM was done in accordance with international diagnostic criteria of CM (Table E1) [7]. Darier' sign was present on skin lesions. The patient experienced pruritus and abdominal pain with diarrhea as mast cell mediator-related symptoms. He also presented with unclassified inflammatory peripheral arthritis for 2 years. Arthritis was not controlled by oral corticosteroids and *methotrexate. HCQ* (4.5 mg/kg/day) was introduced to alleviate joint symptoms. At six months *HCQ* dose was increased to

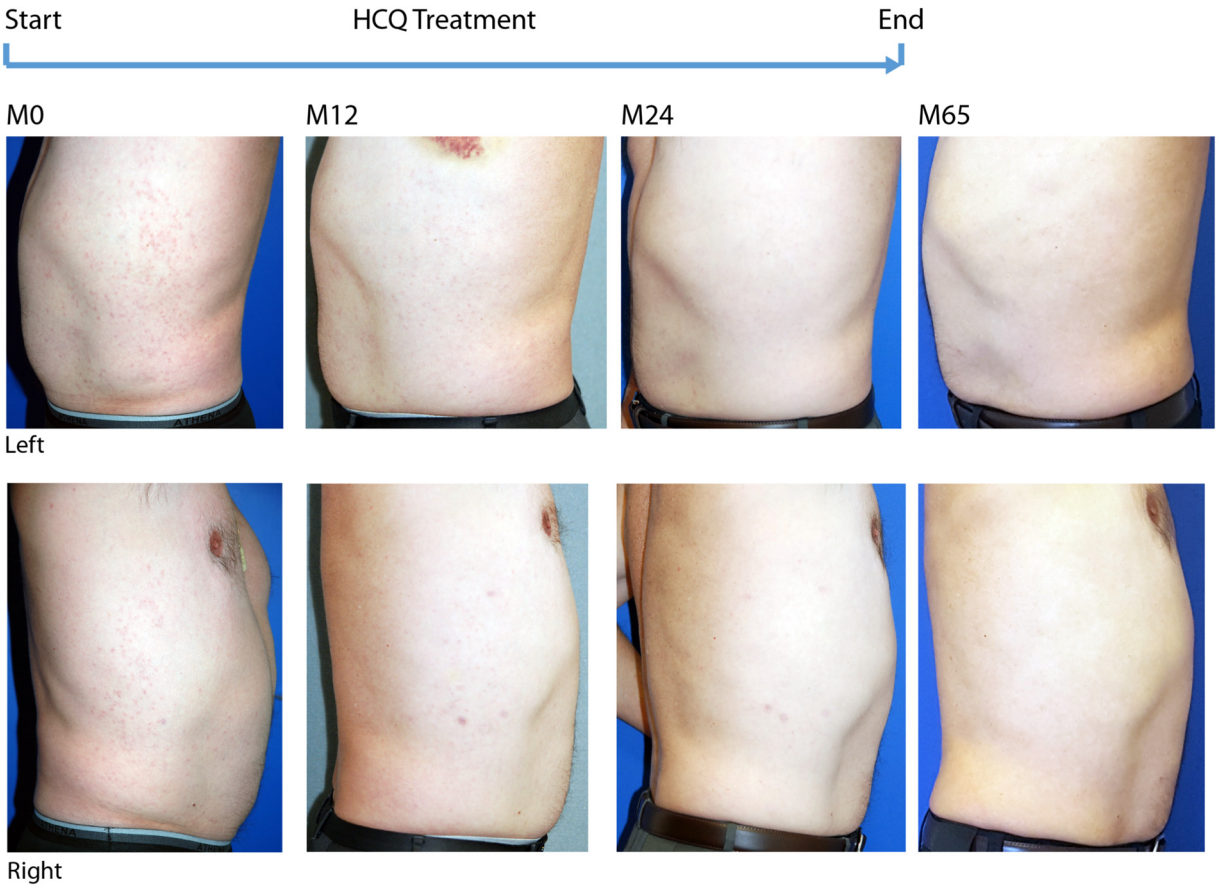

Fig. 1. Cutaneous mastocytosis in patient #1. Cutaneous involvement before *hydroxychloroquine* (*HCQ*) treatment (M0) and after 12 (M12) and 24 (M24) months of *HCQ* treatment and 21 months after withdrawal of therapy (M65). Complete regression of skin lesions was observed after 24 months treatment with *HCQ*.

6.8 mg/kg/day to reach a blood *HCQ* level of 1000 µg/L. Twelve months after the introduction of *HCQ* treatment, a clear improvement of CM lesions was observed (Fig. 1). Two years after the introduction of *HCQ* - and six years after onset of CM - complete disappearance of CM was observed (Fig. 1). Histological examination of a skin biopsy of a lesion of CM showed a significant reduction in the number of mast cells in the skin (Fig. E1) as shown by anti-tryptase antibodies staining (Mast Cell Tryptase, FL-275 antibody, Santa Cruz Biotechnology, Inc., Dallas, USA). The number of skin mast cells per high power field (×40) decreased from 44 at baseline to 20 at 12 months and 17.5 at 24 months. In line with this observation it is worthy to note that T. Shimomatsu et al. recently reported a reduced number of infiltrating mast cells in the dermis of MRL/lpr mice upon *HCQ* treatment [12]. The mast cell mediator-related symptoms were controlled at six months of treatment. The level of serum tryptase was stable (baseline: 5.1 µg/L *versus* 4.85 µg/L at 24 months). *HCQ* was discontinued after 42 months of treatment. No relapse of CM lesions was observed with a follow-up of 22 months.

Other three MCAD patients (extension biological evaluation shown in Table E2) with unclassified inflammatory rheumatism were treated with *HCQ*. It was about of diffuse joint pain with sensation of stiffness the morning at wake up, during 30 to 60 min. The imaging exams showed a non-erosive arthritis and the immunological tests - anti-citrullinated cyclic peptide antibodies and rheumatoid factor - were negative. All three patients had concomitant severe and uncontrolled mast cell mediator-related symptoms. In all patients, six months after *HCQ* treatment initiation, a significant improvement of mast cell activation symptoms - was observed (Table E2 and Fig. E2).

These clinical observations prompted us to investigate the biological effects of *HCQ* treatment on human mast cells (hMCs) *in vitro*. We used primary hMCs derived from $CD34^+$ peripheral blood progenitor cells [13]. hMCs were treated with *HCQ* concentrations ranging from 2.5 to 10 µmol/L. These concentrations are clinically relevant because *HCQ* concentration in the blood of patients treated with 400 mg/day *HCQ* is about 917 mg/L or 3 µmol/L [9] and *HCQ* is expected to accumulate in tissues. This range of concentrations did not exhibit cellular toxicity as tested with a classical toxicity assay after 18 h treatment (Fig. E3, *A*). We observed that such *HCQ* concentrations induced hMC death after 3 to 4 weeks of treatment (Fig. E3, B–C). Annexin V staining showed that ~20% hMCs were apoptotic when treated for three weeks with 10 µM of *HCQ* (data not shown). This effect of *HCQ* was not observed on human fibroblasts treated for 28 days (data not shown).

Because *HCQ* is known to modify lysosome homeostasis, we analyzed the impact of *HCQ* treatment on hMC granule compartment (*i.e.* secretory lysosomes). We focused our study on mast cells treated for 3 weeks because *HCQ* effects appeared after 3 weeks of treatment and hMC survival is still high, notably for the concentrations of 2.5 and 5 µmol/L of *HCQ*. Moreover flow cytometry allowed us to analyze living cells only. We first investigated the expression and the localization of the lysosomal molecule CD63. We found that prolonged treatment with *HCQ* for three to five weeks decreased the intracellular expression level of CD63 and modified the expression pattern of CD63. While untreated hMCs showed an homogeneous cytoplasmic localization of CD63, hMCs treated with *HCQ* exhibited a more discrete and punctuated expression (Fig. 2, *A-B*). We next analyzed the expression and the function of key enzymes stored on mast cell granules such as β-hexosaminidase, chymase and tryptase. We found that *HCQ* treatment did not alter the intracellular levels of tryptase and chymase (as detected by flow cytometry) but dramatically decreased tryptase enzymatic activity. Conversely, the β-hexosaminidase and chymase activities were barely

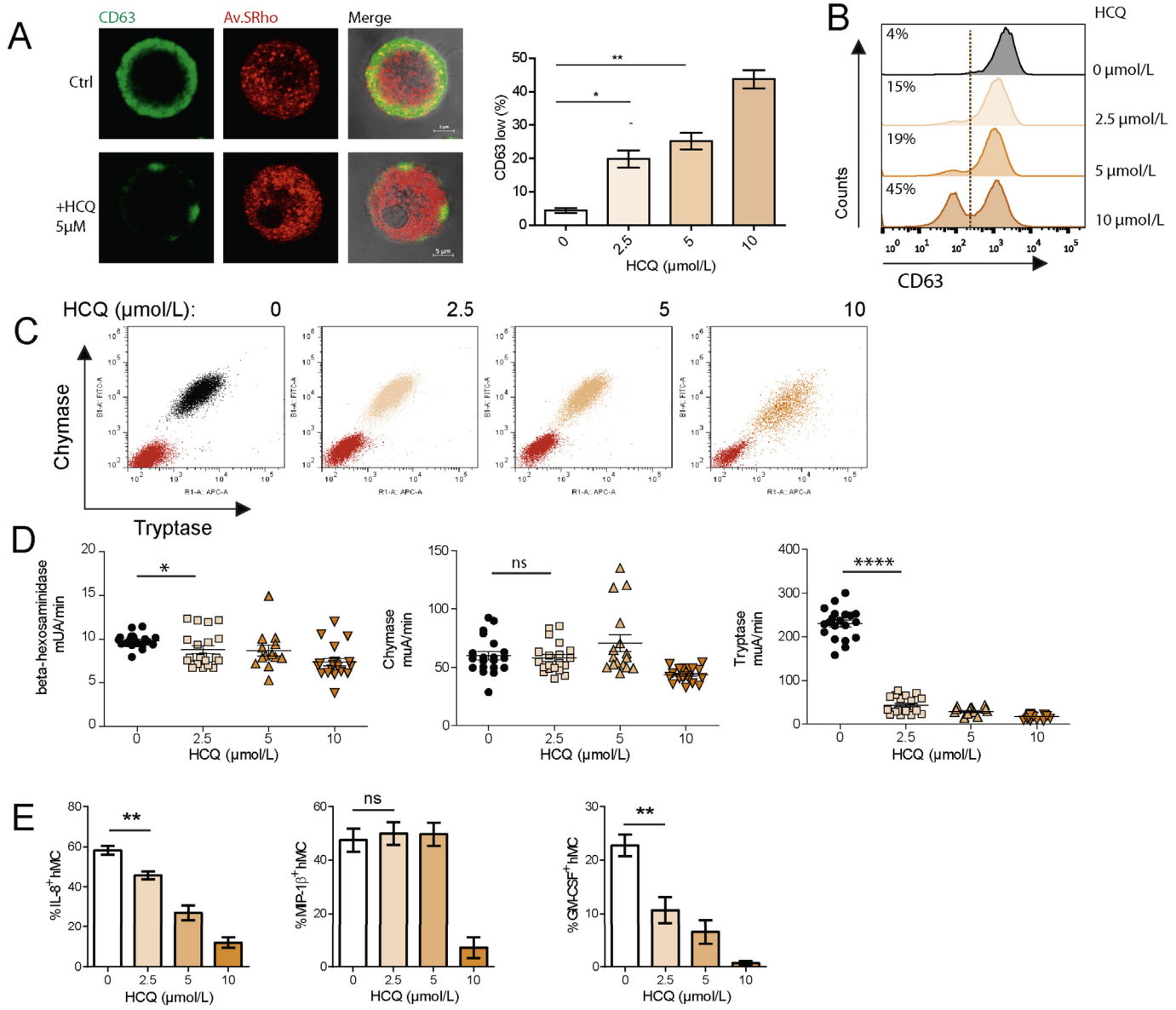

Fig. 2. *Hydroxychloroquine* impairs pro-inflammatory capabilities of human mast cells. Human mast cells were treated for 3 weeks with *HCQ*. A-B: CD63 expression and localization: shown is a representative mast cell stained with anti-CD63 mAb and fluorescent avidin (Av.SRho) to stain granules and quantification of the $CD63^{low}$ phenotype observed among > 100 cells analyzed (A). Intracellular expression of CD63 analyzed by flow cytometry; numbers indicate the percentage of $CD63^{low}$ cells (B). C: Intracellular tryptase and chymase expression as detected by flow cytometry, overlaid are the isotype-matched control staining (red dots). D: mast cell intracellular β-hexosaminidase, chymase and tryptase activities. E: IL-8, MIP-1β and GM-CSF expression following PMA/ionomycin stimulation. *P* values were determined using unpaired *t*-test. ns $P > .05$, * $P < .05$, ** $P < .01$, **** $P < 0$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

affected (Fig. 2, *C-D*). These results indicate that prolonged *HCQ* treatment interferes with lysosome function and leads to the accumulation of non-functional tryptase in mast cell granules. We can hypothesize that lysosomal perturbations induced by *HCQ* impair the association of tryptase with the heparin matrix of the secretory lysosomes because the interaction with heparin is known to be essential for maintaining enzymatic activity.

Because cytokine production is an important component of the mast cell-mediated inflammation, we next investigated the production of inflammatory cytokine/chemokines by flow cytometry. This analysis showed that three weeks treatment with low concentrations of *HCQ* decreased IL-8 and GM-CSF expression by hMCs but did not affect MIP-1β expression (Fig. 2E). It suggests that cytokine/chemokine expression can be differently affected by *HCQ* treatment.

These results indicate that *HCQ* treatment modifies key features of mast cell biology and induces a profound alteration of mast cell granules homeostasis. Namely, it leads to the storage of inactive tryptase and to the decreased expression of key mast cell mediators such as IL-8 and GM-CSF.

Very few studies have reported the impact of *HCQ* on mast cell biology [14,15]. In a pioneer study, Green et al. showed that pre-treatment of rat mast cells with *Chloroquine* (10–1000 μmol/L) decreased their ability to degranulate and to produce prostaglandin D2 [14]. Our *in vitro* study goes further by providing some mechanisms by which *HCQ* impact mast cell responses. It also gives some clues about the efficacy of *HCQ* in the treatment of inflammatory processes in which mast cell play a key role, such as chronic spontaneous urticarial [16]. Our results indicate that *HCQ* might turn out to be a helpful agent in the treatment of MCAD, as it has been suggested by C. Akin [17] and M. Soderberg [18].

Taken together, we may say that, the dramatic reduction of mast cell inflammatory capabilities induced by *HCQ* contribute to the observed efficacy of *HCQ* in MCAD patients. This study paves the road for randomized clinical trials to demonstrate the efficacy of *HCQ* in MCAD.

Author contributions

EE, CP, and CBL wrote the manuscript. EE, SV, and CBL designed the study. CBL recruited patients and collected all clinical data. EE performed *in vitro* analysis and collected all *in vitro* data. ML[3] performed rheumatologic evaluation of patients. PAA performed the serum tryptase assay and the mast cells flow cytometric analysis. CL performed the analysis of bone marrow and skin biopsies. ML[6] performed the analysis to establish the blood *hydroxychloroquine* level. All authors reviewed the final version of the manuscript for intellectual content and approved it.

Conflict-of-interest

The authors report applying for a patent for the use of antimalarials in MCAD. No other potential conflict of interest relevant to this letter was reported.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2018.07.004.

References

[1] L.B. Afrin, Mast cell activation disease and the modern epidemic of chronic inflammatory disease, Transl. Res. 174 (2016) 33–59.
[2] L.B. Afrin, J.H. Butterfield, M. Raithel, G.J. Molderings, Often seen, rarely recognized: mast cell activation disease—a guide to diagnosis and therapeutic options, Ann. Med. 48 (2016) 190–201.
[3] P. Valent, H.P. Horny, L. Escribano, B.J. Longley, C.Y. Li, L.B. Schwartz, G. Marone, R. Nunez, C. Akin, K. Sotlar, W.R. Sperr, K. Wolff, R.D. Brunning, R.M. Parwaresch, K.F. Austen, K. Lennert, D.D. Metcalfe, J.W. Vardiman, J.M. Bennett, Diagnostic criteria and classification of mastocytosis: a consensus proposal, Leuk. Res. 25 (2001) 603–625.
[4] G.J. Molderings, S. Brettner, J. Homann, L.B. Afrin, Mast cell activation disease: a concise practical guide for diagnostic workup and therapeutic options, J. Hematol. Oncol. 4 (2011) 10.
[5] P. Valent, H.P. Horny, M. Triggiani, M. Arock, Clinical and laboratory parameters of mast cell activation as basis for the formulation of diagnostic criteria, Int. Arch. Allergy Immunol. 156 (2011) 119–127.
[6] P. Valent, C. Akin, M. Arock, K. Brockow, J.H. Butterfield, M.C. Carter, M. Castells, L. Escribano, K. Hartmann, P. Lieberman, B. Nedoszytko, A. Orfao, L.B. Schwartz, K. Sotlar, W.R. Sperr, M. Triggiani, R. Valenta, H.P. Horny, D.D. Metcalfe, Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal, Int. Arch. Allergy Immunol. 157 (2012) 215–225.
[7] P. Valent, C. Akin, L. Escribano, M. Fodinger, K. Hartmann, K. Brockow, M. Castells, W.R. Sperr, H.C. Kluin-Nelemans, N.A. Hamdy, O. Lortholary, J. Robyn, J. van Doormaal, K. Sotlar, A.W. Hauswirth, M. Arock, O. Hermine, A. Hellmann, M. Triggiani, M. Niedoszytko, L.B. Schwartz, A. Orfao, H.P. Horny, D.D. Metcalfe, Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria, Eur. J. Clin. Investig. 37 (2007) 435–453.
[8] G.J. Molderings, B. Haenisch, S. Brettner, J. Homann, M. Menzen, F.L. Dumoulin, J. Panse, J. Butterfield, L.B. Afrin, Pharmacological treatment options for mast cell activation disease, Naunyn Schmiedeberg's Arch. Pharmacol. 389 (2016) 671–694.
[9] M. Jallouli, L. Galicier, N. Zahr, O. Aumaitre, C. Frances, V. Le Guern, F. Liote, A. Smail, N. Limal, L. Perard, H. Desmurs-Clavel, D. Le Thi Huong, B. Asli, J.E. Kahn, J. Pourrat, L. Sailler, F. Ackermann, T. Papo, K. Sacre, O. Fain, J. Stirnemann, P. Cacoub, G. Leroux, J. Cohen-Bittan, J. Sellam, X. Mariette, B. Blanchet, J.S. Hulot, Z. Amoura, J.C. Piette, N. Costedoat-Chalumeau, G. Plaquenil Lupus Systemic Study, Determinants of hydroxychloroquine blood concentration variations in systemic lupus erythematosus, Arthritis Rheumatol. 67 (2015) 2176–2184.
[10] M.E. Wewers, N.K. Lowe, A critical review of visual analogue scales in the measurement of clinical phenomena, Res. Nurs. Health 13 (1990) 227–236.
[11] F. Lanternier, A. Cohen-Akenine, F. Palmerini, F. Feger, Y. Yang, Y. Zermati, S. Barete, B. Sans, C. Baude, D. Ghez, F. Suarez, R. Delarue, P. Casassus, C. Bodemer, A. Catteau, F. Soppelsa, K. Hanssens, M. Arock, H. Sobol, S. Fraitag, D. Canioni, A. Moussy, J.M. Launay, P. Dubreuil, O. Hermine, O. Lortholary, A. Network, Phenotypic and genotypic characteristics of mastocytosis according to the age of onset, PLoS One 3 (2008) e1906.
[12] T. Shimomatsu, N. Kanazawa, N. Mikita, Y. Nakatani, H.J. Li, Y. Inaba, T. Ikeda, T. Kondo, F. Furukawa, The effect of hydroxychloroquine on lupus erythematosus-like skin lesions in MRL/lpr mice, Mod. Rheumatol. 26 (2016) 744–748.
[13] R. Joulia, N. Gaudenzio, M. Rodrigues, J. Lopez, N. Blanchard, S. Valitutti, E. Espinosa, Mast cells form antibody-dependent degranulatory synapse for dedicated secretion and defence, Nat. Commun. 6 (2015) 6174.
[14] K.B. Green, H.W. Lim, Effects of chloroquine on release of mediators from mast cells, Skin Pharmacol. 2 (1989) 77–85.
[15] R. Nosal, K. Drabikova, J. Pecivova, Effect of chloroquine on isolated mast cells, Agents Actions 33 (1991) 37–40.
[16] G.E. Reeves, M.J. Boyle, J. Bonfield, P. Dobson, M. Loewenthal, Impact of hydroxychloroquine therapy on chronic urticaria: chronic autoimmune urticaria study and evaluation, Intern. Med. J. 34 (2004) 182–186.
[17] C. Akin, Mast cell activation disorders, J Allergy Clin Immunol Pract 2 (2014) 252–257 e251. (quiz 258).
[18] M.L. Soderberg, The mast cell activation syndrome: a mini review, MOJ Immunol. 2 (1) (2015) 00032.