

Nonclinical evaluation of the potential for mast cell activation by an erythropoietin analog

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ABSTRACT

The erythropoietin analog peginesatide was withdrawn from marketing due to unexpected severe anaphylactic reactions associated with administration of the multi-use formulation. The adverse events occurred rapidly following the first ever administration of the drug with most affected patients becoming symptomatic in less than 30 min. This is most consistent with an anaphylactoid reaction due to direct activation of mast cells. Laboratory evaluation was undertaken using rat peritoneal mast cells as the model system. Initial studies showed that high concentrations of the formulated drug as well as formulated vehicle alone could cause mast cell degranulation as measured by histamine release. The purified active drug was not able to cause histamine release whereas the vehicle filtrate and lab created drug vehicle were equally potent at causing histamine release. Individual formulations of vehicle leaving one component out showed that histamine release was due to phenol. Dose response studies with phenol showed a very sharp dose response curve that was similar in three buffer systems. Cellular analysis by flow cytometry showed that the histamine release was not due to cell death, and that changes in light scatter parameters consistent with degranulation were rapidly observed. Limited testing with primary human mast cells showed a similar dose response of histamine release with exposure to phenol. To provide *in vivo* confirmation, rats were injected with vehicle formulated with various concentrations of phenol via a jugular vein cannula. Significant release of histamine was detected in blood samples taken 2 min after dosing at the highest concentrations tested.

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Introduction

Patients on hemodialysis secondary to kidney failure often require secondary treatment to maintain erythrocyte production. Where the failure is due to iron deficiency, the treatment is with an approved iron supplement. In other cases, direct stimulation of erythropoiesis is needed. There are several FDA approved products that are forms of human erythropoietin. These products have a long clinical track record and a well defined safety profile. On March 27, 2012, a new drug was approved for direct stimulation of erythropoiesis. This drug, peginesatide (Omontys®), is a synthetic peptide that does not have structural homology with human erythropoietin. Peginesatide consists of two identical

21 amino acid peptides covalently linked through sarcosine to a polyethylene glycol (PEG) molecule of ~40 kD. Each peptide has an internal disulfide bond between residues 6 and 15. The intended pharmacological action is stimulation of the erythropoietin receptor (EPOR) to increase production of erythrocytes.

Following the initiation of marketing of this drug, adverse event reports were received of anaphylactic type reactions. The reactions occurred in approximately 0.2% of patients with approximately 1/3 requiring serious medical intervention and with fatal reactions in 0.02% of patients (FDA website, 2013). These reactions occurred rapidly (<30 min) following administration of the first dose of the drug. Peginesatide adverse events were correlated with exposure of new groups of patients in dialysis clinics (Bennett et al., 2014). The drug was administered intravenously via an injection port on the dialysis machine during active dialysis. The drug was withdrawn from the market on February 23, 2013 by the manufacturer. A total of about 25,000 patients were treated with peginesatide prior to its withdrawal (FDA website, 2013).

A more detailed evaluation of individual case reports suggested a clinical picture consistent with an anaphylactoid reaction due to direct activation of mast cells. Anaphylactoid reactions represent non-IgE mediated activation of mast cells and/or basophils resulting in the release

Abbreviations: API, active pharmaceutical ingredient; HBSS, Hanks Balanced Salt Solution; hMCs, human mast cells; MCs, mast cells; MU, multiuse; MUV, multiuse vehicle; PMCs, peritoneal mast cells.

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of histamine, leukotrienes and other chemical mediators of inflammation. While anaphylactoid reactions are rare, many compounds have been shown to have the potential to induce them including radiographic contrast media, quinolone antibiotics, vancomycin, non-steroidal anti-inflammatory drugs (NSAIDs) and opiates (Farnam et al., 2012).

Mast cells are typically found in tissues rather than circulation and are present in normal human heart, particularly the atrial appendage (Sperr et al., 1994) and aorta (DeBruin et al., 2015). Their numbers increase between 1.7 and 6-fold in association with a variety of cardiac pathologies, including those observed in association with renal failure (reviewed in Janicki et al., 2015; DeBruin et al., 2015). Importantly, cardiac mast cells have been reported to play a role in fibrosis and formation of atherosclerotic plaques (Janicki et al., 2015). However, cardiac mast cells are difficult to isolate and while some differences exist between rodent and human mast cell sources (reviewed in Bischoff, 2007) we chose to perform laboratory experiments using purified rat peritoneal mast cells and human mast cells from humanized mice to test the hypothesis of anaphylactoid reaction and to determine whether a specific causative agent could be identified.

Materials and methods

Chemicals

The chemicals used to recreate the pharmaceutical vehicle were obtained from Sigma Chemical Corporation (St Louis, MO) and were of the highest grade available. Cell culture reagents were obtained from Life Technologies (Carlsbad, CA). The mast cell assay reagents Compound 48/80, teicoplanin (Sugimoto et al., 2000) and octyl β -glucoside were obtained from Sigma Chemical Corporation (St Louis, MO).

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee, Center for Drug Evaluation and Research, FDA, and carried out in an AAALAC-accredited facility.

Rats

Female Sprague-Dawley rats (10–12 weeks old) for mast cell donation were purchased from Taconic (Hudson, NY) and were doubly housed. Double cannulated rats used for the *in vivo* studies were also purchased from Taconic and were implanted with jugular vein and femoral vein cannulas by Taconic. Maintenance of the cannulas was performed according to the protocol provided by Taconic. The cannulated rats were single housed. All rats were fed Certified Purina Chow #5002 (Ralston Purina Co., St Louis, MO) and water *ad libitum*.

Mice

Female NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were group housed in individually ventilated cage racks, fed autoclaved LabDiet (St. Louis, MO) formula #5K52, and provided autoclaved acidified water *ad libitum*.

Mouse humanization methodology and testing

Humanization surgery was performed essentially as previously described (Melkus et al., 2006) with differences noted as follows. Bone marrow ablation was completed using a single dose of either Busulfan at 30 mg/kg, i.p. or Treosulfan at 2 g/kg i.p., 24 h prior to i.v. CD34+ stem cell infusion. Isolated CD34+ stem cells were either maintained in culture overnight or frozen and stored in liquid nitrogen and then administered either following implant surgery or three weeks post-surgery. Total number of CD34+ cells injected ranged from $2.2\text{--}2.4 \times 10^5$. Monitoring of humanization began approximately eight weeks after surgery and was assessed using whole blood every

3–4 weeks for over 20 weeks. Humanization analysis by flow cytometry employed RBC Lysis Buffer (Biolegend, San Diego, CA) according to manufacturer instructions. Cells were blocked using TruStain FcX and Human TruStain FcX (Biolegend, San Diego, CA) and stained using monoclonal antibodies against murine CD45 (clone 30-F11), human CD45, CD3, CD19, CD33, CD4 and CD8 (clones HI30, UCHT1, HIB19, WM53, RPA-T4, and RPA-T8, respectively; Biolegend, San Diego, CA). Samples were acquired and analyzed using a FACS Aria III flow cytometer and FACS Diva software (Becton, Dickinson & Co, Franklin Lakes, NJ). CountBright™ absolute counting beads (Life Technologies, Grand Island, NY) were used to determine absolute human cell numbers in addition to the percentage of human cells present. Mice were euthanized and splenocytes and bone marrow cells were isolated using standard techniques.

Rat mast cell isolation

Rat peritoneal mast cells were isolated using a method adapted from Shanahan et al., 1985. Briefly, an untreated rat was euthanized by CO₂ asphyxiation using IACUC approved methodology. The peritoneal cavity was injected with ice cold Hanks Balanced Salt Solution (HBSS) supplemented with 1% heat-inactivated horse serum (HBSS/HS) and 1000 IU/ml of sodium heparin. After 15 min, the peritoneal cells were aspirated and centrifuged at $120 \times g$ for 5 min. Cells were resuspended in 2 ml HBSS/HS and layered over 8 ml of a mixture of 50% Percoll and 50% RPMI 1640 with 10% horse serum. This was centrifuged 30 min at $300 \times g$. Purified rat peritoneal mast cells (PMCs) were recovered in the pellet and washed twice in HBSS/HS. Purity was >95% as assessed by toluidine blue staining.

Human mast cell isolation

Mast cells were isolated from mice with an engrafted human immune system. Human mast cells (hMCs) were isolated from combined spleen and bone marrow cell suspensions using Miltenyi Biotec (San Diego, CA) Fc-block, anti-human CD117-PE antibody (clone A3C6E2), followed by anti-PE microbeads (kit 130-048-801) according to manufacturer instructions. Cells were sorted using a MidiMACS magnetic separator and LS columns. The positive and negative fractions were washed and counted. A post-enrichment flow cytometric analysis was run to determine the percent hMC however, the antibody used to enrich for hMC appears to partially block binding of alternate antibodies used for phenotyping hMC. Therefore, toluidine blue was used to identify hMC and flow cytometry staining was used to identify the other cell types present (Supplemental Fig. 1). The purity of hMC across four mouse isolations from two original tissue donors was $55 \pm 9\%$ as assessed by toluidine blue staining. The remaining cells were primarily human hematopoietic precursors and murine neutrophils. Less than 0.6% of cells present following hMC enrichment were murine mast cells.

Histamine release assay

Purified rat or human MCs were resuspended at a concentration of 5×10^5 /ml in HBSS/HS. Test or control solutions in a volume of 90 μ l were placed in wells of a round-bottomed 96 well plate. Where needed, volume was made up to 90 μ l with HBSS/HS. A volume of 10 μ l of cell suspension was added and mixed. The plate was incubated for 30 min at 37 °C. The plate was centrifuged for 5 min at $120 \times g$ and then 50 μ l of the supernatant was transferred to a different well. The cell pellet was solubilized by adding 5 μ l of a 5% solution of the detergent octyl β -glucoside. The plate was then sealed and frozen at -30 °C for analysis of histamine release by LC–MS (Chimalakonda et al., 2015). Total available histamine was calculated as the sum of the histamine measured in half the supernatant well plus the amount of histamine released in the well with the other half supernatant plus cell pellet. Percent release was calculated at two times the half supernatant value divided by the

total histamine value. Triplicate wells were used for each experimental condition.

Rat histamine release in vivo

Rats were deeply anesthetized with isoflurane. A pretreatment sample was obtained from the jugular vein cannula (JVC). Following this, saline or treatment was administered *via* the JVC in a volume of 100 μ l by rapid push. Post treatment samples were obtained from either the JVC or femoral vein cannula (FVC) at +2 min and *via* the FVC at +5 and +9 min after treatment. A blood volume of 200 μ l was collected into 20 μ l of 0.5 M EDTA. Samples were stored on ice then centrifuged at 300 $\times$ g for 5 min. Plasma was removed to a separate tube and stored at -30°C until analyzed.

LC–MS assay for histamine

This assay was performed as previously described (Chimalakonda et al., 2015). Briefly samples, standards or blanks were mixed with acetonitrile, 0.1% formic acid and 100 ng/ml deuterium-labeled histamine. After centrifugation the supernatant was transferred into sample vials and analyzed on the LC–MS system.

Flow cytometry

Rat PMCs were resuspended in HBSS or MUV in a volume of 300 μ l. At 1 min before each time point, 4 μ l of propidium iodide (PI) solution (50 mg/ml) was added and mixed. Flow cytometry data was collected on 10,000 cells on a Stratified EX1413 instrument (San Jose, CA) using 488 nm excitation. Data were obtained for forward and side scatter as well as PI fluorescence.

Results

Drug composition

Various lots of the recalled multi-use formulations of the drug were extensively analyzed by FDA/CDER laboratories. The drug was found to be manufactured according to the specifications in the drug labeling. In addition, no contaminants were found within the limits of detection for all methods used in this phase of the analysis (internal communication, data not shown). The composition of the multi-use formulation is shown in Table 1 and the single dose formulation in Table 2.

Effect of MU drug or MU vehicle on histamine release from rat PMC

In order to determine if the active pharmaceutical ingredient (API) or vehicle alone could induce direct mast cell degranulation, the MU product and laboratory formulated MU vehicle were tested on rat PMC (Fig. 1). These data show that histamine release is induced from rat MC by the marketed drug formulation and by reformulated vehicle alone, in a dose dependent manner. The amount of release was the same for equal concentrations of drug or vehicle. The effect of pH on histamine release was investigated using three different pH 5.4 buffers

Table 1
Formulation of peginesatide multi-use vials.¹

Component	mg per ml
Peginesatide	10
Sorbitol	47
Phenol	5
L-Methionine	1.5
Glacial acetic acid	0.6
Sodium hydroxide	To adjust pH to 5.4 $\pm$ 0.5

¹ As listed in approved drug label, December 2012.

Table 2
Formulation of peginesatide single use vials.¹

Component	mg per ml
Peginesatide	2–12 (multiple strengths)
Sorbitol	47
Sodium phosphate monobasic	3
Sodium phosphate dibasic	0.12
Polysorbate 20	0.04
Sodium hydroxide	To adjust pH to 6.0 $\pm$ 0.3

¹ As listed in approved drug label, December 2012.

(acetate, citrate and succinate based) and histamine release is not induced by low pH alone (data not shown).

Histamine release by variant MUV formulations

Initial studies demonstrated that vehicle alone could induce direct PMC degranulation, so variant formulations of the MU vehicle were created that had one of the components missing. These were tested for their ability to induce histamine release from rat PMC to identify the component(s) associated with direct mast cell activation (Fig. 2). These data demonstrate that all formulations with phenol present induce histamine release and the variant without phenol does not induce histamine release.

Dose response of histamine release

To confirm that the effect observed in Fig. 2 was due to phenol, a dose response study was performed with phenol in three different buffer systems that included the multi-use vehicle formulation (pH 5.4), citrate buffer (pH 5.4), and Hank's Balanced Salts Solution (pH 7.4). The data in Fig. 3 show that the dose response of histamine release induced by phenol-containing buffers is generally similar with some pH effect.

Histamine release and single versus multi-use formulation of peginesatide

Both single use formulated drug and multi-use formulated drug products were purified over a centrifugal filtration column with a

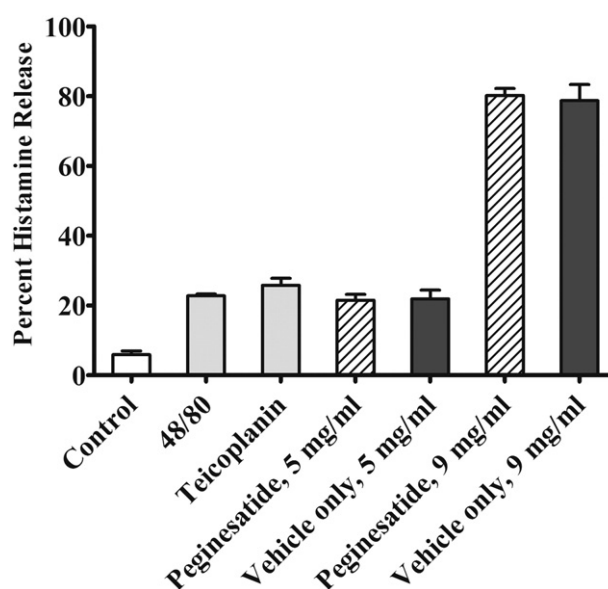


Fig. 1. Effect of MU drug or MU vehicle on histamine release by rat PMC. Control: HBSS only, positive controls (light gray bars): 48/80: Compound 48/80 at 10 μ g/ml, Teicoplanin at 500 μ g/ml. Hatched bars: Peginesatide multi-use formulation. Solid Bars: Lab version of multi-use formulation with no API.

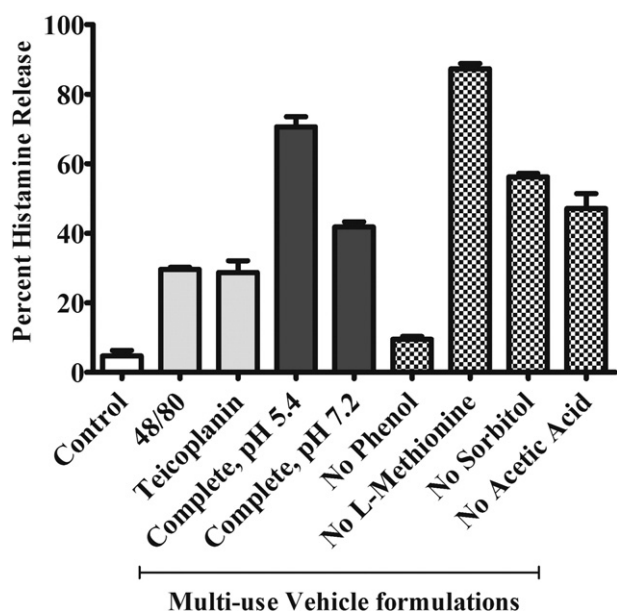


Fig. 2. Effect of the absence of individual MUV components on histamine release from rat PMC. Control: HBSS only, positive controls (light gray bars): 48/80: Compound 48/80 at 10 $\mu\text{g}/\text{ml}$, Teicoplanin at 500 $\mu\text{g}/\text{ml}$. Dotted bar, lab MUV w/o phenol. Solid bars — complete MUV at pH 5.4 or pH 7.2. Hatched bars: MUV without the indicated component.

30 kD molecular weight cutoff. As the pegesatide API has a molecular weight of 45 kD, it should be entirely retained by the filter. The purified vehicle and API in each formulation were tested for their ability to induce release of histamine from rat PMC (Fig. 4). The results demonstrate that multi-use vehicle but not the single use vehicle induces histamine release. Further, the API alone, resuspended in HBSS does not have the ability to induce histamine release.

Effect of MUV on MC viability

In order to address the possibility that observed mast cell degranulation was caused by lysis of cultured mast cells, a study was performed to evaluate the effect of incubation with MUV on the viability of purified rat PMC (Fig. 5). There was minimal effect of the MUV on viability of the PMC as measured by exclusion of propidium iodide indicating that degranulation was occurring *via* interaction with live cells.

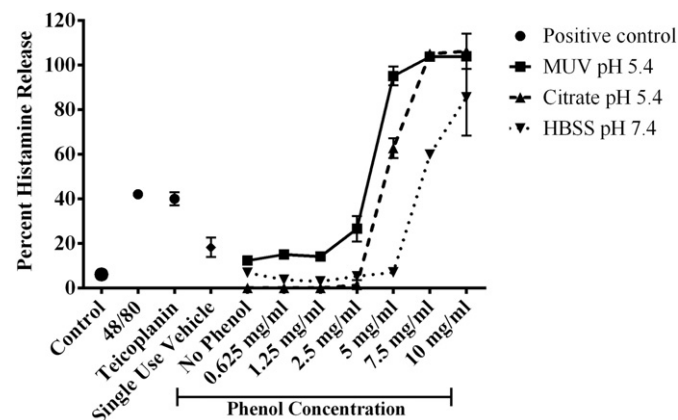


Fig. 3. Dose response of phenol-induced rat PMC histamine release in various buffers. Control: HBSS only, positive controls: 48/80: Compound 48/80 at 10 $\mu\text{g}/\text{ml}$, Teicoplanin at 500 $\mu\text{g}/\text{ml}$. Solid line/squares: MUV at pH 5.4, long dashed line/triangles: citrate buffer at pH 5.4, short dashed line/inverted triangles: HBSS at pH 7.4.

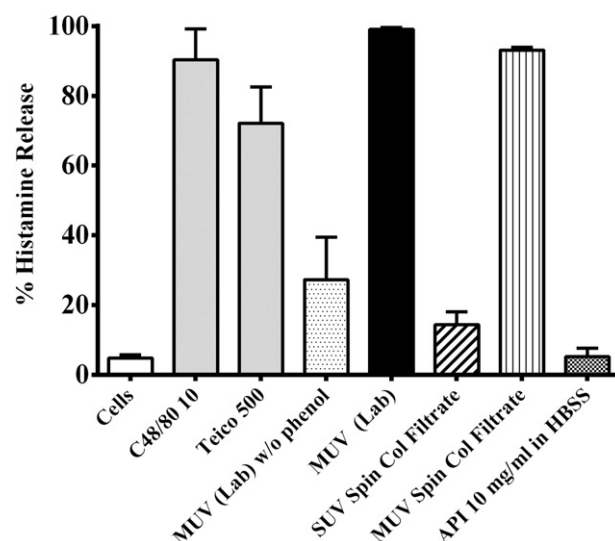


Fig. 4. Effect of purified MUV and purified API on histamine release from rat PMC. Positive controls (light gray bars): 48/80: Compound 48/80 at 10 $\mu\text{g}/\text{ml}$, Teicoplanin at 500 $\mu\text{g}/\text{ml}$. Dotted bar, lab MUV w/o phenol. Solid Bar: lab MUV only. Slanted bar: commercial SUV spin column filtrate. Vertical bar: commercial MUV spin column filtrate. Hatched bar: Purified API at 10 mg/ml in HBSS.

The effect of MUV on MC light scatter

To further evaluate the effect of MUV on rat PMC, a standard forward scatter *versus* side scatter plot was made for selected time points (Fig. 6). The top two plots show the light scatter of control PMC in HBSS at -3 min (Fig. 6A) and $+35$ min incubation (Fig. 6B) and only minor changes are observed. In contrast, the two lower plots show the light scatter of PMC in MUV at 1 (Fig. 6C) or 10 min incubation time (Fig. 6D) and clearly show obvious changes in light scatter characteristics. The observed changes are consistent with those previously reported for PMC degranulation (Hide et al., 1993).

Release of histamine from human MC

In order to confirm that degranulation observed in rat PMC in response to phenol in the MUV, we isolated hMC and tested them in the same culture system. Human mast cells were isolated from pooled spleen and bone marrow cell suspensions of mice engrafted with a

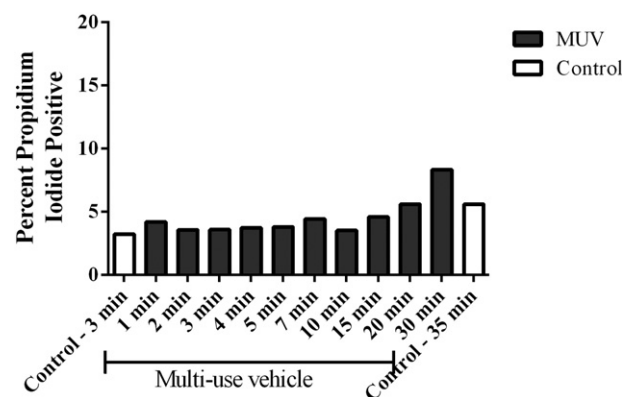


Fig. 5. Effect of exposure to multi-use vehicle on purified rat mast cell viability. Propidium iodide uptake measured by flow cytometry at time points indicated. Open bars — PMC in HBSS. Black bars — PMC in MUV for indicated amount of time.

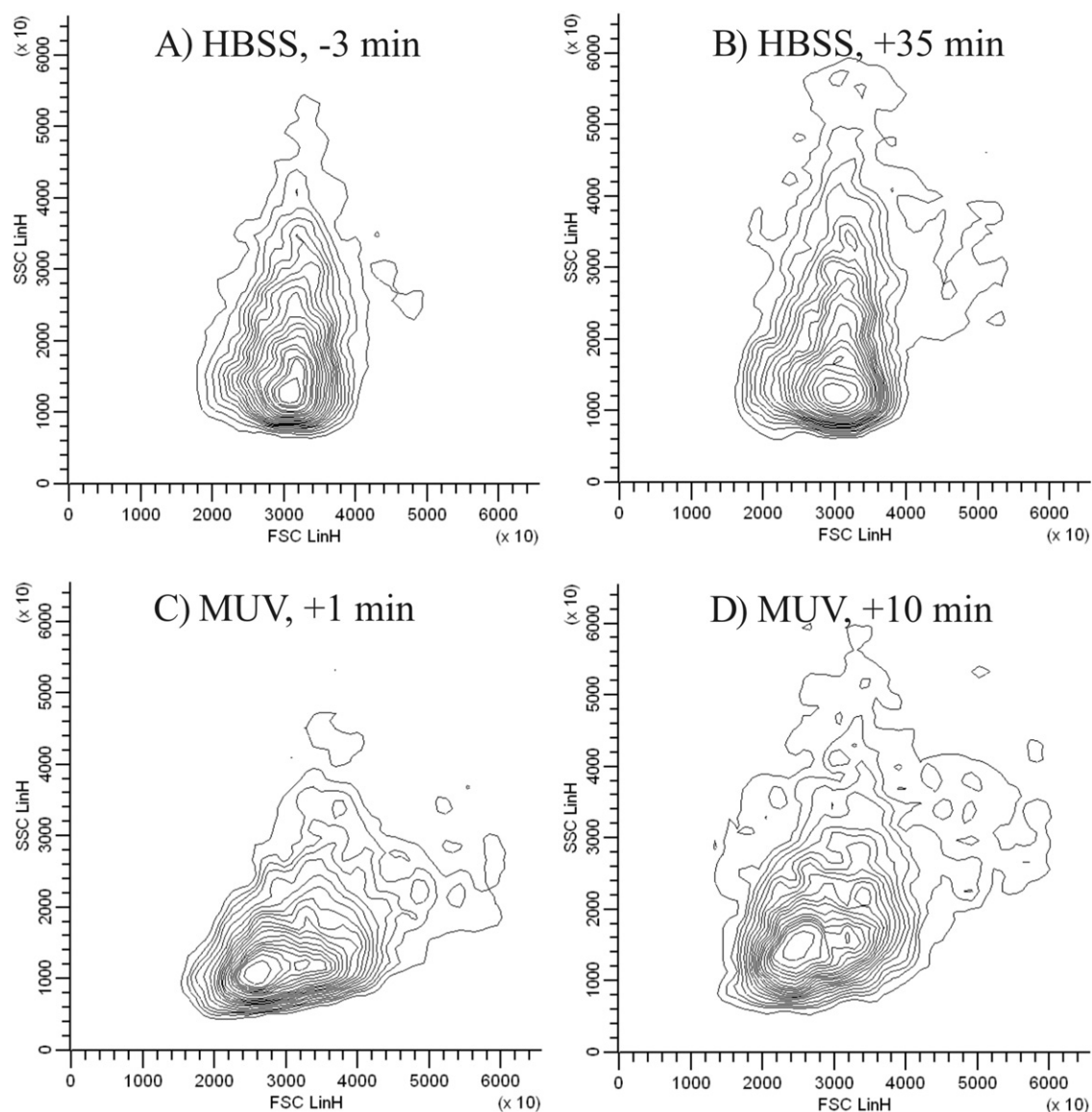


Fig. 6. Effect of MUV on light scatter of purified rat mast cells. Rat PMC were suspended in HBSS or 90% MUV and analyzed by flow cytometry at the indicated times. A: HBSS at -3 min, B: HBSS at $+35$ min, C: MUV at $+1$ min, D: MUV at $+10$ min. FSC: Forward light scatter (narrow angle), SSC: Side light scatter (90°).

human immune system. Four different isolations from two unique donors were tested. The data (Fig. 7) show that hMCs have a similar dose response of histamine release to phenol as compared to rat PMC. In addition, the response of hMC to the standard MUV is shown at a phenol concentration of 5 mg/ml and is clearly positive. We also tested the SUV and the spin-purified API (data not shown) and neither induced release of any histamine from human MC, similar to rat PMC responses.

Release of histamine *in vivo*

To ensure that this was not a purely *in vitro* phenomenon, we attempted to induce histamine release *in vivo* by rapid injection of HBSS with various amounts of phenol *via* a surgically implanted jugular cannula. The results in Fig. 8 show that release of very high amounts of histamine could be induced *in vivo* by injection of a phenol-containing solution in rats at concentrations of 15 mg/ml and 20 mg/ml. Interestingly, one rat exhibited clinical signs of histamine release including

red flushing of the skin, hives and increased respiratory rate that necessitated *i.v.* treatment with diphenhydramine.

Discussion

The studies reported here were part of a larger investigation to evaluate the unexpected adverse events associated with the clinical use of peginesatide. The careful evaluation of the clinical reports showed that the adverse events were highly consistent with an anaphylactic type reaction. Further, nearly all patients who experienced an adverse event did so in their first lifetime exposure to the drug. This suggests that this is more likely to be an anaphylactoid response resulting from direct mast cell degranulation. Histamine release was selected as a widely accepted indicator of mast cell degranulation. Given the large number of mediators released when mast cells degranulate it is likely that histamine is only one of the factors responsible for the actual pathology. Our aim for these studies was to determine whether this effect could be replicated in a laboratory model. The results of the studies showed

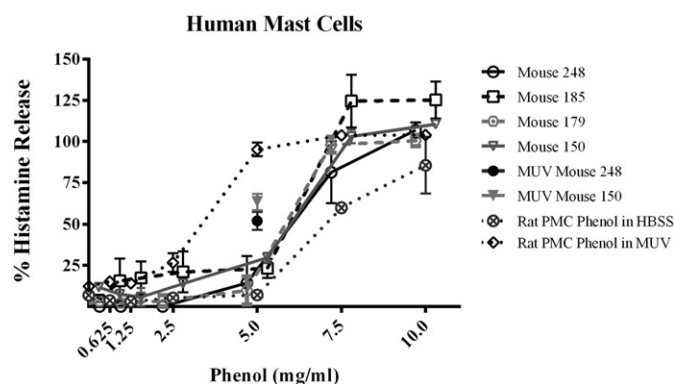


Fig. 7. Histamine release by human primary mast cells in response to phenol-containing solutions. Mice with an engrafted human immune system were used as a source of human mast cells by isolating leukocytes from spleen and bone marrow and sorting cells using a MidiMACS magnet and LS column. Dose response curves for four individual isolations are shown (open black circle, open square, open gray circle, open inverted gray triangle). Individual results for 5 mg/ml treatment from two isolations are shown as solitary points (filled black circle and filled inverted gray triangle). As a comparison, dose response curves for rat PMC results are shown for phenol in either HBSS or MUV (cross-hatched circle and open diamond) from Fig. 3.

that phenol in the multiuse vehicle and not the active drug was able to induce degranulation of rat and human primary MC *in vitro* and rat MC *in vivo*.

Other studies with phenol

Previous studies on the effect of phenol on mast cells are rare. In one study, rats were dosed IP with 1 ml of phenol at 10 mg/ml. When examined 24 h later, the PMCs were normal in number and in ability to degranulate *in vivo* (Macedo et al., 2006). In another study, phenol at various concentrations was reported to cause histamine release from mixed pleural and peritoneal MC (Diel et al., 1981).

Effects of other pharmaceuticals on mast cells

Other pharmaceuticals have been shown to cause direct mast cell degranulation. These include vancomycin (Horinouchi et al., 1993), teicoplanins (Sugimoto et al., 2000), miconazole (Toyoguchi et al., 2000) as well as opiates (Sheen et al., 2007). The release of histamine

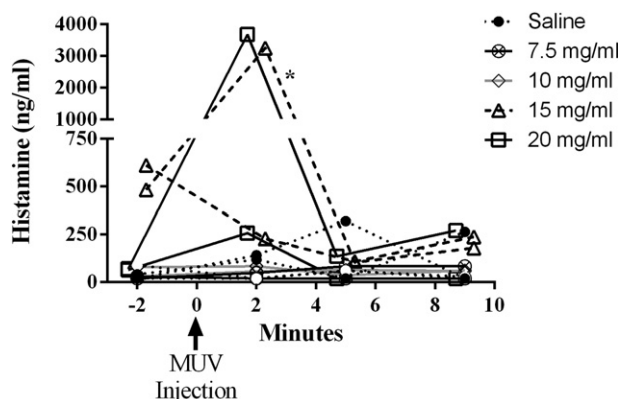


Fig. 8. Effect of varying amounts of phenol in MUV on serum histamine levels in rats. Four saline control and two rats at each dose (7.5, 10, 15, and 20 mg/ml) were injected with an i.v. bolus of the indicated treatment via a jugular vein cannula. Following injection rats had serum histamine assessed at 2, 5 and 9 min post-injection. The asterisk indicates a rat with clinical signs requiring i.v. treatment with diphenhydramine. All rats receiving a specific dose are shown using the same symbols. Legend: saline rats = closed circle with dashed lines; 7.5 mg/ml = open circle with cross-hatch and solid black line; 10 mg/ml = open diamond with light gray line; 15 mg/ml = open triangle with dashed line; and 20 mg/ml = open squares with solid black line.

in vivo has also been observed following treatment with intravenous vancomycin in rats (Shuto et al., 1999). The pretreatment of rats with morphine resulted in increased serum histamine after vancomycin treatment, suggesting the potential for an increased risk of anaphylactoid reactions in patients treated with both drugs (Shuto et al., 1999). The *in vivo* release of histamine has also been observed following IV treatment with vancomycin in humans (Polk et al., 1988). This release was found to be dependent on the rate of infusion suggesting a concentration dependence similar to the observations with phenol in this study. Phenol is used as an excipient in a number of other injectable drug formulations. A few are administered by IV bolus in a peripheral vein, but are not labeled for direct administration *via* dialysis machines. The rate of exposure with IV injection in a peripheral vein is much different than administration during active dialysis where blood is pumped at rates of 200–600 ml/min (Mitra, 2005).

Calculations concerning human mast cells

A question of some relevance to this issue is the number of mast cells that would need to degranulate to produce clinical symptoms. An estimate of this is that the degranulation of 10.7 million MCs would be sufficient to release the same amount of histamine observed in vancomycin-induced Red Man syndrome (Supplemental Table 1). A similar estimate demonstrates that fatal anaphylaxis can result from the release of the amount of histamine exported from degranulation of 46 million MCs (Supplemental Table 2). Further calculations lead to the conclusion that these outcomes could result from degranulation of 1.3% or 5.8% respectively of human cardiac mast cells (Supplemental table 3). An additional potential factor is that mast cell density can be increased between 2 and 10-fold over controls as measured in clinical cardiovascular disease or in a variety of experimental animal models (reviewed in Levick et al., 2011). Finally, it should be noted that human cardiac mast cells are highly concentrated in the atrial appendage, maximizing exposure of hMC to materials arriving from veins used for dialysis (Sperr et al., 1994).

Other sources of histamine

It has been shown recently that human neutrophils contain a small amount of histamine (Alcaniz et al., 2013). The reported histamine content is given as 0.29 pg/cell as compared with 2 pg/cell for human MC (Hogan and Schwartz, 1997). The kinetics of release appear to be much slower than that of mast cells suggesting that neutrophils are less likely to be the cause of the extremely rapid clinical adverse events. In the same study, it was noted that eosinophils and lymphocytes were isolated and found not to have detectable levels of histidine decarboxylase, the enzyme required for histamine production. Basophils in circulation are reported to contain 1 pg/ml of histamine (Casolaro et al., 1989), but we were not able to locate any published data on the effect of phenol on histamine release by basophils.

Summary & conclusions

In conclusion, the studies reported here have established that the multi-use vehicle of peginesatide is able to induce strong degranulation of rat peritoneal mast cells. The single use vehicle and the API are not able to induce rat PMC degranulation. The data have shown that the degranulation activity is due to phenol and that the ability of phenol to induce degranulation is independent of buffer. Studies using flow cytometry have shown that the release of histamine is not due to death of the PMC and that light scatter changes consistent with degranulation are observed. Human MC showed responses highly similar to the rat PMC results. Finally, release of high levels of serum histamine can be induced in an *in vivo* model using cannulated rats. The low patient incidence (0.2%) is probably related to a combination of factors including inherent MC sensitivity as well as disease associated increases in the

number of cardiac MC (reviewed in Levick et al., 2011). These data show how *in vitro* and *in vivo* studies can suggest mechanisms that may be associated with the adverse events caused by the multi-use formulation of peginesatide.

Disclosure statement

All authors were employees of the U. S. Food and Drug Administration when this work was performed. There are no conflicts of interest to declare.

Transparency document

The Transparency Document associated with this article can be found, in the online version.

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Disclaimer

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be considered to represent any agency determination or policy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.taap.2015.06.009>.

References

- Alcaniz, L., Vega, A., Chacon, P., el Bekay, R., Ventura, I., Aroca, R., Blanca, M., Bergstralh, D.T., Monteseirin, J., 2013. Histamine production by human neutrophils. *FASEB J.* 27, 2902–2910. <http://dx.doi.org/10.1096/fj.12-223867>.
- Bennett, C.L., Jacob, S., Hymes, J., Usvyat, L.A., Maddux, F.W., 2014. Anaphylaxis and hypotension after administration of peginesatide. *N. Engl. J. Med.* 370, 2055–2056. <http://dx.doi.org/10.1056/NEJMc1400883>.
- Bischoff, S.C., 2007. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat. Rev. Immunol.* 7 (2), 93–104. <http://dx.doi.org/10.1038/nri2018> (Feb).
- Casolaro, V., Galeone, D., Giacummo, A., Sanduzzi, A., Melilli, G., Marone, G., 1989. Human basophil/mast cell releasability. V. Functional comparisons of cells obtained from peripheral blood, lung parenchyma, and bronchoalveolar lavage in asthmatics. *Am. Rev. Respir. Dis.* 139, 1375–1382.
- Chimalakonda, K.C., Pang, E., Weaver, J.L., Howard, K.E., Patel, V., Boyne II, M.T., 2015. Development and validation of a liquid-chromatography tandem mass spectrometry method to determine *in vitro* and *in vivo* histamine release. *J. Pharm. Biomed. Anal.* 102, 494–499. <http://dx.doi.org/10.1016/j.jpba.2014.10.016> (Oct 23).
- DeBruin, E.J., Gold, M., Lo, B.C., Snyder, K., Cait, A., Lasic, N., Lopez, M., McNagny, K.M., Hughes, M.R., 2015. Mast cells in human health and disease. *Methods Mol. Biol.* 1220, 93–119. http://dx.doi.org/10.1007/978-1-4939-1568-2_7.
- Diel, F., Neidhart, B., Oprea, W., 1981. Application of rat mast cell incubates as a possible short-time test for sensitizing occupational chemicals. *Int. Arch. Occup. Environ. Health* 48, 369–373.
- Farnam, K., Chang, C., Teuber, S., Gershwin, M.E., 2012. Nonallergic drug hypersensitivity reactions. *Int. Arch. Allergy Immunol.* 159, 327–345. <http://dx.doi.org/10.1159/000339690>.
- FDA website, 2013. Recall — Firm Press Release: Affymax and Takeda Announce a Nationwide Voluntary Recall of All Lots of OMONTYS® (peginesatide) Injection Feb 23, URL: <http://www.fda.gov/Safety/Recalls/ucm340893.htm> (accessed June 23, 2014).
- Hide, I., Bennett, J.P., Pizzey, A., Boonen, G., Sar-Sagai, D., Gomperts, B.D., Tatham, P.E.R., 1993. Degranulation of individual mast cells in response to Ca^{++} and guanine nucleotides: an all-or-none response. *J. Cell Biol.* 123, 585–593.
- Hogan, A.D., Schwartz, L.B., 1997. Markers of mast cell degranulation. *Methods* 13, 43–52.
- Horinouchi, Y., Abe, K., Kubo, K., Oka, M., 1993. Mechanisms of vancomycin-induced histamine release from rat peritoneal mast cells. *Agents Actions* 40, 28–36.
- Janicki, J.S., Brower, G.L., Levick, S.P., 2015. The emerging prominence of the cardiac mast cell as a potent mediator of adverse cardiac remodeling. *Methods Mol. Biol.* 1220, 121–139. http://dx.doi.org/10.1007/978-1-4939-1568-2_8.
- Levick, S.P., Meléndez, G.C., Plante, E., McLarty, J.L., Brower, G.L., Janicki, J.S., 2011. Cardiac mast cells: the centrepiece in adverse myocardial remodelling. *Cardiovasc. Res.* 89, 12–19. <http://dx.doi.org/10.1093/cvr/cvq272>.
- Macedo, S.M.D., Lourenco, E.L.B., Porelli, P., Fock, R.A., Ferreira Jr., J.M., Farsky, S.H.P., 2006. Effect of *in vivo* phenol or hydroquinone exposure on events related to neutrophil delivery during an inflammatory response. *Toxicology* 220, 126–135. <http://dx.doi.org/10.1016/j.tox.2005.12.008>.
- Melkus, M.W., Estes, J.D., Padgett-Thomas, A., Gatlin, J., Denton, P.W., Othieno, F.A., Wege, A.K., Haase, A.T., Garcia, J.V., 2006. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nat. Med.* 12 (11), 1316–1322. <http://dx.doi.org/10.1038/nm1431>.
- Misra, M., 2005. The basics of hemodialysis equipment. *Hemodial. Int.* 9, 30–36. <http://dx.doi.org/10.1111/j.1492-7535.2005.01115.x>.
- Polk, R.E., Healy, D.P., Schwartz, L.B., Rock, D.T., Garson, M.L., Roller, K., 1988. Vancomycin and the red-man syndrome: pharmacodynamics of histamine release. *J. Infect. Dis.* 157, 502–507. <http://dx.doi.org/10.1093/infdis/157.3.502>.
- Shanahan, F., Denburg, J.A., Fox, J., Bienenstock, J., Befus, D., 1985. Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. *J. Immunol.* 135, 1331–1337.
- Sheen, C.H., Schleimer, R.P., Kulka, M., 2007. Codeine induces human mast cell chemokine and cytokine production: involvement of G-protein activation. *Allergy* 62, 532–538. <http://dx.doi.org/10.1111/j.1398-9995.2007.01345.x>.
- Shuto, H., Sueyasu, M., Otsuki, S., Hara, T., Tsuruta, Y., Kataoka, Y., Oishi, R., 1999. Potentiation of vancomycin-induced histamine release by muscle relaxants and morphine in rats. *Antimicrob. Agents Chemother.* 43, 2881–2884.
- Sperr, W.R., Banki, H.C., Gundigler, G., Klappacher, G., Grossschmidt, K., Agis, H., Simon, P., Laufer, P., Imhof, M., Radaszkiewicz, T., Glogar, D., Lechner, K., Valent, P., 1994. The human cardiac mast cell: localization, isolation, phenotype, and functional characterization. *Blood* 84, 3876–3884.
- Sugimoto, Y., Iba, Y., Utsugi, K., Kamei, C., 2000. Influences of evernimicin, vancomycin and teicoplanin on chemical mediator release from rat peritoneal mast cells. *Jpn. J. Pharmacol.* 83, 300–305. <http://dx.doi.org/10.1254/jip.83.300>.
- Toyoguchi, T., Ebihara, M., Ojima, F., Hosoya, J., Shoji, T., Nakagawa, Y., 2000. Histamine release induced by antimicrobial agents and effect of antimicrobial agents on vancomycin-induced histamine release from rat peritoneal mast cells. *J. Pharm. Pharmacol.* 52, 327–331.