Bullous allergic drug eruption with presence of myeloperoxidase and reorganization of the dermal vessels observed by using CD34 and collagen IV antibodies

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Citation: Abreu-Velez AM, Klein DA, Smoller BR, Howard MS. Bullous allergic drug eruption with presence of myeloperoxidase and reorganization of the dermal vessels observed by using CD34 and collagen IV antibodies. North Am J Med Sci 2011; 3: 82-84.
Doi: 10.4297/najms.2011.382
Availability: www.najms.org
ISSN: 1947 – 2714

Abstract

Context: Bullous allergic reactions are inflammatory skin disorders, presenting usually as a result of some type of reaction to medication. Case Report: A 67-year-old female was evaluated for the presence of diffuse patches of erythema, microvesiculation, vesicles, crusts, and oozing of sudden appearance on the extremities and on the rest of the body after taking sulfamethoxazole in combination with trimethoprim. Skin biopsies for hematoxylin and eosin examination, as well as for direct immunofluorescence and immunohistochemistry analysis were performed. H&E staining demonstrated classic features. Direct immunofluorescence revealed strong deposits of fibrinogen in the vessels of the skin. The immunohistochemistry stain showed strong positivity of myeloperoxidase within the blister cavity. The distribution of the vessels around the inflammatory process were noticed by using antibodies to CD34 as well to collagen IV. Conclusions: sulfamethoxazole is catalysed by CYP2C9 and/or myeloperoxidase. Thus, myeloperoxidase appears to be of importance in this disorder.

Keywords: CD34, bullous drug eruption, myeloperoxidase, collagen IV.

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Introduction

Bullous or blistering drug eruptions and drug-induced anaphylaxis and hypersensitivity syndromes are amongst the most serious types of adverse drug reactions. Based on the various mechanisms, bullous drug eruptions may be classified into the following categories: spongiotic or eczematous, acute generalized exanthematous pustulosis, fixed drug eruption, erythema multiforme, Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) [1, 2].

As with other bullous disorders, drug-induced blistering reactions take place via a diversity of pathophysiological mechanisms and at different levels within the epidermis and or at the dermoeipidermal junction. Examples of these mechanisms comprise the following: exocytosis and/or spongiosis, formation of subcorneal spongiform pustules, cytolysis and keratinocytic necrosis, antiepidermal antibody formation, deposition of immunoglobulin at the basement membrane zone (BMZ), and photo-induced matrix and collagen alterations that lead to a mechanobullous disorder [1, 2].

Case Report

A 67-year-old Female presented with clinical blisters and sudden prutitus, initially in the extremities that extended to the rest of the body, associated with diffuse patches of erythema, microvesiculation, vesicles, crusts, and oozing. The patients were taking sulfamethoxazole in combination with trimethoprim. A lesional skin biopsy was taken for
hematoxylin and eosin (H & E) analysis. In addition, a direct immunofluorescence (DIF) and immunohistochemistry (IHC) studies were performed.

**DIF**
In brief, skin cryosections were prepared, and incubated with multiple fluorescein isothiocyanate as previously reported [3-6].

**IHC**
It was performed as previously described [3-6].

**Microscopic Description**
Examination of the H&E tissue sections demonstrated a subepidermal blistering disorder. Within the blister lumen, numerous lymphocytes, histiocytes, eosinophils and neutrophils were present. Mast cells were rare. Focal, superficial dermal scarring was present. In addition, the dermis displayed a superficial, perivascular infiltrate of lymphocytes, histiocytes, neutrophils and occasional eosinophils (Figure 1).

![Figure 1a (10x) b (20x) H&E tissue sections demonstrates a subepidermal blistering and within the blister lumen, numerous lymphocytes, histiocytes, eosinophils and neutrophils are present (black arrows). c (10x) and d (40x). IHC positive myeloperoxidase positive inside the blister (red arrows). e. DIF showing positive stain against the superficial dermal vessels under the inflammatory process when using anti-human fibrinogen-FITC1 conjugated (green stain, white arrows). The red stain is antibody to collagen IV corroborating that these are vessels. f. H&E shows reorganization of the vessels around the hair follicles (black arrows). g through i. IHC using CD34, showing how most of the vessels around the inflammatory process are reorganized and loss their normal distribution on the skin (red arrows). j. DIF, showing positive stain with anti-collagen VI antibody (yellowish stain, red arrow) at the base membrane zone (BMZ) of the skin as well as also show reorganization of the superficial and intermediate vessels around the inflammatory process (blue arrows). k. IHC using anti-body against collagen IV (dark stain) shows the stain on the sweat glands, and also show some reorganization of the vessels around the sweat glands with some kind of polarization towards the inflammation (red arrows). l. DIF, showing positive stain with anti-human IgM-FITC1 conjugated to the sweat glands (yellow stain (white arrow). The nuclei were counterstained with Dapi (blue) and the sweat glands with mapped with anti-collagen VI antibody (red stain). DIF results
The test was performed and displayed the following results: IgG(-); IgG3(-); IgG4 (-); IgA(-); IgM(-); IgE (-); complement/C1q (-); complement/C3 (-); albumin (+, weak dermal perivascular) and fibrinogen (++, dermal perivascular). No deposits of complement and or immunoglobulins were seen in the BMZ.

**IHC results**
Immunohistochemistry showed the presence of myeloperoxidase in the entire subepidermal blister. In addition, we also noted alteration in the distribution and reorganization of the dermal vessels being closely located under the blister as determined by the CD34 and collagen IV antibodies (Figure 1). (Usually the superficial vessels not inflamed are located in the upper vascular plexus of the skin, with some space between the epidermis and them by the papillary dermis.

**Discussion**
In general, the incidence of adverse cutaneous reactions to drugs has been estimated at 0.1-2.2% of treatment courses; however, some penicillins and sulfamethoxazole/trimethoprim may have a significantly elevated incidence at 3-5% of treatment courses. Patients infected with human immunodeficiency virus infection may be at greater risk for adverse cutaneous drug reactions. SJS and TEN have are more frequent in those younger than 20 years and older than 65 years [1, 2].

Sulfamethoxazole is also administered in combination with trimethoprim, as co-trimoxazole, for prophylaxis and treatment of HIV-associated infections. Unfortunately, sulfamethoxazole administration is associated with the development of adverse drug reactions in between 1 and 5% of patients. The most common adverse events the development of cutaneous symptoms. Such reactions are thought to be immune mediated. To generate a drug antigen, sulfamethoxazole is metabolised in liver, blood cells and keratinocytes to a hydroxylamine metabolite [2, 7-10]. These reactions are catalysed by CYP2C9 and/or myeloperoxidase. However, polymorphisms in other drug metabolizing enzymes such as myeloperoxidase may be important to be considered.

We conclude that in all blistering diseases it is ideal to perform not only the biopsy for H & E, but also DIF and if possible some immunohistochemistry stains. A full
pathologic understanding of this process seems to require not only the inflammatory cells to possible contribute to induce the blisters, but also requires some reorganization with polarization of the vessels where these inflammatory arrive into the dermis. As shown some of these inflammatory cells (mainly neutrophils released strong myeloperoxidase that can also contribute to the blistering formation). Collagen IV and CD34 (vessels markers), also indicated that the vessels get physically closers to the main inflammatory target (in this case the blister)

In Figure 1, we demonstrate some of the most prominent findings using H&E, DIF and IHC stains.

Acknowledgments
Jonathan S. Jones HT (ASCP), and Lynn K. Nabers HT, HTL (ASCP) at GDA for excellent technical assistance.

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