Case Report

Erythroderma and Epidermal Necrosis Induced by a Type of Proton Pump Inhibitor in Beagle Dogs

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Abstract: Reddish skin covering the entire body (erythroderma) was observed in a preliminary one-week oral toxicity study of a type of proton pump inhibitor in Beagle dogs. Histologically, full-thickness epidermal necrosis accompanied by apoptosis, evidenced by an immunohistochemical positive reaction to cleaved caspase-3, and detachment of the epidermis were observed in the skin. In the epidermis and upper dermis, a slight infiltration of mononuclear cells was seen, which was predominantly positive for MAC387 (macrophages) and partly positive for CD3 (T lymphocytes). These findings have some similarities to drug-induced severe skin reactions such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) and may represent incipient changes of such lesions. The skin lesions observed in these dogs are being considered as a potential animal model of cutaneous drug reactions. (J Toxicol Pathol 2007; 20: 257–261)

Key words: drug-induced, cutaneous drug reactions, erythroderma, epidermal necrosis, dog, proton pump inhibitor

In addition to corrosive agents or skin irritants, systemically administered drugs can induce skin lesions. Cutaneous drug reactions are the most commonly reported adverse drug reaction. They encompass a large variety of clinical types, such as urticaria, maculopapular, bullous, fixed drug eruptions, erythema multiforme and more severe reactions such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)1–4. Although the precise pathogenesis is unknown, many of these are believed to be immune-mediated, and these can be divided into immediate- and delayed-type reactions1,3,5. We found a unique skin lesion in a preliminary one-week oral toxicity study of a new compound in dogs, and herein describe the lesion in detail by histological and immunohistochemical methods and discuss similarities of the lesion to SJS and TEN.

Beagle dogs were purchased from Marshall BioResources (North Rose, NY, USA) and acclimatized to laboratory conditions. They were 9 months old at the commencement of dosing. Three groups of dogs (1/sex/group) were treated with a type of proton pump inhibitor by oral gavage via a flexible catheter at low, mid, and high doses for 7 consecutive days (Day 1 to Day 7). A separate group (1/sex) was treated with a vehicle (0.5% methylcellulose) and served as a control. On the day following the last dosing, all dogs were euthanized and necropsied. This study was authorized by the Animal Ethics Committee of Pfizer Global Research and Development Nagoya Laboratories and was conducted in accordance with the Ethical Guidelines for the Use of Experimental Animals at Pfizer Inc.

Systemic reddish skin was first observed on Day 7 in the high dose treatment dogs. At necropsy on Day 8, reddish skin over the whole body (erythroderma), including the auricle and oral mucosa, was seen in the high dose group (Fig. 1), and there was slight desquamation in the abdomen. Their abdominal skin tissues (not protocol assigned) were taken as a gross abnormality. The reddish changes vanished after exsanguination. The abdominal skin samples were fixed in 10% neutral buffered formalin, trimmed, dehydrated and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE), Masson’s trichrome, periodic acid Schiff (PAS) and alcian blue. They were stained immunohistochemically using the avidin-biotin complex method (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA) for CD3 (1:100, Dako, Glostrup, Denmark), CD79α (1:1000, Dako, Glostrup, Denmark) and MAC387 (1:25000, Abcam, Tokyo, Japan), which are markers for T and B lymphocytes and macrophages, respectively. They were also stained for cleaved caspase-3 (1:400, Daiichi Pure Chemicals, Tokyo, Japan) to detect apoptosis. Since the skin tissues were taken only from the high dose group, skin samples of a vehicle-treated group.
Fig. 1. Gross photographs of the lower jaw (A) and abdomen (B) of a dog in the high dose group. The skins of the lower jaw and oral mucosa (A) and entire abdominal skin where the hair was shaved for necropsy (B) are reddish in color.

Fig. 3. Photomicrographs of upper dermis from a dog in the high dose group. In the dermis, there are many MAC387-positive macrophages (A) and a small number of CD3-positive T lymphocytes (arrows, B). Immunohistochemistry for MAC 387 (A) and CD3 (B).

Fig. 4. Photomicrographs of hair follicles from a dog in the high dose group. There are many single cell necrosis of keratinocytes (arrows) in the external root sheath (A), and they are positive for cleaved caspase-3 (B). HE (A), immunohistochemistry for cleaved caspase-3 (B).
Fig. 2. Photomicrographs of epidermis and upper dermis from dogs in the high dose group showing A) full-thickness epidermal necrosis and mononuclear cell infiltration in the dermis, B) vacuolated keratinocytes and eosinophilic materials (arrows) in the necrotic epidermis, C) swollen keratinocytes with a stratified squamous structure and slight dermal edema, D) cleaved caspase-3 positive keratinocytes in an adjacent section of C, E) slight detachment of necrotic epidermis and reepithelialization (arrows) and F) moderate epidermal detachment. HE (A–C, E, F), immunohistochemistry for cleaved caspase-3 (D).
Microscopically, epidermal necrosis and thinning were observed in both dogs in the high dose group; in most parts, full-thickness epidermal necrosis was seen in which the epidermis was very thin and no nuclei (complete loss of nuclei) or only a single degenerative/necrotizing cell layer was detected (Figs. 2A, 2B). Meanwhile, in some parts, swollen (vacuolated) and degenerative/necrotizing keratinocytes with a stratified squamous structure were observed (Fig. 2C). These degenerative/necrotizing keratinocytes included small vacuoles and eosinophilic materials in the cytoplasm (Figs. 2B, 2C). The eosinophilic materials were not stained red by Masson’s trichrome, and thus they were differentiated from erythrocytes. In addition, the materials were not stained positive by PAS. Immunohistochemistry showed that a number of the degenerative/necrotizing keratinocytes were positive for cleaved caspase-3, which was indicative of apoptosis (Fig. 2D). In the affected epidermis and its adjacent dermis (upper epidermis), a slight infiltration of mononuclear (macrophage- or lymphocyte-like) cells were seen (Figs. 2A, 2B). These cells consisted mainly of MAC387-positive macrophages and sparse CD3-positive T lymphocytes (Fig. 3), while they were negative for CD79α (B lymphocytes). No eosinophils or increased number of mast cells (by alcin blue staining) were observed in the dermis. Some parts of the dermis just beneath the epidermis were slightly edematous with angiogenesis, and slight to moderate detachment of the epidermis, rarely accompanied by reepithelialization, was seen, although no bullae (blistering) was apparent at necropsy (Figs. 2C, 2E, 2F). In the hair follicles, the stratified squamous structure of the external root sheath was more preserved compared with the severe epidermal changes described above. However, a lot of single cell necrosis of keratinocytes was seen that were positive for cleaved caspase-3 (Fig. 4). Many MAC387-positive macrophages were also seen around the hair follicles.

Drug-induced SJS and a more severe (widespread detachment of the epidermis) type of TEN, characterized by a macular exanthema, are rare but serious and potentially life-threatening reactions of the skin. Histopathologically, scattered necrotic keratinocytes in the epidermis are seen in the early stages of SJS/TEN. Late stage lesions reveal confluent “full-thickness” epidermal necrosis, which ultimately results in formation of subdermal bullae. Dermal mononuclear cell infiltration varies from sparse (‘silent dermis’) to dense. Damage to the skin is thought to be mediated by cytotoxic T lymphocytes and mononuclear cells, which induce apoptosis in keratinocytes expressing drug-derived antigens at their surfaces. Keratinocyte apoptosis has been shown to be triggered by caspase cascade either through Fas (CD95, a cell surface death receptor) and its ligand (FasL) or the perforin/granzyme B (cytotoxic granule proteins) pathway and is a hallmark of the early stages of SJS/TEN. The typical interval between the onset of drug therapy and SJS/TEN is between 1 and 3 weeks.

Although there was no severe skin detachment in the dogs at necropsy, the histopathological changes of the epidermal necrosis were similar to SJS/TEN in that total epidermal necrolysis, apoptosis of epidermal keratinocytes, detachment of the epidermis and sparse inflammatory infiltration in the epidermis/dermis were the major findings. These findings for the dogs may represent an incipient to middle period change of SJS/TEN-like lesions followed by the onset of gross skin detachment in later stages. In regard to epidermal or dermal infiltrates, skin biopsies from TEN patients have previously revealed that macrophages are the most numerous cells, although T lymphocytes are predominant in blister fluid. These results are consistent with the immunohistochemical findings in the present study. Although there are other cutaneous drug reactions in humans that require a differential diagnosis to SJS/TEN, such as erythema multiforme, maculopapular drug exanthema, drug-induced pemphigoid and pemphigus, drug-induced linear IgA bullous dermatosis, and fixed drug eruptions, these reactions are dissimilar to the present case in terms of presence or absence of full-thickness epidermal necrosis, degree/type of cell infiltration, edema, erythrocyte extravasation, etc.

In addition to the skin lesions, dose-dependent severe hepatotoxicity was evidenced by increased alanine aminotransferase (ALT) that was approximately 5 times higher at the low dose to 50 times higher at the high dose than the pre-dose values in the present study. Histologically, hepatic changes were characterized by prominent mononuclear cell infiltration (many of them were CD3 or MAC387 positive), moderate hepatocellular necrosis and severe hemorrhage and fibrosis. These findings were primarily seen in the centrilobular areas (data not shown). Since hepatic disorders are complications of drug eruptions including SJS/TEN, the changes induced in the liver in this study may be associated with the skin lesions.

Sulfonamide is one of the most common drugs that cause drug-induced hypersensitivity reactions in humans. Severe sulfonamide-associated skin eruptions, including TEN, erythema multiforme and pemphigus foliaceus, and acute hepatopathy have been reported in dogs and are considered to be a useful animal model for sulfonamide-induced hypersensitivity reactions. The epidermal changes in this study were unpredictable when considering the pharmacological effects of the compound. However, for proton pump inhibitors, fetal TEN due to lansoprazole, erythrodemic reactions due to omeprazole and lansoprazole and allergic contact dermatitis due to lansoprazole have been reported in human patients. Although the information obtained in the present study is limited because of its preliminary properties, if involvement of an immune-related mechanism is confirmed, the epidermal necrosis in the dog could become a useful animal model for mechanistic studies of drug-induced hypersensitivity reactions, including cutaneous drug reactions. It would be an advantage that the
cutaneous or hepatic changes could be induced within a short period (1 week) and also dose-dependently.

In conclusion, systemic erythroderma was induced by a type of proton pump inhibitor in Beagle dogs. Histologically, full-thickness epidermal necrosis accompanied by apoptosis, detachment of the epidermis and slight cell infiltration (macrophages and T lymphocytes) in the dermis were observed. These histology findings resembled those seen in SJS/TEN. The skin lesions observed in these dogs are being considered as a potential animal model of cutaneous drug reactions.

References