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Possible In Vitro Model of Toxic Epidermal Necrolysis

Toxic epidermal necrolysis (TEN) is an acute, life-threatening, and potentially blinding mucocutaneous disease. It was first described by Lyell in 1956. While several factors including a spectrum of medications that may play a role in the pathogenesis of TEN have been reported, the disease mechanism remains unknown. The lack of in vitro and animal models is a major obstacle in investigating the pathogenesis and treatment of TEN. The purpose of this study was to develop an in vitro model for TEN.

Methods. A pool of sera from 3 patients with biopsy-proven, acute TEN was used as a reagent in an organ culture. A pool of sera from 3 age- and race-matched healthy individuals was used as a control reagent. Normal human skin (NHS), normal human conjunctiva (NHC), and normal human buccal mucosa (NHBM) were used as substrates in the organ culture. Informed consent was obtained from all patients and healthy individuals. All experiments were approved by an institutional review board.

The in vitro organ culture investigating the effect of TEN serum on normal human tissue was similar to an earlier technique that investigated the effect of sera obtained from patients with pemphigus vulgaris or ocular cicatricial pemphigoid.

After the incubation had been terminated, pieces of NHS, NHC, and NHBM were embedded in paraffin blocks for routine histopathological analysis.

Results. On light microscopy, specimens of NHS, NHC, and NHBM incubated with TEN serum showed morphologic changes consistent with TEN (Figure 1). These changes were observed in all epithelial cell layers of NHS and NHBM after 72 hours and in NHC after 48 hours of incubation. Extensive shedding of the epithelial cells, leaving only the basal layer, was observed in NHS and NHBM incubated in normal human serum for the same period.

In addition, a separation between the basal epithelial cell layer and basement membrane zone was observed in NHS and NHBM after 72 hours of incubation. The separation was not observed in NHC during the incubation period of up to 96 hours. On the other hand, unlike NHS and NHBM, areas of frank acantholysis and epithelial shedding were observed in NHC after 48 hours of incubation with TEN serum. The epithelium of NHS, NHC, and NHBM cultured in normal human serum remained intact (Figure 1). Histopathological features in biopsy specimens of skin and conjunctiva from a patient during an acute phase of TEN were similar to those observed in NHS and NHC incubated in the pool of TEN sera (Figure 2).

Comment. The results of this study demonstrate the in vitro effect of sera obtained from patients in the acute phase of TEN on normal human tissues typically affected by the disease. The histopathological changes induced in vitro were similar to those observed in the biopsied tissues of patients with TEN. Necrosis involving the full thickness of the epithelium was observed in NHS, NHC, and NHBM incubated with TEN serum. Similar findings have been previously described in the skin and conjunctival biopsy specimens of patients with TEN.

Significant pathological findings were observed in NHC after shorter incubation times compared with NHS and NHBM, suggesting that conjunctival epithelium is more susceptible to injury by the pathogenic components in TEN serum. It is possible that this plays a role in induction of chronic cicatizing conjunctivitis, seen in approximately one-third of patients who recover from the acute phase of TEN. Unlike conjunctiva, skin and buccal mucosa typically heal within a few weeks after the acute phase of TEN and do not exhibit clinically evident chronic inflammation.
Figure 2. Hematoxylin-eosin staining of a skin biopsy specimen (A) and a conjunctival biopsy specimen (B) obtained from a patient during an acute stage of toxic epidermal necrolysis (original magnification ×160). Note the separation between the basal epithelial cell layer and the basement membrane zone in the skin as well as acantholysis of the conjunctival epithelium.

In summary, this study could represent an in vitro model that may allow for further investigation of disease mechanisms and therapeutic interventions in TEN.

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Elevation of Intraocular Pressure in Patients With Uveitis Treated With Topical Difluprednate

Difluprednate ophthalmic emulsion, 0.05% (Durezol), is a topical corticosteroid approved for postoperative inflammation. Its efficacy has been demonstrated in several phase 3 clinical studies.1-3 Compared with topical prednisolone acetate, 1%, for the treatment of anterior uveitis, difluprednate, 0.05%, dosed 4 times a day is reported to be as effective as prednisolone acetate, 1%, dosed 8 times a day.4 Each of the phase 3 studies described a small number of patients who developed increased intraocular pressure (IOP) (≥21 mm Hg; ≥10 mm Hg from baseline), without further quantification.5-8 All patients responded to topical IOP-lowering medications.5 We report the occurrence of large elevations in IOP in a cohort of patients receiving difluprednate for uveitis.

Methods. With approval of the institutional review board at the University of Illinois at Chicago, medical records of consecutive patients treated with difluprednate ophthalmic emulsion between November 1, 2008, and October 17, 2010, were reviewed. Collected information included age, race, sex, diagnosis, level of inflammation, and IOP (measured with Goldmann applanation tonometry). The classifications of uveitis and level of inflammation were based on the Standardization of Uveitis Nomenclature criteria.6

Results. A total of 46 eyes of 27 patients were treated with difluprednate for a mean of 16.4 weeks (range, 1-46 weeks). The mean age was 34 years (range, 6-63 years). Uveitis was anterior in 15 patients (56%), intermediate in 1 patient (4%), posterior in 1 patient (4%), and panuveitis in 10 patients (37%). Twenty-six patients had chronic disease. Prior to initiation of difluprednate treatment, 17 patients (63%) were using Pred Forte (prednisolone acetate, 1%), 8 patients (30%) were using generic prednisolone acetate, 1%, and 2 patients (7%) were not using topical steroids. All patients who were originally receiving Pred Forte or generic prednisolone acetate were switched because of persistent anterior chamber (AC) inflammation in at least 1 eye. The numbers of patients who displayed an elevation in IOP greater than or equal to 10, 15, and 20 mm Hg are shown in the Table, as are the numbers of patients who had a peak IOP greater than or equal to 30, 40, or 50 mm Hg.

The mean baseline IOP before difluprednate treatment was 13.4 mm Hg (range, 0-27 mm Hg). An initial increase of at least 5 mm Hg was measured at a mean of 4.9 weeks (range, 1-16 weeks), with a mean time to peak elevation...