

Acute Generalized Exanthematous Pustulosis Due to Tetrazepam

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■ Abstract

Tetrazepam is a benzodiazepine that is widely used in Spain as a muscle relaxant, with occasional cutaneous side effects. We report a patient who developed a generalized pruriginous cutaneous reaction compatible with acute generalized exanthematous pustulosis (AGEP) due to tetrazepam. Patch tests with bromazepam, diazepam, and tetrazepam were negative at 48 and 72 hours; however, the tetrazepam patch showed a positive reaction at 10 days. Immunohistochemical studies revealed a mononuclear infiltrate composed of CD4⁺ and CD8⁺ T lymphocytes. Analysis of interleukin (IL) 8 expression by quantitative polymerase chain reaction revealed increased *IL-8* mRNA levels in patch test-positive skin. Lymphoblast transformation test (LTT) was positive with tetrazepam but not with diazepam. Positive patch test and LTT suggested that tetrazepam-specific lymphocytes might be responsible for a T cell-mediated reaction. These results support previous data suggesting an important role for IL-8 and drug-specific T cells in the pathogenesis of AGEP and imply that the reaction was specific to tetrazepam with no cross-reactivity to other benzodiazepines.

Key words: Acute generalized exanthematous pustulosis. Benzodiazepines. Drug reaction. Interleukin-8. Lymphocyte transformation test. Patch test. Tetrazepam.

■ Resumen

El tetrazepam es una benzodiazepina ampliamente utilizada en España como relajante muscular con la que se han descrito algunas reacciones alérgicas con afectación predominantemente cutánea. Presentamos una paciente con una reacción cutánea pruriginosa compatible con una pustulosis exantemática aguda generalizada por tetrazepam. Las pruebas epicutáneas con bromazepam, diazepam y tetrazepam fueron negativas. Sin embargo, el tetrazepam resultó positivo a los 10 días. Estudios histológicos revelaron un infiltrado mononuclear compuesto por linfocitos T CD4⁺ y CD8⁺. Mediante la reacción de polimerasa en cadena cuantitativa se detectó en piel afecta un incremento en los niveles de mRNA específico de interleucina (IL) 8. El test de transformación linfoblástica (TTL) resultó positivo con tetrazepam pero no con diazepam. Las pruebas epicutáneas y el TTL positivos sugieren que linfocitos específicos de tetrazepam podrían ser los responsables de una reacción mediada por células T. Los resultados apoyan datos previos y sugieren que IL-8 y linfocitos específicos frente al fármaco sospechoso desempeñan un papel importante en la patogénesis de la pustulosis exantemática aguda generalizada y que la reacción fue específica a tetrazepam, sin presentar reactividad cruzada con otras benzodiazepinas.

Palabras clave: Pustulosis exantemática aguda generalizada. Benzodiazepinas. Alergia a fármacos. Interleucina-8. Test de transformación linfoblástica. Pruebas epicutáneas. Tetrazepam.

Introduction

Acute generalized exanthematous pustulosis (AGEP) is an uncommon eruption characterized by acute, extensive formation of sterile nonfollicular pustules, fever, and peripheral blood leucocytosis. In a few cases, the etiology of AGEP appears to be a viral infection (enterovirus or parvovirus B19)

or a hypersensitivity reaction to mercury, but most cases of AGEP (90%) have been described in association with the intake of drugs, in particular antibacterial agents such as aminopenicillins [1]. Positive skin patch test and lymphocyte transformation test (LTT) suggest involvement of T cells [2].

Tetrazepam is a benzodiazepine widely used as a muscle relaxant and frequently prescribed in Spain. The most

common adverse reactions to the drug are of a neurological and gastrointestinal nature, and skin manifestations are rare [3]. Maculopapular exanthema [4-6], erythematous rash [5,7-10], urticarial eruption [6], erythema multiforme [6], photodermatitis [11], eczema [12], and Stevens-Johnson syndrome [6,13] have all been reported in association with the use of tetrazepam, probably as a result of delayed cell-mediated hypersensitivity. Epicutaneous patch testing is a useful tool to confirm tetrazepam allergy, particularly in cases with a high degree of sensitization and severe skin reactions [6].

We describe a woman who presented with AGEP after administration of tetrazepam. Patch testing with tetrazepam and other benzodiazepines was performed to identify cross-reactivity. The immunohistochemical study of the positive patch test and analysis of cytokine profiles and lymphocyte proliferation are reported.

Case Description

A 48-year-old woman was referred to our department because of a suspected adverse drug reaction. Four years previously she had been treated with tetrazepam for cervical arthralgia and had a severe generalized pruriginous reaction that lasted 20 days and resolved upon discontinuation of the drug. In September 2002 she was prescribed tetrazepam again together with codeine to treat a cold and 10 hours after the first dose she developed fever above 38°C and a similar generalized pruriginous reaction with erythema located on the trunk and proximal extremities. The reaction was more severe and longer lasting than the previous one and was treated with antihistamines; however, it resolved upon discontinuation of both drugs. As the patient had shown good tolerance of codeine since the reaction, tetrazepam was suspected to be the culprit drug.

Skin prick tests and patch tests were performed 4 months



Figure 1. Positive patch test on day 10 with Myolastan (left picture), 1% and 5% tetrazepam in petrolatum (right picture, upper patches), and 1% and 5% tetrazepam in aqueous solution (right picture, lower patches). Patch tests to lorazepam (left picture, L) and diazepam (left picture, D) were negative.

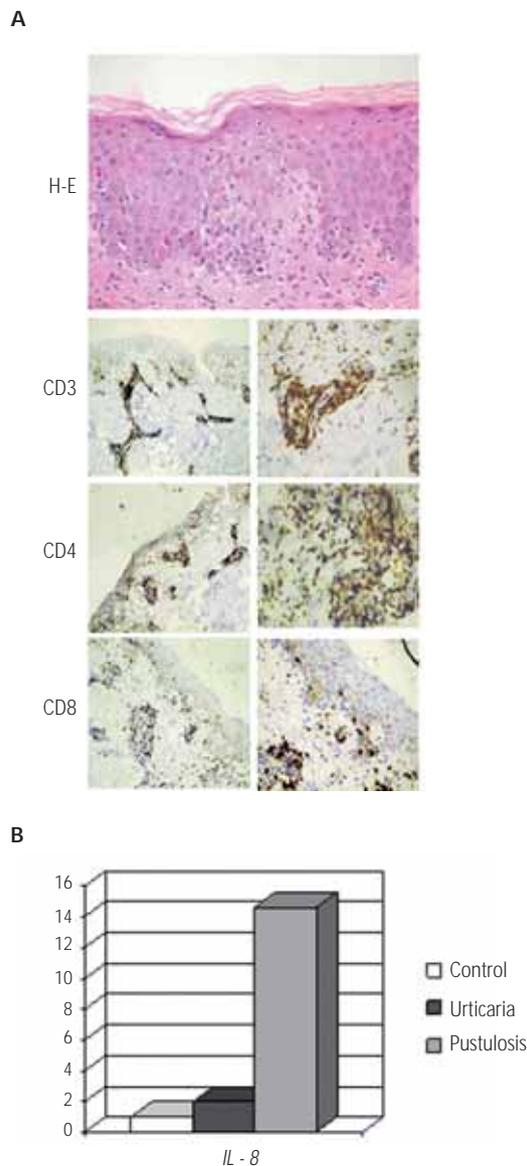


Figure 2. A. Hematoxylin–eosin (H-E) staining and immunohistochemical study of a skin biopsy from a positive patch test to tetrazepam at 10 days. CD3, CD4, and CD8 immunostaining of the lymphocyte infiltrate in a skin biopsy from the positive epicutaneous test to tetrazepam is shown. Original magnification, $\times 100$ (left column) and $\times 400$ (right column and H-E). B. Quantitative reverse-transcriptase polymerase chain reaction analysis of interleukin (IL) 8 mRNA expression in skin biopsies. Results are normalized to β -2-microglobulin mRNA levels. Data are shown as fold induction relative to IL-8 expression in healthy skin.

after the last reaction. Skin prick tests were performed with several benzodiazepines: pure bromazepam powder, pure diazepam powder, pure tetrazepam powder, 5% tetrazepam in aqueous solution, 5% tetrazepam in petrolatum, and Myolastan. Patch tests were performed with pure lorazepam powder, pure diazepam powder, pure tetrazepam powder, 1% and 5% aqueous tetrazepam, 1% and 5% tetrazepam in petrolatum, and Myolastan. The prepared drugs were applied on the

upper back using the Leukotest patch (Beiersdorf, Hamburg, Germany) and the reactions were read at 48 and 96 hours and scored according to the guidelines of the International Contact Dermatitis Research Group [14]. Single-blind, placebo-controlled oral challenge test was done with increasing doses of diazepam, using lactose as placebo. Skin prick tests and oral challenge with diazepam (10 mg) were negative. Patch tests were negative at 48 and 72 hours and were removed. However, on day 10 the patient came back complaining of itching on the back, and the spots where patch tests with Myolastan, 1% and 5% aqueous tetrazepam, and 1% and 5% tetrazepam in petrolatum were located showed a positive reaction with erythema and papules (Figure 1). No signs of reaction were found at the sites of patch tests with other benzodiazepines. Patch tests were performed on 2 healthy control subjects, with negative responses.

Histological analysis of a punch biopsy of affected skin from the positive patch test showed a prominent mononuclear infiltrate and necrosis of keratinocytes (Figure 2A, upper panel). The immunohistochemical study revealed that the infiltrate was composed mainly of T lymphocytes, as shown by the strong positive staining for CD3, with a perivascular infiltrate composed primarily of CD4⁺ T cells and exocytosis of CD8⁺ lymphocytes (Figure 2A).

Total RNA was extracted from affected skin and interleukin (IL) 8 mRNA expression was analyzed by quantitative reverse-transcriptase polymerase chain reaction. The results showed a marked increase in *IL-8* mRNA levels in tetrazepam patch test-positive skin, compared to healthy skin or urticaria-affected skin used as an additional control (Figure 2B).

LTT was performed in triplicate in the presence of different concentrations of drugs, ranging from 5 to 500 µg/mL. The cultures were incubated for 5 days at 37°C in 5% CO₂ and 1 µCi ³H-thymidine was added to each well 18 hours before cell harvesting. Phytohemagglutinin and tetanus toxoid were used as positive controls. The stimulation index (SI) was calculated as the ratio between the mean counts per minute in cultures with antigen and those obtained without antigen. An SI greater than

2 was regarded as a positive response. The assay was positive upon stimulation of the patient's peripheral blood mononuclear cells (PBMCs) with 10 µg/mL tetrazepam, showing an SI greater than 4, but not with diazepam (SI < 2). Negative results were obtained when LTT was performed with PBMCs from a healthy donor tolerant to tetrazepam (Figure 3).

Discussion

This severe reaction in our patient was caused by tetrazepam, as confirmed by the clinical characteristics and the in vivo tests. Immunological studies were consistent with a diagnosis of AGEF. AGEF is an uncommon eruption most often provoked by drugs, by acute infections with enterovirus, or by mercury [15,16]. Tetrazepam is a drug involved in a few cases of adverse cutaneous reactions, but there are no previous reports of this unusual reaction to tetrazepam.

Recent studies have suggested that T cells are involved in the etiology of this clinical entity [2]. Positive patch tests with tetrazepam and positive LTT also suggest that there are tetrazepam-specific memory T cells that may be responsible for a T cell-mediated cutaneous reaction [17,18]. Immunohistochemistry of the lesions in the positive epicutaneous reaction confirmed the presence of a perivascular T-cell infiltrate. It has been suggested that local IL-8 production in the skin is a key factor in the development of AGEF [2,19]. We demonstrated that the local production of IL-8, a chemokine classically involved in the recruitment and differentiation of neutrophils, was increased in our patient as compared to healthy skin or urticaria-affected skin.

The positive patch test result with tetrazepam confirms that patch testing is a useful tool to assess tetrazepam allergy, particularly in cases with a high degree of sensitization and severe skin reactions, although the optimal test concentration for tetrazepam drug allergy has still to be determined. The patch test with tetrazepam was performed with aqueous solutions and preparations in petrolatum, and concentrations ranged from 1% to 100%. We recommend a dilution of 1% in petrolatum for the diagnosis of severe skin reactions to tetrazepam. In some cases, patch testing on residual lesional skin may be useful, as described in a patient with a maculopapular rash due to tetrazepam [10]. The positive patch test at 10 days suggests that reading at 72 hours may not be sufficient and a further reading may be necessary in some patients [14]. To our knowledge this is the first case described of a positive epicutaneous test reading with tetrazepam at 10 days. Patch tests with other benzodiazepines were negative, and oral administration of diazepam was tolerated. The results of epicutaneous and in vitro tests along with the negative oral challenge to diazepam suggest that the reaction was specific to tetrazepam with no cross-reactivity to other benzodiazepines with structural homology to that drug. Positive LTT results have been previously reported in AGEF, but to our knowledge, this is the first case in which a positive LTT was obtained with benzodiazepines. These data, in agreement with previous reports [12,20], suggest that there is little cross-reactivity among benzodiazepines. However, Kämpgen et al [8] obtained a positive patch test to diazepam in a patient with

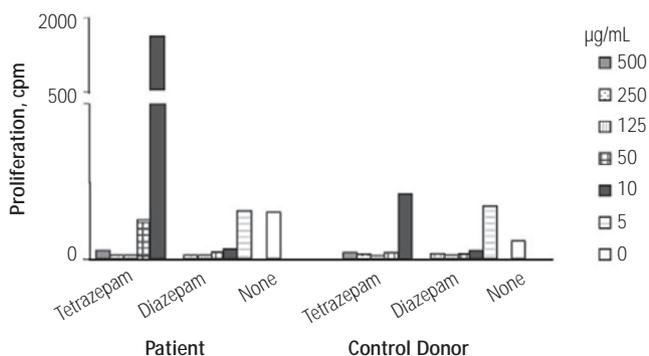


Figure 3. Drug-specific proliferation of peripheral blood mononuclear cells. A sample of 2×10^5 cells from the patient or from a tolerant control donor were stimulated with different concentrations of tetrazepam or diazepam for 5 days. Proliferation was detected by ³H-thymidine incorporation. cpm indicates counts per minute.

tetrazepam allergy, and García Bravo et al [12] also reported a positive patch test to both drugs, explained by concomitant sensitization. Patch test with other benzodiazepines is useful in order to offer a therapeutic alternative, but, in our opinion, tolerance must always be confirmed by oral challenge.

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