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Outcomes for Nitazoxanide Treatment in a Case Series of Patients with Primary Immunodeficiencies and Rubella Virus-Associated Granuloma

Ludmila Perelygina[1] · David Buchbinder[2] · Morna J. Dorsey[3] · Marc Eloit[4] · Fabian Hauck[5] · Timo Hautala[6] · Despina Moshous[7,8] · Ignacio Uriarte[9] · Elena Deripapa[10] · Joseph Icenogle[1] · Kathleen E. Sullivan[11]

Abstract

Purpose Nitazoxanide was recently reported as having in vitro effectiveness against the rubella virus. Immunodeficiency-related vaccine-derived rubella occurs in some patients who have an inherited immunodeficiency and who received the MMR vaccine. This study investigated the in vivo effectiveness of nitazoxanide therapy.

Methods This is a retrospective analysis of seven patients treated with nitazoxanide as salvage therapy for immunodeficiency-related vaccine-derived rubella infection. The patients were recruited from an ongoing rubella detection surveillance project.

Results Seven patients with persistent rubella were treated with nitazoxanide and one demonstrated significant clinical improvement. Two additional patients exhibited diminished viral capsid production with one patient having transient slowing of progression. The cohort overall generally had low T cell counts and had a high burden of comorbidities. There were three deaths. Two deaths were from PML and one was related to hematopoietic stem cell transplantation.

Conclusions Nitazoxanide has limited in vivo anti-viral effects for immunodeficiency-related vaccine-derived rubella. Most patients did not exhibit clinical improvement.

Keywords Granulomas · chronic inflammation · vaccine · nitazoxanide · MMR

✉ Kathleen E. Sullivan
  sullivank@email.chop.edu

1 Division of Viral Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA, USA

2 Division of Hematology, CHOC Children's Hospital, Orange, CA, USA

3 Department of Pediatrics, Division of Allergy, Immunology, and Blood and Marrow Transplant, Benioff Children's Hospital, University of California, San Francisco, 1975 4th Street, San Francisco, CA 94158, USA

4 Institut Pasteur, Laboratory of Pathogen Discovery, Biology of Infection Unit, Inserm U1117, Paris, France

5 Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU, Munich, Germany

6 Research Unit of Internal Medicine, University of Oulu and Oulu University Hospital, Kajaanintie 50, 90220 Oulu, Finland

7 Department of Pediatric Immunology, Hematology and Rheumatology, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Children's Hospital, Paris, France

8 Laboratory "Genome Dynamics in The Immune System," INSERM UMR1163, Université Paris Descartes Sorbonne Paris Cité, Institut Imagine, Paris, France

9 Immunology Unit, The Child's and Mother Hospital, Vitorio Tetamanti, High School of Medicine, Mar del Plata National University, Castelli 2450 Mar del Plata, 7600 Buenos Aires, Argentina

10 Department of Immunology, Center for Pediatric Hematology, Oncology, Immunology, Moscow, Russia

11 Division of Allergy Immunology, The Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, 3615 Civic Center Blvd, Philadelphia, PA 19104, USA

Introduction

Rubella virus is a single-stranded RNA virus and is an important human pathogen because of its ability to cause fetal malformations during in utero infection [1]. It is not thought to represent a dangerous pathogen for immunocompetent adults, although persistent infections in immune-privileged body sites have been described. Indeed, rubella-associated arthritis/arthralgia can develop in adults due to persistent vaccine-strain infection [2]. Fuch's uveitis is also associated with persistent infection with rubella virus, and a few cases of uveitis have been observed with vaccine-strain rubella [3, 4]. These cases demonstrate the ability of the rubella virus to evade immune eradication/control even in immunocompetent individuals although the reasons for this are not defined. Recently, patients with primary immunodeficiency have been identified with persistence of vaccine-strain rubella associated with chronic inflammation [5, 6]. Immunodeficiency-related vaccine-derived rubella virus (iVDRV) was initially identified in patients with cutaneous granulomas. More recently, iVDRV has been detected in other sites of chronic inflammation [5, 6].

To date, there are no known treatments that are highly effective. In most cases, the inflammation has progressed relentlessly unless hematopoietic stem cell transplantation (HSCT) has been performed with resolution of the immunodeficiency, although one patient has been reported to have responded to topical steroid treatment [7]. Rubella virus is insensitive to type I interferons and no other antiviral therapy is of proven benefit. The inability of specific maternal and fetal antibody to clear rubella virus infection in the fetus also suggests that humoral immunity alone is not sufficient in eliminating the persisting virus [8]. Thus, most of the tools utilized for chronic viral infections in immunodeficient patients are not thought to be effective for rubella virus.

Rubella virus replication can be inhibited in vitro with the drug nitazoxanide [9]. Nitazoxanide is approved for the treatment of parasites, protozoa, and anaerobic bacteria. It has been demonstrated to have antiviral activities and is being tested as a therapeutic intervention for hepatitis C, influenza, rotavirus, and norovirus. Nitazoxanide has a favorable adverse event profile and is used frequently in children for the treatment of parasitic disease. The mechanism of its anti-viral action is not understood but it may act in a cell-intrinsic fashion to limit replication. We recently demonstrated that nitazoxanide inhibited rubella virus replication in human umbilical vein endothelial, Vero, and A549 cells [9]. The inhibitory concentration was consistent with serum levels obtained during therapeutic use. Analysis of the effect of nitazoxanide revealed multiple mechanisms of inhibition at both early and late stages of infection. We therefore retrospectively analyzed the effect of nitazoxanide treatment in a case series of immunodeficient patients with persistent iVDRV at our institutions.

Methods

For this study, seven patients with a primary immunodeficiency and persistent iVDRV were identified. Ongoing surveillance for iVDRV in the USA at the CDC and in France at the Institut Pasteur for rubella capsid and genome [5, 6] led to definition of the initial iVDRV cohort and submitting physicians identified nitazoxanide use. Based on in vitro efficacy, limited adverse events, poor prognosis, and absence of other proven interventions, clinicians at each site decided to institute therapy with nitazoxanide. This is a retrospective study of outcomes of the seven patients. A structured case report form was used to collect standardized data from each physician. No statistical analysis was performed.

Results

All patients had a significant T cell-oriented immunodeficiency and most had low T cell counts in peripheral blood (Table 1). Only patients 3 and 4 had normal T cell counts. T cell function was not systematically assessed. Natural killer cell counts (NK cells) were generally normal. The age of onset of inflammatory lesions ranged from 15 months of age to 16 years, and all patients had previously received the MMR vaccine months to years prior to symptoms. All patients had skin involvement that was described as initially psoriasiform or resembling pyoderma gangrenosum (Fig. 1). The cutaneous granulomas spread relentlessly and in one case led to amputation and in another case led to surgical excision in an effort to control the spread. Neither effort at surgical control of the lesions was successful. Empiric treatments directed at mycobacteria or fungi were not successful. Immune suppression appeared to help in two cases but did not lead to resolution. In the most severe cases, multiple organs were ultimately affected. Rubella capsid protein was detected in all patients, and virus RNA was recovered from patients 2, 4, and 5. Different iVDRV strains with multiple non-overlapping mutations were identified in the skin biopsies by sequencing viral genomes. Survival was generally dismal, and progressive multifocal leukoencephalopathy (PML) was the final cause of death in two patients. HSCT was curative in one patient with resolution of skin lesions. Immunoglobulin was used uniformly without benefit.

Nitazoxanide was used largely as salvage therapy in these extremely ill patients. Rubella capsid protein became undetectable (Fig. 2) in two patients (patients 4 and 5) but without clinical improvement although progression may have stabilized. In one case, clear clinical improvement coincided with the institution of nitazoxanide (patient 7). In the other four cases, nitazoxanide had no obvious benefit (Table 1).

Table 1 Clinical summary of cases

	Patient 1	Patient 2	Patient 3	Patient 4
Diagnosis	DNA Ligase IV	Ataxia Telangiectasia	Ataxia telangiectasia	Ataxia Telangiectasia
MMR vaccine	12 m	24 m	12 m and 17 m	18 m
Age of onset of inflammatory process	15 m	30 m	39 m	10 years
Sites of chronic inflammation	Skin Liver Intestine	Skin	Skin	Skin
Sites where rubella was detected by immunofluorescence or PCR	Skin Liver	Skin	Skin	Skin
CD3 count	380 cells/uL	240 cells/uL	3074 cells/uL	3085 cells/uL
CD4 count	73 cells/uL	153 cells/uL	2450 cells/uL	1091 cells/uL
CD19 count	21 cells/uL	77 cells/uL	178 cells/uL	0 cells/uL (post-rituximab)
NK cell count	432 cells/uL	184 cells/uL	1158 cells/uL	560 cells/uL
Previous treatments	Immune modulatory: topical steroids, no improvement	Antimicrobial: Cryotherapy Erythromycin Imiquimod No improvement Immune modulatory: Tacrolimus cream Hydroxycholorquine Topical steroids No improvement Oral prednisone led to some improvement	Immune modulatory: Topical steroids NSAID No improvement	Immune modulatory: Abatacept No improvement
Treated with IVIG	+	+	+	+
Response to nitazoxanide	Dose: 32 mg/kg/day Given for norovirus- for 5 weeks with no improvement in skin lesions	Dose: 7 mg/kg/day Tried for 1 year without improvement	Dose: 11 mg/kg/day Tried for 5 months without improvement	Dose: 22 mg/kg/day Slowing in progression and loss of rubella in skin on biopsy but subsequent involvement of bone
Outcome	HSCT with ultimate death at d + 135 although with improvement in lesions	Progression of granulomas with persistent rubella on repeat biopsy	HSCT with improvement in lesions (currently d + 140)	Alive

	Patient 5	Patient 6	Patient 7
Diagnosis	CID (unknown genetics)	Coronin1A	Cartilage hair hypoplasia
MMR vaccine	5 years and 9 years	3 years and 5.7 years	1 year
Age of onset of inflammatory process	13 years	13 years	15 years
Sites of chronic inflammation	Skin	Skin and underlying bone	Skin Liver Kidney

Table 1 (continued)

Sites where rubella was detected by immunofluorescence or PCR	Skin	Skin	Larynx / Palate / Bone marrow / Skin / Liver / Kidney
CD3 count	453 cells/uL	464 cells/uL	550 cells/uL
CD4 count	189 cells/uL	224 cells/uL	450 cells/uL
CD19 count	312 cells/uL	24 cells/uL	120 cells/uL
NK cell count	105 cells/uL	144 cells/uL	120 cells/uL
Previous treatments	Antimicrobial: Linezolid Clarithromycin Rifampin Isoniazid Ethambutal Voriconazole Posaconazole Interferon No improvement Amputation of finger- continued local spread	Antimicrobial: Cotrimoxazole, Azithromycin, Fluconazole, Amoxicillin-clavulanic acid, Ciprofloxacin No improvement Immune modulatory: Topical steroids No improvement Surgical excision-local recurrence	Antimicrobial: Azathioprine Azithromycin No improvement Immune modulatory; Mycophenolic acid No improvement Cyclosporine and steroids-partial response
Treated with IVIG	+	+	+
Response to nitazoxanide	Dose: 16 mg/kg/day Tried for 5 months without clinical improvement although rubella became undetectable by immunohistochemistry	Dose: 25 mg/kg/day Tried for 4 months, no improvement	Dose: 20 mg/kg/day After 2 months, lesions in the skin, liver, and kidney improved and improvement was sustained until death
Outcome	Deceased from PML	Alive without HSCT (age at last F/U 18 years)	Deceased from PML

Fig. 1 Immunodeficiency-related vaccine-derived rubella virus (iVDRV) clinical features. **a** Patient 5 with typical granulomatous inflammation. **b** Patient 5 demonstrating relentless progression over 1 year. **c** Patient 6 in 2016 after surgical excision. **d** Patient 6 after 2.5 months of nitazoxanide. **e** Patient 3 right leg prior to stem cell transplant. **f** Legs of patient 3, legs 20 days after transplantation


Discussion

This study provides critical information on treatment approaches for clinicians caring for immunodeficient patients with this challenging complication. The recent identification of iVDRV in granulomatous inflammation has led to increasing recognition; however, there no known therapeutic approach. Nitazoxanide may have provided some limited benefit in our study. One patient had dramatic resolution, only to die later of PML (patient 7). Similarly, one patient may have stabilized with diminished rubella capsid detection, but this patient also succumbed to PML (patient 5). Patient 4 was

thought have slowed in the progression of lesions but did not resolve any existing lesions. Our model of this condition describes an interplay between acquired mutations that allow escape from T cell control and compromised T cell function. We hypothesize that the administration of live attenuated vaccine rubella in patients with T cell compromise allows prolonged persistence. The additional time for replication allows the acquisition of mutations that favor persistence in M2 macrophages. The acquired mutations further facilitate escape from T cell control. If nitazoxanide is beneficial, it may require a threshold of competent T cells, may require longer treatments, or it may be effective against iVDRV strains that have

Fig. 2 Regression of viral capsid expression after nitazoxanide. **a** Patient 5 immunohistochemistry rubella capsid detection (red) in granuloma (CD206 in green is an M2 macrophage marker). DAPI is provided as a nuclear stain. **b** Patient 5 after 2 months of nitazoxanide treatment (NTZ). There is no detectable rubella capsid
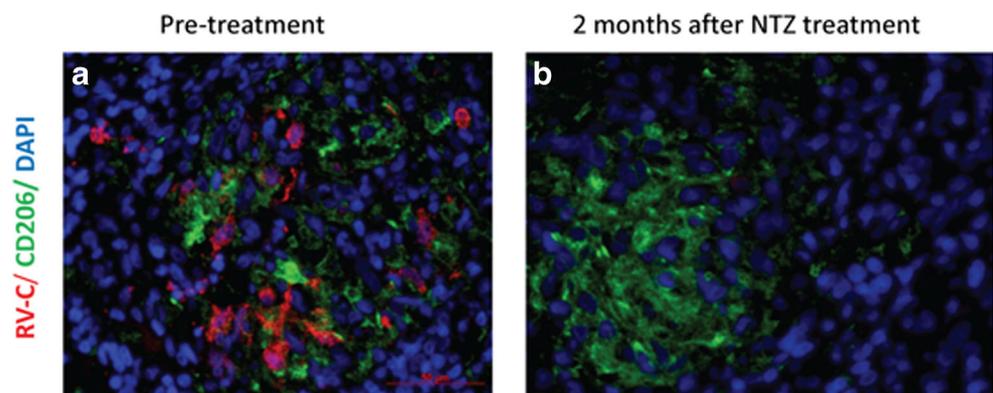
specific replication characteristics. These data do not provide strong support for efficacy of nitazoxanide as salvage therapy; however, the drug has limited adverse events and may provide limited benefit. The question of whether it might provide more clinical effectiveness earlier in the infection is unanswered. This study also highlights a second finding. The patients who are susceptible to iVDRV are extremely immunodeficient. Definitive treatment with HSCT had not been performed in these patients for a variety of reasons, but is underway for patient 3 (currently d + 140) and may provide the only curative strategy. The poor outcomes in this study document the uphill battle faced by patients with combined immunodeficiency who are not transplanted [10]. Out of two long-term survivors in this small study, one had an HSCT and the second one is 18 years old at the time of this report and is clinically stable but with ongoing inflammatory iVDRV disease.

Conclusions

In summary, nitazoxanide may have provided some benefit in 3/7 patients with persistent iVDRV. Additional study may define the variables related to responsiveness.

Compliance with Ethical Standards

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, US Department of Health and Human Services.

Conflict of Interest The authors declare that they have no conflicts of interest.

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