Original Article

MUTATIONS ASSOCIATED WITH RESISTANCE TO TMS IN PNEUMOCYSTIS JIROVECII AND THEIR IMPACT ON PATIENTS IN INTENSIVE CARE UNIT

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ABSTRACT
Pneumocystosis is a frequent opportunistic infection among patients with AIDS. Lately the uncertainty about the resistance to TMS has grown since mutations in the DHPS gene that would be related to this phenomenon have been characterized. However, the real relationship with clinical evolution is unknown. With the objective of knowing the prevalence of these mutations and inferring their implication, they were studied by PCR and RFLP in 30 LBA samples from patients with aids and their presence was related to the days of hospitalization, the requirement of MRA and death. A very high percentage of isolates with mutations was found. This makes its relationship with morbidity and mortality unlikely. However, in patients without mutations the difference in hospitalization days was notorious although the small number of patients with strains without mutations makes it difficult to obtain definitive conclusions.

Keywords: Pneumocystosis, TMS resistance, DHPS mutations.

INTRODUCTION

Pneumocystosis is a serious opportunistic infectious disease, with almost exclusively pulmonary manifestations, distributed worldwide, that has had a strong association with AIDS since the beginning of the pandemic. It is caused in humans by Pneumocystisjirovecii (PJ), a noncultivable fungus that presents high stenoxenism, characteristics that make its detection very difficult for the certainty diagnosis that is only achieved by visualizing the microorganism from bronchoalveolar lavage samples, with the risks and complications involved in this procedure and the need for a trained observer (1).

Pneumocystosis affects different groups of immunocompromised patients with a prevalence ranging from 10 to 50%, with differences in clinical manifestations, the development of the disease depending on the underlying condition, and also differences in mortality. In recent years with advances in medicine, the survival of patients with immunosuppressive pathologies has increased and due to the use of immunomodulatory drugs, new groups susceptible to suffering from pneumocystosis have appeared. However, in developing countries, AIDS is still the main cause associated with pneumocystosis due to the large number of patients who do not have access to highly active antiretroviral therapy (HAART) and opportunistic disease prophylaxis, and also due to the Unfavorable socioeconomic conditions that generate lack of adherence to said treatments (2,3).

In recent years, unfortunately, the number of patients with PJP has not decreased despite the availability of HAART and prophylaxis. This has installed worldwide uncertainty and fear due to the presence of strains of P. jirovecii resistant to trimethoprim-sulfamethoxazole (TMS), the drug of choice for prophylaxis and treatment of this mycosis (4, 5).

TMS works by inhibiting the synthesis of tetrahydrofolic acid, necessary for the production of purines. Trimethoprim interferes in the action of dihydrofolate reductase (DHFR) and sulfa in dihydropteroatosynthase (DHPS) as shown in Table 1.

Various researchers have described mutations in both enzymes that are believed to be related to resistance to TMS. However, mutations in DHFR are very many, highly variable, and their implication is still highly controversial.

In the case of DHPS, two point mutations have been characterized in codons 55 and 57 where a change from threonine to alanine and proline to serine, respectively, is generated. These changes in the protein alter the target site of action of sulfa drugs and therefore would be related to resistance to this drug, as has been seen in other microorganisms. Despite numerous molecular studies, the real implication that these mutations would have on the evolution of patients and possible therapeutic failure is unknown (6,7).

TARGET

To know the prevalence of mutations associated with sulfa resistance in PJ and to relate their presence with parameters of clinical evolution in patients admitted to intensive care to infer the in vivo impact of these mutations.

MATERIALS AND METHODS

Thirty bronchoalveolar lavage (BAL) samples from 30 patients admitted to intensive care at the Hospital de Infectious FJ Muñiz with AIDS and pulmonary pneumocystosis confirmed by concordant clinical manifestations and the microscopic observation of elements compatible with PJ in said sample were studied. Of them, 20 were men and 10 women. DNA extraction from the BAL was carried out by means of extraction columns and subsequently the amplification by polymerase chain reaction (PCR) of the DHPS gene was carried out using two rounds of amplification with specific primers designed in-house.

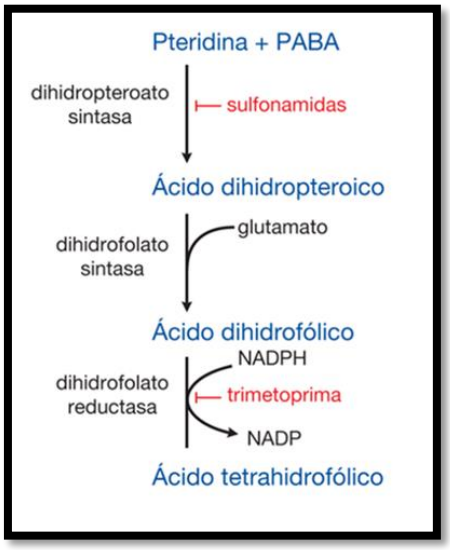

Figure 1: Intervention of trimethoprim and sulfonamides in the tetrahydrofolic acid synthesis pathway.

Once the amplicon had been obtained, the study of the polymorphisms in the restriction fragments (RFLP) with the enzymes AcI and HaeIII with a cleavage site at codons 55 and 57 respectively of the DHPS gene was carried out (8, 9). the presence of point mutations in said codons. The observed genotype was then related to the following clinical parameters: number of days of hospitalization in intensive care, requirement for mechanical ventilation (MRA), and death.

RESULTS

90% (27) of the patients studied had mutations in codon 55; 20% (6) in 57; 93.3% (28) at least one mutation; 16.7% (5) both and 6.7% (2) neither. The analysis of the clinical parameters according to each of the aforementioned profiles is shown in Table 1.

Presence of mutations	N (%)	Median days of ICU hospitalization (range)	ARM requirement (%)	Death (%)
Mutant codon 55, wild codon 57	22 (73.3)	20 (5-65)	8 (36.4)	6 (27.3)
Codon 55 mutant, codon 57 mutant	5 (16.6)	20 (10-32)	1 (20)	0
Codon 57 mutant, codon 55 wild	1 (3.3)	13	0	0
No mutations	2 (2.7)	9 (7-14)	0	0
With at least one mutation	28 (93.3)	21 (5-65)	9 (32.1)	6 (21.4)

Table 1. Clinical parameters associated with the presence of mutations
MRA: mechanical ventilation
ICU: intensive care unit

DISCUSSION

The high percentage of patients with P. jirovecii strains with at least one mutation (93.3%) far exceeds that reported by other authors in other countries around the world (10, 11). This could be due to the particular population of our center, characterized by patients with low adherence to treatments and multiple relapses in opportunistic pathologies. On the other hand, it could be considered that there is an ecological niche in this hospital environment with a high concentration of strains with mutations. The observed values inevitably lead us to question the impact that the presence of mutations in the target site of action of sulfa drugs has in vivo since, clinically, treatment failure is usually markedly less than what the presence of mutations would suggest. . The fact that the carriage of strains with mutations in codon 55 is so common among the population studied makes it difficult to assign it a relevant role in mortality and morbidity. The mortality observed is the usual one expected for pneumocystosis for the population of patients in intensive care, also taking into account that in general they are patients with other concomitant opportunisms. Regarding the codon 57 mutation, the 6 patients who presented it evolved in a similar way to the rest, but more data is necessary to draw reliable conclusions. However, in patients without mutations, the difference in the days of hospitalization (12), the requirement for MRA and death is notable, although the small number of patients in this group makes it difficult to obtain results of

statistical validity. Finally, given the very high percentage of isolates with mutations, it is difficult to know if they are irrelevant in the evolution of patients or if there is a niche in our center with a high prevalence of mutant strains and there are not enough patients with pneumocystosis at the expense of strains without mutations to assess whether they have a less severe presentation and faster recovery. In the future, it would be interesting to make a comparison with patients from other centers or even from other countries where the mutation carrier rates are markedly lower. It is imperative to continue this study with a greater number of samples and with a more exhaustive analysis of the clinical evolution of the patients as well as their history of exposure to TMS and their history of hospitalization. This will give us tools to elucidate the origin of the presence of mutations and put into debate the risk factors for this phenomenon as well as the possibility of taking new behaviors in the approach of patients with pneumocystosis since, for example, currently there is no there are clear recommendations in our country regarding the isolation of these patients (13).

CONFLICTS OF INTEREST

None of the authors declares any conflict of interest in relation to this publication.

REFERENCES

1. Calderón Sandubete E, de Armas Rodríguez Y, Capó de Paz V. Pneumocystisjirovecii : one hundred years of history. Rev Cubana MedTrop 2011; 63: 97-116.

2. Morris A, Lundgren JD, Masur H, Walzer PD, Hanson DL, Frederick T, et al. Current Epidemiology of Pneumocystis Pneumonia. Emerg Infect Dis. 2004; 10: 1713-1720.

3. Calderón EJ, de Armas Y, Panizo MM, Wissmann G. Pneumocystisjirovecii pneumonia in LatinAmerica. A public health problem? Expert Rev Anti Infect Ther. 2013; 11: 565-570.

4. Helweg-Larsen J, Benfield T, Eugen-Olsen J, Lundgren J, Lundgren B. Effects of mutations in Pneumocystis carinii dihydropteroate synthase gene on outcome of AIDS-associated P. carinii pneumonia. Lancet 1999; 354: 1347–51.

5. Huang L, Crothers K, Atzori C, Benfield T, Miller R, Rabodonirina M, et al. DihydropteroateSynthase Gene Mutations in Pneumocystis and SulfaResistance.EmergInfectDis, 2004; 10: 1721-1728.

6. Calderón E, de la Horra C, Montes-Cano MC, s Respaldiza, Martín J, Varela JM. Genotypic resistance to sulfonamides in patients with Pneumocystisjiroveci pneumonia . Med Clin (Barc) 2004; 122: 617-9.

7. Ponce CA, Chabé M, George C, Cárdenas A, Durán L, Guerrero J, et al. High Prevalence of Pneumocystis joveciiDihydropteroate Synthase Gene Mutations in Patients with a First Episode of Pneumocystis Pneumonia in Santiago, Chile, and Clinical Response to Trimethoprim-Sulfamethoxazole Therapy. Antimicrob Agents Chemother. 2017; 61 (2) e01290-16.

8. Helweg-Larsen J, Eugen-Olsen J, Lundgren B. Rapid Detection of Dihydropteroate Polymorphism in AIDS-related Pneumocystis carinii Pneumonia by Restriction Fragment Length Polymorphism. Scand J Infect Dis 2000; 32: 481-483.

9. Mogoye B, Du Plessis D, Poonsamy B, Frean J. Characterization of Pneumocystis jirovecii DHPS genotypes using a simple, inexpensive restriction fragment length polymorphism analysis. South Afr J Infect Dis 2015; 30: 46-50.

10 Suárez I, Roderus L, van Gumpel E, Jung N, Lehmann C, FätkenheuerC, et al. Low prevalence of DHFR and DHPS mutations in Pneumocystis jirovecii strains obtained from a German cohort. Springer-Verlag Berlin Heidelberg 2017. doi: 10. 1007 / s15010-017-1005-4

eleven Sheikholeslami MF, Sadraei J, Farnia P, ForozandehMoghadam M, Emadikochak H. Dihydropteroate synthase gene mutation rates in Pneumocystis jirovecii strains obtained from Iranian HIV-positive and non-HIV-positive patients. MedMycol, 2015, 53, 361–368

12. Nahimana A, Rabodonirina M, Bille J, Francioli P, Hauser PM. Mutations of Pneumocystis jirovecii DihydrofolateReductase Associated with Failure of Prophylaxis. Antimicrob. Agents Chemother. 2004; 48: 4301-4305.

13. Hauser P, Nahimana A, Taffe P, Weber R, Francioli P, Bille J, et al. Interhuman Transmission as a Potential Key Parameter for Geographical Variation in the Prevalence of Pneumocystis jirovecii Dihydropteroate Synthase Mutations. Clin Infect Dis. 2010 Aug 15; 51 (4): e28-33.