NITAZOXANIDE FOR THE TREATMENT OF *GIARDIA DUODENALIS* INFECTION: A COMPARATIVE TRIAL WITH SECNIDAZOLE

Giardia duodenalis Enfeksiyonlarının Tedavisinde Nitazoksanid: Seknidazol İle Karşılaştırmalı Bir Çalışma

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Geliş Tarihi: 19.06.2010
Kabul Tarihi: 01.09.2010

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ABSTRACT

Objective: Nitazoxanide is a 5-nitrothiazolyl derivative with broad-spectrum activity against numerous intestinal protozoa, helminths and anaerobic bacteria licensed in the United States for the treatment of *Cryptosporidium* spp. and *Giardia duodenalis*. The aim of this study was to compare the efficacy and safety of nitazoxanide (NTZ) versus secnidazole (SNZ) in the treatment of *giardia*sis.

Method: A randomized controlled open-label trial was carried out at the Cuban Institute of Gastroenterology in adults with confirmed *Giardia duodenalis* mono-infection. 125 patients were randomly assigned to receive either NTZ [500 mg two times daily for three days (n= 62)] or SNZ [2 g single dose (n= 63)]. The evaluation of the efficacy was based on parasitological response. All patients were asked to provide three faecal samples on days 3, 5, and 10 after treatment completion. Patients were considered to be cured, if no *Giardia* trophozoites or cysts were found in any of the three post-treatment faecal specimens evaluated by direct wet mounts and/or after Ritchie concentration techniques.

Results: The frequency of cure was a little higher for NTZ [95.2%- (59/62)] than for SNZ [93.7%- (59/63)] but the difference was not statistically significant (P>0.05). Bitter taste was only reported in SNZ treated group were as yellowish coloration of the urine and rash were only reported in NTZ treated group. Nausea and headache were more common in patients treated with SNZ (P<0.05).

Conclusion: NTZ, for three days, is as efficacious as a single dose SNZ in the treatment of *giardia*sis in adults.

Key Words: *Giardia duodenalis* infection, drug therapy, secnidazole, nitazoxanide, Cuba

ÖZET

Amaç: Nitazoksanid (NTZ), çeşitli intestinal protozoalar, helmintler ve anaerob bakterilere karşı etki gösteren geniş spekturumlu, Amerika Birleşik Devletleri'nde *Cryptosporidium* spp. ve *Giardia duodenalis* tedavisi için ruhsatlandırılmış 5-nitrotiazol türevi bir ilaçtır. Bu çalışmada, *Giardia*zis tedavisinde nitazoksanid kullanımının güvenilirlik ve etkinlik yönünden seknidazol (SNZ) ile karşılaştırılması amaçlanmıştır.

Yöntem: Randomize kontrollü açık-etiketli bu çalışma Küba Enstitüsü Gastroenteroloji Kliniğinde, sadece *Giardia duodenalis* enfeksiyonu olan yetişkin hastalarda yürütülmüştür. 125 hasta rastgele olarak NTZ [3 gün, günde 2 kez 500 mg (n= 62)] ya da SNZ [2 g / Tek doz (n= 63)] tedavisi uygulanmak üzere belirlenmiştir. Etkinlik değerlendirmesi parazitolojik cevaba göre yapılmıştır. Tedavinin tamamlanmasından sonraki 3., 5. ve 10. günlerde tüm hastalardan fekal örnek verilmesi istenmiştir. Tedavi sonrası alınan her üç dışkı örneğinin hiçbirinde Nativ-Lugol ve/veya Ritchie konsantrasyonu teknikleriyle *Giardia* trofozoitleri veya kistleri tespit edilemeyen hastalar tedavi edilmiş olarak değerlendirilmiştir.

Bulgular: Tedavi oranı NTZ [%95.2 - (59/62)] uygulanan grupta SNZ [%93.7 - (59/63)] uygulanan gruba göre biraz daha yüksek olmakla beraber istatistiksel olarak anlamlı bulunmamıştır (P>0.05). Acı tat alma sadece SNZ uygulanan grupta sarımsı renkte idrar çıkarma ile beraber görülürken döküntü oluşumu sadece NTZ tedavisi verilen grupta görülmüştür. Bulantı ve başağrısına SNZ tedavisi verilen grupta daha sık rastlanmıştır (P<0.05).

Sonuç: Yetişkinlerde 3 günlük NTZ uygulaması *giardia*zis tedavisinde tek doz SNZ verilmesi kadar etkili bulunmuştur.

Anahtar Sözcükler: *Giardia duodenalis* enfeksiyonu, *Giardia duodenalis* tedavisi, seknidazol, nitazoksanid, Küba

INTRODUCTION

Giardia duodenalis an important cause of diarrheal disease all over the world, resides in the small intestinal lumen in close opposition to epithelial cells (1). The World Health Organization (WHO) has estimated that 3000 million people live in places where the rate of *giardia*sis is around 30%, and suggests that there are almost 1000 million cases of *giardia*sis; contributing to 2.5 million deaths annually from diarrheal disease (2).

For several years some drugs such as quinacrine or the 5-nitroimidazole metronidazole (MTZ) have been used for chemotherapy against this protozoan parasite, however, different pre-clinical and clinical investigations revealed that anti*giardia*l chemotherapy may be complicated by emergence of *giardia*l resistance (3-5).

Nitazoxanide (NTZ) is a 5-nitrothiazolyl derivative with broad-spectrum activity against numerous intestinal protozoa, helminths and anaerobic bacteria licensed in the United States for treatment of *Cryptosporidium* spp. and *G. duodenalis* (5). In-vitro studies have confirmed the efficacy of NTZ in the treatment of *giardia*sis demonstrating that this drug and its derivative, tizoxanide, are 2.5 and 50 times more efficacious than albendazole and MTZ against *Giardia* isolates (6).

On the other hand clinical studies also demonstrated the effectiveness of NTZ in *G. duodenalis* infections. Ortiz JJ et al., (7) in 2001 reported that NTZ was as efficacious as a standard 5-day course of metronidazole in treating *giardia*sis and controlling diarrhoeal episodes. Similar results were presented by Rossignol JF et al., (8) in a randomized, double-blind, placebo-controlled study carried out the same year.

The aim of this study was to determine the efficacy and safety of NTZ versus secnidazole (SNZ) in the treatment of *giardia*sis. This kind of study should be valuable in view of the fact that the use of NTZ is not limited to the treatment of symptomatic soil transmitted helminthic infections, but also in the large scale control and prevention of morbidity in people living in endemic areas where *Giardia* is also sometimes prevalent.

PATIENTS AND METHODS

Study setting

The study, a randomized controlled open-label trial, was carried out at the Institute of Gastroenterology, Havana City, Cuba, between January and June 2008.

Enrolment and subject selection

The subjects were adults who visited the centre, seeking treatment for symptomatic, acute *G. duodenalis* infection, with or without diarrhoea. A standardized questionnaire was used to record clinical signs and symptoms before starting treatment and at the end. In addition, a physical examination was carried out. The criteria for inclusion were: (a) mono-infected with *G. duodenalis* (proven by microscopic examination of faecal samples, as direct wet mounts and/or after Ritchie concentration) (9), (b) able to take oral medication, (c) not known to have contraindications to NTZ or SNZ, with no history of disease other than *giardia*sis, and (d) who had not received any anti-parasitic chemotherapy in the previous 4-weeks. Additionally, those who were not able to attend follow-up examinations were excluded from the study.

Ethics

Ethical clearance was granted by the Research and Ethics Committee of the Cuban Institute of Gastroenterology. The enrolment also required that the agreement model were signed by patients, after being fully informed about the aim of the study and the characteristics of the drugs under investigation. The doctors signed the agreement model as well as the patients. The Protocol was kept with the code (IGE-12-2008) at the Research Department of the Cuban Institute of Gastroenterology. A full copy of that protocol was also kept at the specialized library of the Institute.

Experimental design

The sample size for each treatment group (n) needed to ensure sufficient statistical power (80%) to reject the null hypothesis that NTZ and SNZ are not equally effective (in terms of a parasitological cure) with a significant level of 5%, was calculated according to Armitage and Berry (10). The following equation was used:

$$n = \left(\frac{Z_{2\alpha}\sqrt{2\pi(1-\pi)} + Z_{2\beta}\sqrt{\pi(1-\pi_1)+\pi_2(1-\pi_2)}}{\pi_1 - \pi_2} \right)^2$$

Where:

π_1: denotes the proportion of population cured with standard treatment.

π_2: denotes the proportion of population cured with the assayed treatment.

$Z_{2\alpha} = 1.96$

$Z_{2\beta} = 0.842$

One hundred and twenty two patients were required. The patients enrolled were divided into two treatment groups using a computer-based randomization table to receive either: NTZ (Omniparax ®) 500 mg two times daily for three days or SNZ (Secnidazol gal ®) 2 g as a single dose. Omniparax ® and Secnidazol gal ® are trademarks of Laboratorios López, S.A. de C.V. El Salvador.

Treatment allocations were kept in envelopes, which were opened only on admission to the study, after obtaining the signed agreement model, availability for follow-up examinations, and all inclusion and exclusion criteria were checked. Each envelope was labelled beforehand. Patients and those providing the treatments were not blinded to the treatment allocation because the drugs look very different and the number of tablets to take varied. However, to overcome this weakness, the laboratory personnel who analysed the faecal samples were unaware of the treatment allocation.

Assessment of compliance

Comprehensive oral instructions regarding the study were given to all patients. All of them were investigated for compliance to treatment, and one of the following requirements was considered to indicate treatment non-compliance: (1) failure to attend the follow-up visits; (2) not taking one or more dose at the

instructed level and duration; (3) discontinued the drug without first asking the consent of the doctor.

Follow-up

Treatment efficacy was determined based on parasitological cure rate for the therapy assessed. To avoid apparent treatment failure due to re-infection, patient were asked to provide three faecal samples on days 3, 5, and 10 after treatment completion. A patient was only considered to be cured if no *Giardia* trophozoites or cysts could be found in any of the three post-treatment faecal specimens.

In case of treatment failure

All cases in which recommended medication failed were provided with rescue treatment using metronidazole at 250 mg given three times daily for 7 days.

Evaluation of safety

All data related to safety were monitored and recorded. Adverse drug reaction was defined as all noxious and unintended responses that did not exist beforehand, or those signs and symptoms that were present at the inclusion but became more serious following the commencement of the treatment. Unexpected adverse drug reaction was defined as an adverse drug reaction which was not consistent with the product information in terms of nature or severity. Serious adverse drug reaction was defined as those resulting in death or life threatening events. All adverse drug reactions were graded as mild, moderate, or severe.

Data management and statistical analysis

The data regarding the parasitological response and adverse events were noted on pre-designed record forms and subsequently analysed to determine the frequency of each response/effect using EpiInfo 6.0 software (Public Health Domain software, CDC, Atlanta, GA, USA). The statistical significance of differences between mean values was determined using the Student's t-test. Where appropriate, Fisher exact test was used to establish the significance of differences in proportions.

RESULTS

A total of 125 patients were included on the study, 62 in the NTZ-treated group and 63 in the SNZ-treated group. Two patients were withdrawn, one on each group, because completed the treatment assigned but did not bring the three post-treatment faecal samples. All data were analysed by intention to treat in order to guarantee the external validity of the study. The two treatment groups were similar with respect to sex, race and mean age (p>0.05).

The frequency of parasitological cure after NTZ was a little higher 59 out of 62 (95.2%) than that obtained with SNZ 59 out of 63 (93.7%) but the difference was not statistically significant (p=0.7134) [odds ratio; 1.33 (I.C): 0.24-7.91]. (Table 1).

Tablo 1. Cure rates and drug-related adverse events reported by treatment groups

Treated	Nitazoxanide group (n= 62)		Secnidazole group (n= 63)*	
	n	(%)	n	(%)
Cure rate	59	(95.2)	59	(93.7)
Any adverse event	26	(41.9)	34	(54.0)
Abdominal pain	5	(8.1)	1	(1.6)
Nausea	1	(1.6)	11	(17.5)†
Bitter taste	-		27	(42.9)
Yellowish color-ation of the urine	19	(30.6)	-	
Headache	6	(9.7)	16	(25.4)†
Rash	3	(4.8)	-	

* One patient could have had more than one adverse event.

† Statistically significant (P< 0.05).

Both treatments were well tolerated with only mild, transient and self-limited adverse events. Twenty six patients (41.9%) from NTZ treated-group and 34 patients (54%) from SNZ treated-group experienced at least one adverse event; none of them was considered to be serious (p=0.1781) [odds ratio; 1.62 (I.C): 0.75-3.51]. Bitter taste was only reported in SNZ treated group [27/63 (42.9%)] were as yellowish coloration of the urine [19/62 (30.6%)] and rash [3/62 (4.8%)] were only reported in NTZ treated group. Nausea (p=0.0026) [odds ratio; 12.90 (I.C): 1.63-276.24] and headache (p=0.0210) [odds ratio; 3.18 (I.C): 1.05-9.97] were more common in patients treated with SNZ (Table 1).

DISCUSSION

Despite reductions in mortality worldwide, diarrhoea still accounts for more than 2 million deaths annually (11). In the United States, an estimated 211 million to 375 million episodes occur each year being responsible for more than 900,000 hospitalizations and 6000 deaths annually (12). Empirical antibiotic treatment in adults who presents with severe, community-acquired diarrhoea reduces the average duration of illness by one to two day; however, the potential benefits must be weighed against the potential harm, such as prolonged faecal shedding of certain pathogens, the increased risk of relapse, and the increased risk of complications (13).

Frequently recognized as a common cause of intestinal discomfort the flagellated enteric protozoa *G. duodenalis* can be associated with long-term consequences on growth and development and is the most commonly detected parasite in the intestinal tract of humans (1,14). Given the increasing incidence of clinical treatment failures and the demonstration of the parasite resistance at laboratory level many researchers have been evaluating different drugs alternatives (3-5,14).

Nitazoxanide, recently licensed in the United States for treatment of *Cryptosporidium* spp. and *G. duodenalis*, is also a safe and effective option in the treatment of patients with chronic hepatitis C (15), viral gastroenteritis (16), and *Clostridium difficile* colitis infections (17).

Different clinical trials demonstrated the usefulness of this drug in *G. duodenalis* infections, most of them showing clinical efficacies over than 80%. The parasitological cure after NTZ In the present study was 95.2% higher than the 80.4% reported by Rodríguez-García R et al., (18) in Mexican children but similar to the 94% reported by Abaza et al., (19) in 1998 in Egypt.

Other authors demonstrated the effectiveness of NTZ against *G. duodenalis* infections. Ortiz JJ et al., (7) in 2001, reported that NTZ was as efficacious as a standard 5-day course of metronidazole (85% and 80% respectively) treating *giardia*sis and controlling diarrhoeal episodes in children from Northern Peru similar to the 81% presented by Rossignol JF et al., (8) in a randomized, double-blind, placebo-controlled study carried out the same year. NTZ is also a useful option in patients with acquired immunodeficiency syndrome. In that way Abboud P et al., (20) reported a case of metronidazole- and albendazole-resistant *giardia*sis that was successful treated with NTZ. In Cuba, a randomized controlled open-label trial, carried out at the Institute of Gastroenterology of Havana City in 2007 (21), showed an efficacy of 78.4% after using NTZ.

The parasitological cure after SNZ (93.7%) in this study was a little lower than the 98% reported by Di Prisco MC et al., (22) in an open, noncomparative study in Venezuelan children but similar to the 91.3% reported by Cimerman B et al., (23) in a randomized, open-label, clinical trial performed with Brazilian children. Both studies again demonstrated the usefulness of SNZ against this intestinal pathogen.

Adverse events notified in both treatments groups were all mild, transient, and self-limiting. No previously undescribed adverse events occurred, and none of the patient included needed to discontinue

treatment or receive additional drugs as a result of an adverse event. The adverse events notified generally occurred at frequencies similar to those observed in previous trials using the same drugs.

Two studies carried out by Rossignol JF et al., (16) and, Favennec L et al., (24) demonstrated that NTZ was a safe drug. Similar to those results Ortiz JJ et al., (7) in a trial comparing the efficacy and safety of NTZ and metronidazole in the treatment of diarrhoea caused by *G. duodenalis* in children from Northern Peru evidenced that NTZ was safe with only mild and self-limited adverse events reported. In the present study yellowish coloration of the urine and rash were only notified in NTZ treated group. Others adverse events were reported but similar in frequency to those notified in the other clinical trials using the same drug (7,16,24).

SNZ, on the other hand, is considered to be a safe drug in almost all clinical trials carried out over the world. For that reason and for its high efficacy demonstrated in many trials the scientist identifies that drug as one of the golden standards to treat *G. duodenalis* infections (14). In the present study like in others bitter taste, nausea and, headache were the adverse event more frequently notified (22,23).

One possible weakness in the current study was that for practical reasons it was conducted in an open fashion. As the two drug treatments look very different and the number of tablets to take daily varied it was impossible to make the study blind. This could be a limitation and consequently, despite well-defined pre-study criteria for evaluating efficacy and safety, evaluation of the treatment response and possible cause of adverse events could have been somewhat biased; but it could not have influenced the major result (eradication of *Giardia* infection) because the efficacy analysis was done by the laboratory department where those checking post-treatment faecal samples were unaware of the treatment allocation and had no clinical involvement with the paediatric patients or their parents.

The management of *G. duodenalis* infection has been considered by many clinicians as a problem mainly in tropical and subtropical settings. The results obtained in the present work suggest that NTZ, for three days, is as efficacious as a single dose SNZ in the treatment of *giardia*sis in adult patients.

ACKNOWLEDGEMENTS

The authors wish to thank Niurka Santos and Gisela Orvera for their technical assistance and Dr. Enrique Arús Soler chairman of the Cuban Institute of Gastroenterology for fruitful discussions.

DECLARATION OF INTEREST

This study was supported in part by a grant from Laboratorios López, S.A. de C.V. El Salvador. That laboratory behalf its representative in Havana city warranted the drugs and the external quality control of the activity. Cuban Institute of Gastroenterology was responsible for the internal quality control and supports most part of the study materials and salaries.

REFERENCES

1. Ringqvist E, Palm JE, Skarin H, Hehl AB, Weiland M, Davids BJ, et al. Release of metabolic enzymes by *Giardia* in response to interaction with intestinal epithelial cells. Mol Biochem Parasitol, 2008; 159 (2): 85-91.
2. Upcroft P, Upcroft J. Drugs target and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev, 2001; 14: 150-64.
3. Sterk M, Müller J, Hemphill A, Müller N. Characterization of a *Giardia lamblia* WB C6 clone resistant to the isoflavone formononetin. Microbiol, 2007; 153 (Pt 12): 4150-8.

4. Müller J, Ley S, Felger I, Hemphill A , Müller N. Identification of differentially expressed genes in a *Giardia lamblia* WB C6 clone resistant to nitazoxanide and metronidazole. J Antimicrob Chemother, 2008; 62(1): 72-82.

5. Aslam S, Musher DM. Nitazoxanide: clinical studies of a broad-spectrum anti-infective agent. Future Microbiol, 2007; 2: 583-90.

6. Cedillo-Rivera R, Chávez B, González-Robles A, Tapia A, Yépez- Mulia L. In vitro effect of nitazoxanide against *Entamoeba histolytica*, *Giardia intestinalis*, and *Trichomonas vaginalis* trophozoites. J Eukaryot Microbiol, 2002; 49(3): 201-8.

7. Ortiz JJ, Ayoub A, Gargala G Chegne NL, Favennec L. Randomized clinical study of nitazoxanide compared to metronidazole in the treatment of symptomatic *giardia*sis in children from Northern Peru. Aliment Pharmacol Ther, 2001; 15(9): 1409-15.

8. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* or *E. dispar*: a randomized, double-blind, placebo-controlled study of nitazoxanide. J Infect Dis, 2001; 184(3): 381-4.

9. García LS, Bruckner DA. Macroscopic and microscopic examination of faecal specimens. In: Diagnostic medical parasitology. Washington, DC: American Society for Microbiology, 1993, pp. 501-40.

10. Armitage P, Berry G. Statistical methods in medical research, 2nd ed. Oxford: Blackwell Scientific, 1987.

11. Thielman NM, Guerrant R. Acute infectious diarrhea. N Engl J Med, 2004; 350: 38-47.

12. Herikstad H, Yang S, Van Gilder TJ, Vugia D, Hadler J, Blake P, et al. A population-based estimate of the burden of diarrhoeal illness in the United States: FootNet, 1996-7. Epidemiol Infect, 2002; 129: 9-17.

13. Wistrom J, Jertborn M, Ekwall E. Empiric treatment of acute diarrheal disease with norfloxacin: a randomized, placebo-controlled trial. Ann Intern Med, 1992; 117: 202-8.

14. Escobedo AA, Cimerman S. *Giardia*sis: A pharmacotherapy review. Expert Opinion on Pharmacotherapy, 2007; 8:1885-902.

15. Rossignol JF, Kabil SM, El-Gohary Y, Elfert A, Keeffe EB. Clinical trial: randomized, double-blind, placebo-controlled study of nitazoxanide monotherapy for the treatment of patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther, 2008; 28(5): 574-80.

16. Rossignol JF, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. Aliment Pharmacol Ther, 2006; 24(10): 1423-30.

17. Musher DM, Logan N, Hamill RJ, Dupont HL, Lentnek A, Gupta A, et al. Nitazoxanide for the treatment of *Clostridium difficile* colitis. Clin Infect Dis, 2006; 43(4): 421-7.

18. Rodríguez-García R, Rodríguez-Guzmán LM, Cruz del Castillo AH. Effectiveness and safety of mebendazole compared to nitazoxanide in the treatment of *Giardia* lamblia in children. Rev Gastroenterol Mex, 1999; 64(3): 122-6.

19. Abaza H, El-Zayadi A, Kabil SM. Nitazoxanide in the treatment of patients with intestinal protozoan and helminthic infections: a report on 546 patients in Egypt. Curr Ther Res, 1998; 59, 116-21.

20. Abboud P, Lemée V, Gargala G, Brasseur P, Ballet JJ, Borsa-Lebas F et al. Successful treatment of metronidazole- and albendazole-resistant *giardia*sis with nitazoxanide in a patient with acquired immunodeficiency syndrome. Clin Infect Dis, 2001; 32(12): 1792-4.

21. Escobedo AA, Alvarez G, González ME, Almirall P, Cañete R, Cimerman S, et al. The treatment of *giardia*sis in children: single-dose tinidazole compared with 3 days of nitazoxanide. Ann Trop Med Parasitol, 2008; 102(3): 199-207.

22. Di Prisco MC, Jiménez JC, Rodríguez N, Costa V, Villamizar J, Silvera A, et al. Clinical trial with secnidazole in a single dose in Venezuelan children infected by *Giardia intestinalis*. Invest Clin, 2000; 41(3): 179-88.

23. Cimerman B, Camilo Coura L, C Salle JM, Gurvitz R, Rocha RS, Bandeira S, et al. Evaluation of secnidazole gel and tinidazole suspension in the treatment of giardiasis in children. Braz J Infect Dis, 1997; 1(5): 241-7.

24. Favennec L, Jave Ortiz J, Gargala G, Lopez Chegne N, Ayoub A, Rossignol JF. Double-blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from Northern Peru. Aliment Pharmacol Ther, 2003; 17(2): 265-70.