Preliminary report

TREATMENT OF *CLOSTRIDIUM DIFFICILE*- ASSOCIATED DISEASE

Predrag Stojanovic, Branislava Kocic*, Gordana Randjelovic*, Dobrila Stankovic-Djordjevic*, Biljana Miljkovic-Selimovic*, Snezana Antic-Mladenovic**, Kristina Stojanovic*** and Tatjana Babic***

Clostridium difficile is a Gram-positive, spore-forming, anaerobic bacillus that is widely distributed in the environment, but is found as a part of the normal large bowel flora in approximately only 3% of normal adults. *C. difficile* produces two protein exotoxins: toxin A and toxin B. Both toxins are responsible for causing the sings and symptoms of disease.

C. difficile is now thought to be responsible for a spectrum disease, ranging from asymptomatic colonization, to diarrhea of varying severity, to life-threatening colitis, often as a consequence of antibiotic exposure. This spectrum has become known as "*C. difficile* associated disease (CDAD)".

Treatment of *Clostridium difficile* associated disease demands cessation of the offending antibiotic specific therapy (vancomycin, metronidazole), anion exchange resins and probiotics (*Lactobacillus spp., Saccharomyces boulardii*). (*Acta Medica Medianae 2007;46(2):31-34.*

Key words: *Clostridium difficile, CDAD, treatment*

Faculty of Medicine in Nis*
Public Health Institute in Nis, **
Clinical Center in Nis***

Correspondence to: Predrag Stojanovic
Faculty of Medicine
81 Dr Zoran Djindjic Blvd.
18000 Nis, Srbija
Tel.: 018/326384
E-mail:pedjamicro@bankerinter.net

Introduction

Clostridium difficile is a Gram-positive, spore-forming, anaerobic bacillus that is widely distributed in the environment, but is found as a part of the normal large bowel flora in approximately only 3% of normal adults. *C. difficile* produces two protein exotoxins: toxin A and toxin B. Both toxins are typically responsible for causing the sings and symptoms of disease (1).

C. difficile is now thought to be responsible for a spectrum of diseases, ranging from asymptomatic colonization, to diarrhea of varying severity, to life-threatening colitis, often as a consequence of antibiotic exposure. This spectrum has become known as "*C. difficile* associated disease (CDAD)" (2).

Infections caused by *C. difficile* can have a lethal outcome. This fact requires urgent therapeutic treatment, which means discontinuing of the prior antimicrobial therapy, using ethiologic and probiotic therapy, and in some clinical forms of CDAD additional therapeutic procedures (3).

Discontinuing a prior antimicrobial therapy is the first procedure in the treatment of CDAD. The results of three independent studies indicate the spontaneous withdrawal of all the symptoms in 15-23% of patients over a period of 48-72 hours after discontinuing a prior antimicrobial therapy (4).

Etiological and therapeutic approaches of CDAD consist of initiation of specific antibiotics to eradicate *C. difficile* (vankomycine, metronidazole, bacitracine etc.).

Vankomycine is glycopeptides, which was primarily administered as therapy choice in staphylococcus associated enterocolitis and diarrhea followed by therapy of klindamycine, before *C. difficile* was known as the main cause of broad clinical spectrum of CDAD (5). Soon after *C.difficile* was confirmed as etiological factor of CDAD, many studies were performed concerning the optimal therapeutically treatment with vankomycine for management of infections associated with *C.difficile*.

Between 1977 and 1980, the clinician used to prescribe vankomycine during 7-14 days as a therapy of choice in patients with proven CDAD. Of all treated patients, up to 90% experienced clinical improvement. The studies performed after 1980 confirmed that oral administration of vankomycine was effective in 86-100% of patients (6,7). Because the oral use of vankomycine has incomplete absorption, the concentration in stool is up to 3100 μg/g (8, 9). Therefore, it is most probably that in vitro diagnosed resistance has little clinical importance.

Metronidazole is a derivative of nitroimidazole. It is antibiotic of choice for treatment of amebas, but from the early eighty metronidazole has been considered to be an appropriate antibiotic for CDAD (10). The occurrence of metronidazole resistance in C. *difficile* were reported during nineties, last millennium, from some laboratories in Hong Kong (MIC 64 μg/ml) and

Fran-ce (3% isolates) with MIC from 8- 32 μg/ml (11).

Using disk diffusion method, Pelaez et al. (12) confirmed metronidazole resistance in 26 from 415 of all isolates (MIC ≥ 32 μg/mL). Sanches et al. reported that susceptibility of *C. diffficile* to metronidazole was similar to the isolates obtained from patients who have shown metronidazole therapeutic benefit to those in whom the ordinate metronidazole therapy failed (13). Currently performed researches on healthy subjects have shown complete resorption of metronidazole in digestive tract, so it can not be detected in stool. However, the concentration of this antibiotic is significantly higher in liquid or unformed stool than in normal stool ($p<0.5$). This occurrence might be because the fast peristalsis of digestive tract which leads to incomplete absorption or recurrent drug filtration through colon inflammatory mucosa (14, 15).

During the last 25 years, metronidazole and vancomycin have been frequently used as therapy for CDAD (16,17).

Treatment of CDAD with metronidazole and vancomycin has some defaults, the first of all allergic reactions and adverse effects during the therapy. The US Centers for Disease Control and Prevention recommends that metronidazole can be used as first-line therapy for CDAD, which can be replaced with other antibiotic if there is not any effect after 2-3 days (18).

Bacitracin is a mixture of polypeptids isolated from Bacillus subtilis cultures and was successfully used to treat isolated cases of CDAD in the 1980s and it was subsequently compared with vancomycin. The results of three clinical studies showed that there was no difference between those drugs in the therapeutical effect, and success was 76-100% (19, 20, 21).

Teicoplanin and fusidic acid have been shown to have similar efficacy to oral vancomycin and metronidazole. A European study (22) prospectively compared oral vancomycin, metronidazole, teicoplanin, and fusidic acid in 119 patients with CDAD and found that 93-96% were clinically cured for all regimens.

Nitazoxanid is used for treatment of protozoan and helminths diseases in US. It blocks anaerobic metabolism and has effect to anaerobic bacteria (*in vitro* to *C. difficile*; MIC90 0.06-0.5 μg/ml). In humans, two thirds of orally applicated drugs is eliminated by stool as active metabolit tizoxanide (MIC90 0.06 μg/ml for *C. difficile*.) This metabolic product is found in concentration of 200 μg/ml in human bile after oral administration with 1000 mg, resulting in high drug concentration in intestinal lumen (23,24).

OPT-80 is a new macrolid with strong *in vitro* effect to *C. difficile*. Mechanism of its action is based on interruption of RNA synthesis by inhibition of RNA-polimerase (25).

Ramoplanin is new lipoglikozopeptid antibiotic against Gram-positive microorganisms. It blocks pre-peptidoglikan, named Lipid II, and affects cell wall syntheses. Ramoplanin shows bactericide activity against huge range of strains of *C. difficile* (25).

Anion resins, for example, cholestipol and cholestyramin bind toxins of *C.difficile* but still with little therapeutic benefit. Investigations have confirmed that these substances can bind drugs used as etiological therapy for CDAD (eg vancomycine) (26).

Tolevamer is the agent developed to neutralize *C. difficile* toxin in human digestive tract. Active component of tolevamer (GTI 160-246) is highly soluble anion polymer with huge molecular mass that cannot be resorbed in circulation but has good therapeutic effect in *C. difficile*–associated colitis experimented on hamsters (27).

Use of probiotics–non-pathogenic microorganisms

Mechanism of probiotic effect has to be further elucidated. These mechanisms probably involve stimulation of immune system, competition of nutritive ingredients, inhibition of pathogens for epithelia and mucosa, as well as production of antibacterial substances (28). Up date, only therapeutic effect of Lactobacillus (L) rhamnosus strain GG and Saccharomyces (S) boulardii have been investigated.

L. rhamnosus strain GG was isolated by Gorbach et al. in 1987. By passage, it reaches the intestinal tract and persist there for several days. *L. rhamnosus* strain GG adheres to mucosa cells, competes for nutrients and products substances that inhibits other bacteria (29). Competition for receptors between *L. rhamnosus* strain GG and pathogens was studied on Caco-2 cells and results were suggested that this probiotic is excellent to pathogen microorganisms (30).

S. boulardii creates protease which makes receptors inactivated for toxin A in animals, elevates levels of secretory IgA and IgA antitoxin A, competes for binding sites in ileum of rabbits and poses ability to block *C. difficille* to adhere to cell *in vivo*. (31).

McFarland et al. (32, 33) studied therapeutic use of S. boulardii in combination with vancomycine and metronidazole in 60 patients with recurrent CDAD. CDAD was reactivated in 35% of patients, but in 66% of patients there was a marked improvement ($p=0.04$).

Antiperistaltic drugs, as loperamid and diphenoxylate, should be avoided in therapeutic treatment of CDAD (34).

Human immunoglobulins (200-500 mg/kg) are applied in the treatment of particular cases of CDAD with different success. Anti *C.difficile* bovine antibodies neutralize toxin B effect on cells in cytotoxicity test, and they can be used in treatment and prevention of colitis caused by *C.difficile* in rodents (35, 36).

Treatment of recurrent CDAD

Most frequently, extended etiological antimicrobial therapy is applied, but it is not efficient because of microorganism sporulation. Vancomycin should be applied in rhythmic doses or in many minor doses, in order of *C.difficile* spores

germination, which would enable the effects of antibiotics. In some cases, recurrent CDAD is successfully treated by organisms that composed the normal colon microflora. Usually, ten different species of aerobes and anaerobes are given, and it is considered that Bacteroides spp. play the most important role (37, 38).

Therapy of asymptomatic persons: asymptomatic carriers are at low risk for CDAD and for that reason none of therapeutic procedures is applied (39). Annual unadjusted and age-adjusted attack rates and mortality rates indicate a slight but steady increase in both men and women. The disease has been registered in both sexes after 30 years of age, its incidence is highly increased after the age of 44 and it reaches its maximum in patients older than 70. The acute myocardial infarction affected women who were older than the disease-struck men. The mean twenty-eight-day case-fatality after acute myocardial infarction was higher in women than in men. A decrease of case-fatality has been registered in women since 2003.

References

1. McFarland LV, Stamm WE. Review of Clostridium difficile associated diseases. Am J Infect Control 1986; 14: 99-109.
2. Borriello SP. Pathogenesis of Clostridium difficile infection. J Antimicrob Chemother 1998; 41(Suppl. C):13-9.
3. Kuijper EJ. Coignard B. Emergence of Clostridium difficile-associated disease in North America and Europe. Clinical Microbiology and Infectious disease 2006; 12: 2-18.
4. Olson MM, Lee JT. Ten years of prospective Clostridium difficile-associated disease surveillance and treatment at the Minneapolis VA Medical Center, 1982-1991. Infect Control Hosp Epidemiol 1994; 15: 371-81.
5. Khan MY, Hall WH. Staphylococcal enterocolitis: treatment with oral vankomicin. Ann Intern Med 1966; 65: 1-8.
6. Bartlett JG. Treatment of antibiotic-associated pseudomembranous colitis. Rev Infect Dis 1984; 6: 235-41.
7. Jamal WY, Mokaddas EM, Verghese TL, Rotimi VO. In vitro activity of 15 antimicrobial agents against clinical isolates of Clostridium diffcile in Kuwait. Int J Antimicrob Agents 2002; 20: 270 - 4.
8. Pelaez T, Alcala L, Alonso R, Rodriguez-Creixems M, Garcia-Lechuz JM, Bouza E. Reassessment of Clostridium diffcile susceptibility to metronidazole and vancomycin. Antimicrob Agents Chemother 2002; 46: 1647 - 50.
9. Tedesco F, Markham R, Gurwith M, Christie D, Bartlett JG. Oral vancomycin for antibiotic-associated pseudomembranous colitis. Lancet 1978;2: 226-8.
10. Dzink J, Bartlett JG. In vitro susceptibility of Clostridium diffcile isolates from patients with antibiotic-associated diarrhea or colitis. Antimicrob Agents Chemother 1980; 17: 695 - 98.
11. Wong SS, Woo PC, Luk WK, Yuen KY. Susceptibility testing of Clostridium diffcile against metronidazole and vancomycin by disk diffusion and E test. Diagn Microbiol Infect Dis 1999; 34: 1 - 6.
12. Pelaez T, Alcala L, Alonso R, Rodriguez-Creixems M, Garcia-Lechuz JM, Bouza E. Reassessment of Clostridium diffcile susceptibility to metronidazole and vancomycin. Antimicrob Agents Chemother 2002; 46: 1647 - 50.
13. Sanchez JL, Gerding DN, Olson MM, Johnson S. Metronidazole susceptibility in Clostridium dif.cile isolates recovered from cases of C. Diffcileassociated disease treatment failures and successes. Anaerobe 1999;5:205-8.
14. Bolton RP, Culshaw MA. Faecal metronidazole concentrations during oral and intravenous therapy for antibiotic associated colitis due to Clostridium diffcile. Gut 1986;27;1169-72.
15. Friedenberg F, Fernandez A, Kaul V, Niami P, Levine GM. Intravenous metronidazole for the treatment of Clostridium diffcile colitis. Dis Colon Rectum 2001;44:1176-80.
16. Cherry RD, Portnoy D, Jabbari M, Daly DS, Kinnear DG, Goresky CA. Metronidazole: an alternate therapy for antibioticassociated colitis. Gastroenterology 1982;82:849-51.
17. Teasley DG, Gerding DN, Olson MM. Prospective randomised trial of metronidazole versus vancomycin for Clostridium-diffcile-associated diarrhoea and colitis. Lancet 1983;2:1043-6.
18. Fekety R. Guidelines for the diagnosis and management of Clostridium diffcile-associated diarrhea and colitis. American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol 1997;92:739-50.
19. Young GP, Ward PB, Bayley N. Antibiotic-associated colitis due to Clostridium diffcile: double-blind comparison of vancomycin with bacitracin. Gastro-enterology 1985;89:1038-45.
20. Dudley MN, McLaughlin JC, Carrington G, Frick J, Nightingale CH, Quintiliani R. Oral bacitracin vs vancomycin therapy for Clostridium diffcile-induced diarrhea. A randomized double-blind trial. Arch Intern Med 1986;146:1101-4.
21. Chang TW, Gorbach SL, Bartlett JG, Saginur R. Bacitracin treatment of antibiotic-associated colitis and diarrhea caused by Clostridium diffcile toxin. Gastroenterology 1980;78:1584-6.
22. Wenisch C, Parschalk B, Hasenhundl M, Hirschl AM, Graninger W. Comparison of vancomycin, teicoplanin, metronidazole, and fusidic acid for the treatment of Clostridium diffcile-associated diarrhea. Clin Infect Dis 1996;22:813-8.
23. McVay CS, Rolfe RD. In vitro and *in vivo* activities of nitazoxanide against Clostridium diffcile. Antimicrob Agents Chemother 2000;44:2254-8.
24. Aslam S. Treatment of Clostridium difficile-associated disease: old therapies and new strategies. Lancet Infect dis 2005;5:549-57.
25. Ackermann G. In vitro activity of OPT-80 against Clostridium difficile. Antimicrob Agents Chemother 2004;48:2280-2.
26. Mogg GA, Arabi Y, Youngs D. Therapeutic trials of antibiotic associated colitis. Scand J Infect Dis Suppl 1980;22:41-5.

27. Kurtz CB, Cannon EP, Brezzani A. GT160-246, a toxin binding polymer for treatment of Clostridium difficile colitis. Antimicrobial Agents & Chemotherapy 2001;45:23-40.
28. Aas J, Gessert CE, Bakken JS. Recurrent Clostridium diffcile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. Clin Infect Dis 2003;36:580-5.
29. Gorbach SI, Chang TW, Goldin B. Successful treatment of relapsing Clostridium difficile colitis with Lactobacillus GG. Lancet 1987;11:1519.
30. Surawics C. probiotics, antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in humans. Best practice abd research Clinical Gastroenterology 2003;5:775-83.
31. Pothoulakis C, Kelly CP, Joshi MA. Saccharomyces boulardii inhibits Clostridium difficile toxin A binding and enterotoxicity in rat ileum. Gastroenterology 1993;104: 108-15.
32. McFarland LV. Surawicz CM. Prevention of Saccharomyces boulardii comparated with placebo. American Journal of Gastroenterology 1995;90:439-48.
33. Shwan A. Sjolin S. Relapsing Clostridium difficile enterocolitis cured by rectal infustion of normal faeces. Scandinavian Journal of Infections Diseases 1994;16:211-5.
34. Elinav E, Planer D, Gatt ME. Prolonged ileus as a sole manifestation of pseudomembranous enterocolitis. Int J Colorectal Dis 2004;19:273-6.
35. Lyerly DM, Bostwick EF, Binion SB, Wilkins TD. Passive immunization of hamsters against disease caused by Clostridium diffcile by use of bovine immunoglobulin G concentrate. Infect Immun 1991;59:2215-8.
36. Babcock GJ, Coccia JA, Esshaki DJ. Human monoclonal antibody against toxin a protects hamsters from Clostridium diffcile disease. 42nd Annual Meeting of the Infectious Disease Society of America, Boston, Sept 30–Oct 3, 2004; Abstract 567.
37. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. BMJ 2002; 324:1361 - 4.
38. Leung DY, Kelly CP, Boguniewicz M, Pothoulakis C, LaMont JT, Flores A. Treatment with intravenously administered gamma globulin of chronic relapsing colitis induced by Clostridium diffcile toxin. J Pediatr 1991;118:633-7.
39. Johnson S, Homann SR, Bettin KM. Treatment of asymptomatic Clostridium diffcile carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. Ann Intern Med 1992;117:297-302.

TERAPIJA OBOLJENJA IZAZVANIH *CLOSTRIDIUM DIFFICILE*

Predrag Stojanović, Branislava Kocić, Gordana Ranđelović, Dobrila Stanković-Đorđević, Biljana Miljković-Selimović, Snežana Antić-Mladenović, Kristina Stojanović i Tatjana Babić

Clostridium difficile je anaerobna Gram-pozitivna, sporogena vrsta prisutna u zemlji i kolonu digestivnog trakta životinja, 3% zdrave dece i odraslih ljudi. *C. difficile* izaziva patološka stanja u digestivnom traktu lučenjem dva egzotoksina, enterotoksina A i citotoksina B, koji prouzrokuju dijareju i kolitis. Smatra se odgovornim za niz različitih stanja i to od asimptomatske kolonizacije, dijareja različite težine do po život opasnih pseudomembranoznih kolitisa. Danas su oboljenja koja izaziva poznata kao bolesti povezane sa prisustvom *C. difficile* (*Clostridium difficile* associated disease - CDAD).

Infekcije izazvane *C. difficile* mogu se završiti smrću, što nalaže hitan terapijski tretman koji podrazumeva prekid antibiotske terapije koja je prethodila infekciji, primenu etiološke i probiotske terapije a kod nekih kliničkih oblika CDAD i dodatnih terapijskih sredstava.

Prekid antibiotske terapije koja je prethodila infekciji je prva mera u lečenju CDAD. Etiološki terapijski pristup CDAD zahteva primenu efikasnih antibiotika kojima se uništava *C. difficile* (vankomicin, metronidazol, bacitracin itd.). Anjonske smole npr. colestipol i cholestyramin vezuju toksine *C. difficile* ali sa nedovoljno kliničkog efekta. Mehanizam dejstva probiotika nije u potpunosti razjašnjen. Mogući mehanizmi uključuju stimulaciju imunog sistema, kompeticiju za nutritivne supstance, inhibiciju adherencije patogena za epitel i mukoze, kao i produkciju antimikrobnih supstanci. *Acta Medica Medianae 2007;46(2):31-34.*

***Ključne reči**: Clostridium dificile, CDAD, terapija*