2. Kempf W (1980) Schneider, F.: Sugar Analysis, ICUMSA Methods. Official and Tentative Methods Recommended by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA), herausgeg. vom. Starch - Stärke 32 (9):325-326. doi:10.1002/star.19800320914

3. Asadi M (2007) Beet-sugar handbook. In. Wiley-Interscience, pp 333-335

4. Wrolstad RE (2012) Food Carbohydrate Chemistry. In. John Wiley & Sons, p 84

5. Russell NJ, Gould GW (2003) Food preservatives. In. Kluwer Academic/Plenum Publishers, p 87

6. Chichester CO, Mrak EM, Schweigert BS (1986) Advances in food research. In. Academic Press, p 23

7. Poel PW, Schiweck H, Schwartz TK, Foundation BSD (1998) Sugar technology: beet and cane sugar manufacture. In. Verlag Dr Albert Bartens KG, p 484

8. keramat J, khorvash M (2002) The chemical composition of 12 varieties of Iranian dates. journal of science and technology of agricalture and natural resources 6:189-197

THE IMPACT OF MEDICAL DRUGS ON THE MORTALITY RATE OF BIFIDOBACTERIUM STRAINS

Temitayo O. Obanla*, Sulaiman O. Aljaloud**, Salam A. Ibrahim*, Mohammad Ali Shariati***

****Food Microbiology Laboratory, North Carolina Agricultural and Technical University, Greensboro, NC, USA***

*****College of Sports Sciences and Physical Activity, King Saud University, Riyadh, Saudi Arabia***

******Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.***

Abstract: *The objective of this study was to determine the impact of commonly administered medical drugs on the death rate of Bifidobacterium. Four strains of Bifidobacterium (B.breve, B.longum, B.infantis, B.adolescentis, and B.bifidium) and eight medical drugs (Aleve, Aspirin, Tylenol, Hydrochlorothiazide, Lisinopril, Metformin, Metoprolol, or Glipizide) were used in the study. One tablet of each medical drug was ground*

and then dissolved into batches of 10 mL MRS broth. Samples were inoculated with 1 mL overnight grown cultures and incubated at 37ºC for 16 h. Bacterial populations were determined immediately after exposure to medical drugs and after 2 h of incubation. Our result showed a decrease in Bifidobacterium population by an average of 3.0 ± 0.25 log CFU/mL compared to the control due to exposure to tested drugs. Exposure to arthritis drugs (Aspirin®, Tylenol®, and Aleve®) resulted in the highest mortality rate in the Bifidobacterium population with an average of 3.91 ± 0.32 log CFU/mL. No additional decrease in bacterial populations was obtained after 2 h of incubation. These findings suggest that the intake of medical drugs may decrease the viability of probiotics bacteria and thus adversely affect these beneficial bacteria's contribution to human health.

Introduction

The gastrointestinal tract harbors complex and diverse strains of microorganisms. These microbes are important to health. Gut microflora play important roles in human health and in disease prevention (Hebuterne, 2003). The health benefits of gut microflora include metabolizing polysaccharides, activating the immune system, reducing the prevalence of atopic eczema later in life, contributing to the inactivation of pathogens in the gut, improving the immune response in elderly people, and regulating host–signaling pathways (Biagi et al; 2012, Brestoff and Artis 2013, Song et al; 2012). Probiotics may contribute to human health by improving the immune system and by improving the functionality of the gastrointestinal tract against pathogenic organisms without any adverse effects on the host (Gill, et al; 2000, Gill, 2003, Song et al; 2012). Probiotics have a direct impact on digestion, which can also increase the nutritional value of fermented dairy products (Hayek et al; 2013). However, the aging process may affect human gut microflora composition and probiotic functionality (Biagi et al; 2012, Woodmansey, et al; 2004). The mechanisms behind the changes in gut microflora as age increases can only be speculated. For example, elderly people had reduced levels of bacteroides and bifidobacteria compared to younger people (Woodmansey et al; 2004), and bifidobacteria exhibited reduced adhesion to the intestinal mucus of elderly people (Ouwehand et al; 2002). Knowledge of age related changes in the gastrointestinal tract and in gut microflora are important in the treatment and prophylaxis of diseases, and in maintenance of health among the elderly (Woodmansey, 2007). In addition to aging, other factors such as diet, lifestyle, and temporary illnesses could change the gut microflora.

Drugs might also interact with the gut microflora and modify their com-

position (Gill et al, 2001, Woodmansey et al; 2004). Antibiotic drugs which used to kill pathogenic bacteria can also kill other beneficial bacteria. For example, *Bifidobacterium* strains were found to be sensitive to several antibiotics including: penicillins: penicillin G, amoxicillin, piperacillin, ticarcillin, imipenem and other anti-Gram-positive antibiotics (Moubareck, 2005). However, other medications may also interact with probiotic survival or functionality, and reduce the health benefits. Elderly patients (65 or older) could be at high risk of having drug interactions with probiotic bacteria since most of them take medications (Tiihonen, et al; 2008). Arthritis, hypertension, and diabetic medications are among the mostly commonly taken drugs by elderly patients. However, the interactions between these medical drugs and probiotic bacteria have not been investigated. The purpose of this study was to investigate the effect of commonly administered medications on the death rate of bifidobacteria.

Materials and Methods

Culture activation and preparation

Four strains of bifidobacteria, *B. adolescentis* (ATCC15704), *B. bifidum* (ATCC29521), *B. longum (*ATCC15708), and *B. breve* (ATCC15700), were used in this study. The strains were obtained from the culture collection of the Food Microbiological and Biotechnology Laboratory at North Carolina A&T State University, Greensboro, North Carolina. The strains were activated by transferring 100 μL of the stock culture to 10 mL lactobacilli MRS broth (Neogen, Lansing, MI) and incubated at 37°C for 24 h. Bacterial cultures were streaked on MRS agar and incubated at 37°C for 48h. One isolated colony of each strain was propagated three times separately in MRS broth at 37˚C overnight for the drug treatment assay.

Medical drugs

The medical drugs (Table 1) were purchased from a local drug store in Greensboro, North Carolina. A prescription from a licensed medical personnel was obtained to purchase diabetic and hypertension drugs. The medical drugs were stored in a secure place at room temperature until needed.

Assay procedure

Overnight grown cultures of each strain were centrifuged at 4°C (7800 ×g) for 10 min using Thermo Scientific Sorvall RC 6 Plus Centrifuge (Thermo Scientific Co., Asheville, NC, USA). The pellets were washed with a sterilized 0.1% peptone water solution (Bacto peptone, Becton Dickinson, Sparks, MD, USA) and the cells were suspended in 10 mL of peptone water. Batches of 9 mL MRS broth were vigorously mixed with one crushed tablet of each drug then inoculated with 1 mL of suspended cells and incubated for 16 h at 37°C. Bacterial populations were determined at 0 and 2 h of incubation.

Bacterial enumeration

Bacterial populations were determined by plating onto MRS agar. In this procedure, a series of 10-fold dilutions in 0.1% peptone water was made and 100 μL of appropriate dilutions were surface plated onto triplicate MRS agar plates and incubated at 37◦C for 48 h. Plates with colonies ranging between 25–250 were considered for colony counting to determine the bacterial populations.

Statistical analysis

The statistical analysis of the results was performed by the SAS Institute Inc., 2010 software and the significance threshold was set at 5% ($P < 0.05$). Paired-sample t-tests were applied to compare data and exposure time among study groups of medications.

Result and Discussion

The interaction between commonly administered medical drugs (Hydrochlorothiazide, Glipizide, Lisinolril, Metformin, Metoprolol, Tylenol, Aspirin, and Aleve) on *Bifidobacterium* strains was studied. Fig 1 shows the survival of *Bifidobacterium* strains immediately after exposure to medical drugs (0 h) and after 2 h of incubation at 37ºC respectively. In control samples, *Bifidobacterium* strains remained at original populations with an average of 10.33±0.06 log CFU/mL. The exposure of *Bifidobacterium* strains to medical drugs reduced the bacterial population immediately to an average of 7.29±0.65 log CFU/mL. There was an average of 3.04±0.59 log CFU/mL reduction in the bacterial populations due to the exposure to medical drugs. Tylenol, Aspirin, and Aleve showed a higher mortality effect on *Bifidobacterium* strains compared to other tested medical drugs. Populations of *Bifidobacterium* strains were reduced to averages of 6.53±0.05, 6.55±0.02, and 6.44±0.02 due to exposure to Tylenol, Aspirin, and Aleve respectively. After 2 h of incubation, the average population of *Bifidobacterium* strains in the control was 10.36±0.06 log CFU/mL. In the presence of medical drugs, the average bacterial population was reduced to an average of 7.18±0.69 log CFU/mL. Bacterial populations after 2 h of incubation were similar to that of initial exposure time. These results indicated that common medical drugs could kill *Bifidobacterium* strains. However, the effect of medical drugs on different *Bifidobacterium* strains is not strain specific.

The mortality effect of medical drugs used for arthritis (Aspirin, Aleve, Tylenol) was higher than those used for hypertension (Hydrochlorothiazide, Lisinopril, Metoprolol) and diabetes (Metformin, Glipizide). The mortality effect of arthritis medications could be explained as a side effect of their anti-inflammatory properties. For example, Aspirin is an NSAID (nonsteroidal anti-inflammatory drug) and it is often used to treat arthritis, toothaches, and other pains aggravated by inflammation (Singh, 2013). However, Aspirin is noted for

its undesirable side effects such as gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in high doses. NSAID drugs could affect the microbial metabolism of gut microbiota and increase the aerobe microbes in the elderly (Tiihonen, et al; 2008). Such anti-inflammatory properties of arthritis drugs may alter the structure of the bacterial cell wall and result in cell death. NSAID drugs are the most prescribed drugs in the world, but their use is associated with several side effects including gastrointestinal injury and peptic ulceration (Singh, 2013). Aspirin is among the most prominent members of this group of drugs. In addition, most of these drugs are available over the counter in most countries (Singh, 2013). In 2001, NSAID drugs accounted for 70 million prescriptions and 30 billion over-the-counter sold in the United States (Singh, 2013). In this paper, we are reporting the mortality effect of NSAID drugs on bifidobacteria. These drugs may also interact with other beneficial bacteria in the human gut and reduce their population thereby adversely affecting to health and well-being.

Conclusion

The effect of commonly used medical drugs among elderly patients on the death of *Bifidobacterium* was studied. Our results indicated that common medical drugs could kill *Bifidobacterium* strains in laboratory medium immediately upon exposure to the drug. Further incubation of *Bifidobacterium* in the presence of medical drugs did not reduce the bacteria population. In addition, the effect of medical drugs on the survival of *Bifidobacterium* strains may vary among drugs. Drugs used to treat arthritis such as Tylenol, Aspirin, and Aleve have a higher mortality effect compared to other medications tested. In addition, the effect of medical drugs on *Bifidobacterium* strains is not strain specific. Thus, this research suggests that elderly citizens will need to consume food supplemented with probiotics and take probiotics supplements on a daily basis in order to maintain their gastrointestinal health.

Table 1 - List of medications used in this study

Medical drug Generic Name	Active ingredient
Aleve	Naproxene
Aspirin	Acetylsalicylic acid
Tylenol	Acetaminophen
Hydrochlorothiazide	Hydrochlorothiazide,Valsartan
Lisinopril	Lisinopril
Metformin	Metformin hydrochloride
Metoprolol	Metoprolol tartrate
Glipizide	Glipizide

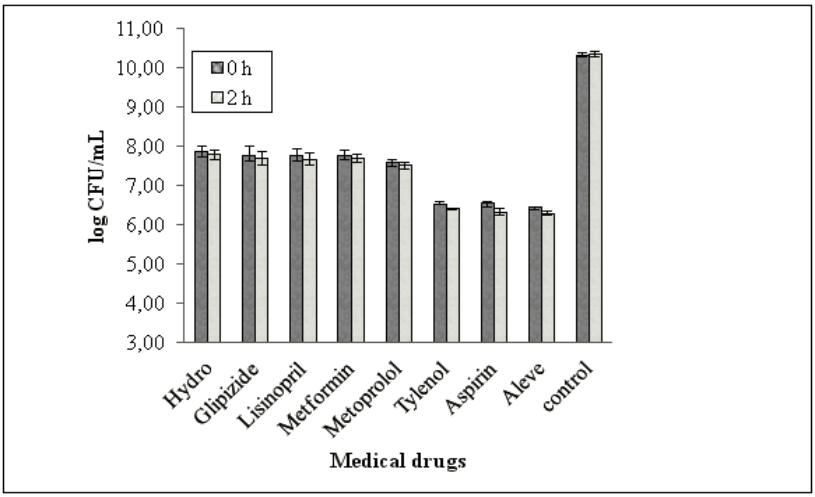

Figure 1 - Average population **of Bifidobacterium strains immediately after exposure to medical drugs (0 h) and after 2 h of incubation at 37ºC. Values are the mean ± standard error (n=3).**

References

1. Biagi E, Candela M, Turroni S, Garagnani P, Franceschi C, Brigidi, P. 2013. Ageing and gut microbes: perspectives for health maintenance and longevity. Pharmacol Res 69(1):11-20.

2. Brestoff JR, David A. 2013. Commensal bacteria at the interface of host metabolism and the immune system. Nat Immunol 14 (7):676-684.

3. Gill HS, Rutherfurd KJ, Cross ML. 2001. Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes. J Clin Immunol 21(4): 264-271.

4. Gill HS, Rutherfurd KJ, Prasad J, Gopal PK. 2000. Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). Brit J Nutr 83(02):167–176.

5. Gill HS.2003. Probiotics to enhance anti-infective defences in the gastrointestinal tract. Best Practice Rese Cl Gas 17(5), 755–773.

6. Hayek SA, Shahbazi A, Worku M, Ibrahim SA. 2013. Enzymatic activity of *Lactobacillus* grown in a sweet potato base medium. Brit Microbiol

Res J 4 (5):509-522.

7. Hébuterne, X. 2003. Gut changes attributed to ageing: effects on intestinal microflora. *Current Opinion in Clinical Nutrition & Metabolic Care*, *6*(1):49-54.

8. Moubareck C, Gavini F, Vaugien L, Butel MJ, Doucet-Populaire F. 2005. Antimicrobial susceptibility of bifidobacteria. *J Antimicrob Chemoth* 55:38–44.

9. Ouwehand AC, Salminen S, Isolauri E. 2002. Probiotics: An overview of beneficial effects. *Antonie Van Leeuwenhoek* 82(1-4): 279–289.

10. Singh S. 2013. An overview of NSAIDs used in anti-inflammatory and analgesic activity and prevention gastrointestinal damage. *J Drug Discov The* 1 (08).

11. Song D, Ibrahim S, Hayek S. 2012. Recent Application of Probiotics in Food and Agricultural Science. In: Rigobelo EC (ed) Probiotics. 1 edn. InTech, New York, pp 3-36

12. Tiihonen K, Tynkkynen S, Ouwehand A, Ahlroos T, Rautonen N. 2008. The effect of ageing with and without non-steroidal anti-inflammatory drugs on gastrointestinal microbiology and immunology. Brit J Nutr 100(1):130–137.

13. Woodmansey EJ .2007. Intestinal bacteria and ageing. J Appl Microbiol 102(5):1178–1186.

14. Woodmansey EJ, McMurdo ME, Macfarlane GT, Macfarlane S. 2004. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. *Appl Environ Microbiol* 70(10), 6113–6122.