Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
C M B Association Publisher

Original Research

High-fat diet stimulates the gut pathogenic microbiota and maintains hepatic injury in antibiotic-treated rats

Sooad Al-Daihan, Abir Ben Bacha, Abeer M Al-Dbass, Mona Awad Alonazi, Ramesa Shafi Bhat*

Biochemistry Department, Science College, King Saud University, Riyadh, Saudi Arabia

Correspondence to: rbhat@ksu.edu.sa

Received May 14, 2017; **Accepted** January 25, 2018; **Published** January 31, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.1.18

Abstract: The gut and the liver are closely linked to each other, as changes in the gut microbiota can play a significant role in the development of many liver diseases. Gut bacteria respond rapidly to changes in diet and thus can affect the liver through their metabolites. The impact of a high lipid diet on the liver in the presence of an altered gut flora modulated by ampicillin was investigated. The study was performed on 30 male Western albino rats randomly divided into 3 groups: control (phosphate buffered saline treated), group II (ampicillin 50 mg/kg for three weeks to induce microbiota alterations and fed on standard diet) and group III (same dose of ampicillin and fed on a lipid rich diet). Stool samples were collected for qualitative determination of bacteria. Serum hepato-specific markers, in addition to Glutathione (GSH), Lipid peroxidase (MDA), Glutathione-S- transferase(GST), and vitamin C in liver tissues, were measured. Altered gut microbiota significantly increased the level of the hepato-specific marker MDA and reduced the GST, GSH and vitamin C levels. However, animals fed a lipid rich diet displayed a more significant shift in hepatic markers and antioxidants. Moreover, a new switch in composition of the gut bacteria was observed by feeding the lipid rich diet. Our study showed that bacterial overgrowth in the gut can be associated with liver dysfunction and that a high lipid diet can promote the overgrowth of some liver damaging microflora during antibiotic treatment.

Key words: Ampicillin; Gut microbiota; High lipid diet; Liver; Oxidative stress.

Introduction

The gut is normally inhabited by rich and diverse micro-organisms which significantly influence our health and act together in many physiological processes in the body (1). Usually, the assortment of gut microbiota is simple at birth but progressively becomes more complex with age and is surely affected by life style and diet (2-4). The normal gut microbiota is also influenced by many antimicrobial agents, as antibiotic therapy can also affect the commensal inhabitants in addition to the target pathogen in the body, which can result in an overgrowth of certain pathogenic bacteria in the gut. β-lactam antibiotics are the safest and most effective drugs, among which ampicillin is widely used (5). Ampicillin usage results in accumulation of the drug in bile which then is directly excreted in the gut, initiating disruption of the normal intestinal microflora (6,7). Typically, microbes in the gut affect the liver though their metabolites, as most of the liver's blood supply is from the portal vein. In normal conditions, some bacterial strains can also enter the liver by crossing the intestinal barrier and can initiate liver injury (8). Depending upon the quality and diversity of the gut bacterial, microbiota can also interact with the host cell gene expression by inserting DNA in the nuclear genome (9).

Diet plays a major role in altering the gut microbiota and ranks as a main factor responsible for the composition and metabolism of colon microbiota. Gut microbes produce numerous small molecules or metabolites, which are mostly dependent on the diet of the host. The gut environment, such as gut transit time and pH, is directly affected by the type of food eaten. The quality and quantity of the three main macronutrients including carbohydrates, proteins and fats can significantly affect the composition of the microbiota in the gut (10). Changes in diet at any stage of life can affect the gut microbiota. A high lipid diet can alter the composition of gut flora (11-13) by decreasing the levels of bifidobacteria and supporting the growth of endotoxin producers (14). Many studies have confirmed that a high lipid diet results in decreased Bifidobacterium spp. in the gut with increases in body weight, fat mass, insulin resistance, and low-grade inflammation (15).

Collectively, the above mentioned studies show that gut microbiota can affect the liver through their metabolites, and diet plays a major role in altered gut microbiota composition. However, the effect of a lipid rich diet on altered gut flora is lacking in the literature. The present study evaluates the effect of a high lipid diet on antibiotic-induced altered gut bacteria in rats and scrutinizes its effect on the liver.

Materials and Methods

Animals and diet

Thirty male Western albino rats (100-150 g) were provided by King Saud University Riyadh. The animals were kept in normal temperature and humidity conditions. Rats were divided into three groups, each consisting of ten rats. The normal control group was fed a standard diet with phosphate buffered saline only, group

two was given ampicillin 50 mg/kg for three weeks (to induce microbiota alterations) and fed a standard diet, and the third group was treated with the same dose of ampicillin and converted to a high lipid diet.

Diet

The high fat diet was prepared by mixing corn oil (30%) with standard rodent chow and left overnight until all oil was absorbed and then used directly (16).

Ethics approval

All animal experiments were conducted with the approval of King Saud University Riyadh, Saudi Arabia.

Sample collection

Serum

Blood collected from the retro-orbital plexus was used to obtain serum by centrifugation at 3,000 rpm for 10 min for investigation of transaminases, alkaline phosphatase and lactate dehydrogenase.

Tissue

Liver tissue was collected and washed with cold normal saline and then homogenized in ten volume/weight of buffer (50 mM Tris-HCl, 1.15% KCl pH 7.4). The homogenate was then centrifuged at 3000 rpm for 10 min. Supernatant obtained was used for various biochemical assays.

Biochemical analyses

Serum sample

The serum was used for the estimation of hepatic marker enzymes, namely serum aspartate aminotransaminase and serum alanine aminotransaminase by the method of Reitman and Frankel (17). Serum alkaline phosphatase activity was measured following the method of King and Armstrong (18). Lactate dehydrogenase was assayed using the lactate-to-pyruvate kinetic method described by Henry et al (19).

Liver tissue

Method described by Ruiz-Larrea *et al* (20) was used to measure lipid oxidation by the formation of thiobarbituric acid reactive substances (TBARS). Vitamin C level was estimated according to the method described by Jagota and Dani (21). Method of Beutler *et al* (22) was used to measure glutathione by using 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) and sulfhydryl compounds. An assay kit from Biovision, USA using GST-catalyzed reaction between glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) was used to measure Glutathione S-transferase activity (23).

Table 1. Estimation of altered microorganisms in the rat intestinal track after treatment.

Bacteria	Group I (Control)	Group II (Amp +St)	Group III (Amp +HLD)
Escherichia coli	+	+	+
Staphylococcus spp.	+	+	+
Bacillus spp.	-	+	+
Klebsiella pneumoniae	-	+	+
Proteus vulgaris	+	+	+
Candida tropicalis	-	-	+
Rhizobium radiobacter	-	-	+
Enterococcus spp.	-	-	+

Amp ampicillin; St standard diet; HLD high lipid diet; + present;- absent.

Microbiological examination

Cecal contents collected in sterile tubes were cultured on plates and continuously scrutinized for seven days. Standard bacteriological techniques were used to identify microbiota after two days of incubation at 37 °C (24).

Statistical analysis

The values are expressed as the mean ± S.D. All statistical comparisons between the control group and the Amp and Amp + HLD groups were performed using one-way analysis of variance (ANOVA) tests with Dunnett's test for multiple comparisons. For the statistical analysis, Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) was used. Significance was assigned at the level of $P < 0.05$.

Results

Altered fecal microbiota in ampicillin-treated and ampicillin-treated fed with HLD rats are shown in Table 1. Fecal samples collected from the control group showed the presence of *Escherichia coli, Staphylococcus spp., and Proteus vulgaris*. Three weeks of antibiotic treatment introduced *Klebsiella pneumonia* and *Bacillus spp.,* whereas antibiotic treatment in the presence of HLD introduced *Candida tropicalis, Rhizobium radiobacter and Enterococcus spp.* In addition to *K.*

Table 2. Mean ± S.D. of all the measured liver enzymes in treated groups compared with those in the control group.

Parameter	Group	Mean ± S.D.	Percent change
AST (U/L)	Control	45± 3	100
	AMP	56± 1.3**	123
	AMP+HLD	73.3± 3.3*	163
ALT (U/L)	Control	72.2± 2.8	100
	AMP	81.2± 4.2*	112
	AMP+HLD	83± 2.1*	114
ALP (U/L)	Control	99.2± 0.1	100
	AMP	150.2± 4.8*	151
	AMP+HLD	152.4±2.8*	153
LDH (U/L)	Control	167.3± 12.6	100
	AMP	186± 6.3*	111
	AMP+HLD	192± 3*	114

Values with asterisks were significantly different from control values [(*p<0.05), (**p<0.01)]. AST serum aspartate aminotransaminase; ALT serum alanine aminotransaminase; ALP serum alkaline phosphatase; LDH lactate dehydrogenase; AMP ampicillin; HLD high lipid diet; S.D. standard deviation of the mean.

Table 3. GSH (µg/ml), MDA (µmol/ml), and vitamin C (µg/ml) concentrations with GST (U/ml) activities in the liver homogenates of treated groups compared with those of the control group.

Parameter	Group	Mean ± S.D.	Percent change
GSH (µg/ml)	Control	103± 8	100
	AMP	76.8±1.6*	75
	AMP+HLD	71.3 ± 1.3*	69
MDA (µmol/ml)	Control	0.15± 0.03	100
	AMP	0.18± 0.01*	120
	AMP+HLD	0.17± 0.01*	113
Vit C (µg/ml)	Control	62± 3	100
	AMP	39± 2*	63
	AMP+HLD	53± 1.7*	86
GST (U/ml)	Control	45± 3	100
	AMP	19±6*	42
	AMP+HLD	4.9±1.9 NS	11

Values with asterisks were significantly different from control [(*p<0.05), (NS: not significant)]. GSH glutathione; MDA malondialdehyde; GST Glutathione-S-transferase; ;AMP Ampicillin; HLD high lipid diet; S.D. standard deviation of the mean.

*pneumonia a*nd *Bacillus spp.*

Table 2 shows significant (p<0.05) increases in AST (+123%), ALT (+112%), ALP (+151%) and LDH (+111%) levels in the serum of ampicillin treated rats when compared with those serum levels of the control group. However, HLD during ampicillin treatment induced more changes in serum hepato-specific markers with increased values of AST (163%), ALT (114%), ALP (153%) and LDH (114%) compared with those of the untreated control group.

Table 3 shows a significant (p<0.05) depletion of glutathione (25%) with ampicillin treatments compared to control glutathione levels. HLD during the treatment produced a 31% decrease in glutathione (P<0.05). Ampicillin given with a normal diet induced more MDA (120%) in the rat liver than did ampicillin with HLD (113%). Among the other two parameters, significant decreases in vitamin C (36%) and GST (58%) levels were recorded in the liver of ampicillin-treated rat pups. However, the HLD during the same treatment induced a non-significant depletion of GST (89%).

Discussion

The guts of nearly all animals consist of microbes which play important roles in host development and physiology. The liver is continuously being affected by the intestinal microbiota, and their metabolites can distress the host's metabolism. Disturbed gut flora has a direct influence on intestinal function and can indirectly affect the liver (8). In the current study, a 3-week antibiotic treatment strongly changed the gut microbiota in animals fed a standard diet, resulting in the overgrowth of *K. pneumoniae* and *Bacillus spp.* On the other hand, a HLD during the same treatment induced the overgrowth of *C. tropicalis, R. radiobacter and Enterococcus spp.* in addition to these two bacteria (Table 1). Our results suggest that diet plays a major role in shaping the gut microbiota, which in turn may result in host metabolic disorders (10, 25). Overgrowth of *K. pneumonia* may be because this bacterium has the β-lactamase enzyme and can easily overgrow in the presence of β-lactam antibiotics (26). Overgrowth of ampicillin-resistant *Klebsiella* species with ampicillin has also been reported in humans (27). *K. pneumoniae are* usually associated with liver abscesses (28). Foods rich in lipids result in the overgrowth of a number of pro-inflammatory and pathogenic gut microbes through the formation of taurine-conjugated bile acids (29).*C. tropicalis* increases in the gut of rats fed a HLD during antibiotic treatment is well known to cause inflammatory bowel disease which in turn can cause liver injury (30). High fat diet also promoted the overgrowth of S*treptococcus R. radiobacter* in addition to C. *tropicalis.* Overgrowth of such bacteria has been shown to be related to many chronic liver diseases and hepatic dysfunction (31). This can contribute to the increase in hepatic marker enzymes in high lipid fed rats.

The effects of bacterial overgrowth on oxidative stress were examined through monitoring hepatic GSH levels, MDA, vitamin C concentrations and GST (Table 3). Overgrowth of *K. pneumoniae* seems to be associated with significant increased MDA activities and concomitant decrease in GST, GSH, and vitamin C levels in the liver. Overgrowth of *C. tropicalis* and *Streptococcus R. radiobacter* in group fed with ampicillin+ high lipid diet further increased the liver dysfunction by increasing MDA and LDH levels with a related decline in GST, GSH, and vitamin C levels (Table 3). Antibacterial agents may cause liver damage (32,33). Normal human cells can be damaged by clinical levels of antibiotics through oxidative stress. Actually, antibiotics prompt the formation of toxic reactive oxygen species (ROS) in bacterial cells which can result in liver damage (34). Our results are supported by some recent findings that antibiotics can increase the expression of oxidative stress markers in blood (34), which is due to up-regulated expression of genes involved in antioxidant defense mechanisms. A high lipid diet results in substantial changes in both bacterial growth and the level of hepatic markers compared to those with a normal diet. Bacteria can produce some hepatotoxic substances and toxic substances metabolized by the liver only (3). Thus, the quantity, quality, and composition of bacteria in the intestine can definitely affect the liver. The portal vein and the hepatic artery supply blood to the liver. The portal blood contains products of digestion and microbial products derived from the gut microbiota. The liver, therefore, is the first site of exposure and filtration of microbial products from the gut, which may evoke inflammatory reactions that contribute to the progression of liver disorders (36). The current study showed that bacterial overgrowth in the gut can be associated with liver dysfunction and that a high lipid diet can promote the overgrowth of some liver damaging microflora during antibiotic treatment.

Competing interests

The authors declare no competing interests.

Acknowledgements

This research project was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges in King Saud University.

References

1. Compare D, Coccoli P, Rocco A, Nardone OM, De Maria S, Cartenì M, Nardone G .Gut-liver axis: the impact of gut microbiota on nonalcoholic fatty liver disease. Nutr Metab Cardiovasc Dis. 2012; 22(6), 471-6 28
2. Relman DA Microbiology learning about who we are. Nature.2012; 486, 194–5.
3. Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L , et al.High protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. Am J Clin Nutr. 2011; 93, 1062–72.
4. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al Dominant and diet-responsive groups of bacteria within the human colonic microbiota . ISME J.2011; 5, 220–30.
5. Edlund C, Stark C, Nord, C. E. The relationship between an increase in β-lactam activity after oral administration of three new cephalosporins and protection against intestinal ecological disturbances. Journal of Antimicrobial Chemotherapy1994; 34, 127–38.
6. Nakaya R, Chida T. Sibaoka H. Antimicrobial agents and intestinal microflora. Bifidobacteria Microflora 1981; 1, 25–37.
7. Acred P, Brown D M, Turner D H, Wilson M J. Pharmacology and chemotherapy of ampicillin—a new broadspectrum penicillin. British Journal of Pharmacology 1962; 18, 356–69.
8. Tu YC, Lu MC, Chiang MK, Huang SH, Peng HL, Chang HY, et al Genetic requirements for Klebsiella pneumoniae–induced liver abscess in an oral infection model. Infect. Immun.2009;77, 2657-71.
9. Saint-Georges-Chaumet Y, Attaf D, Pelletier E, Edeas M. Targeting microbiota-mitochondria inter-talk: Microbiota control mitochondria metabolism. Cell Mol Biol (Noisy-le-grand). 2015 Sep 26; 61(4):121-4.
10. Scott KP, Gratz SW, Sheridan PO, Flint HJ , Duncan SH .The influence of diet on the gut microbiota. Pharmacol Res. 2013; 69, 52– 60
11. Turnbaugh PJ, Hamady M , Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al A core guts microbiome in obese and lean twins. Nature 2009; 457, 480–484.
12. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr. 2010;104(6), 919–929
13. Hildebrandt MA , Hoffmann C, Sherrill-Mix SA. High-fat diet determines the composition of the murine gut microbiome independently of obesity Gastroenterology 2009; 137(5), 1716–1724.
14. Delzenne NM, Neyrinck AM, Bäckhed F, Cani PD .Targetinggut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol. 2011; 7(11),639–46
15. Cani, P. D., Neyrinck, A. M., Fava, F., Knauf, C. et al., Selectiveincreases of bifidobacteria in gut microflora improvehigh-fat-diet-induced diabetes in mice through amechanismassociated with endotoxaemia. Diabetologia. 2007;50, 2374–2383.
16. Lian JH, Xiang YQ , Guo L, Hu WR, Ji W , Gong BQ. The use of High-Fat/Carbohydrate Diet-Fed and Streptozotocin-Treated Mice as a Suitable Animal Model of Type 2 Diabetes Mellitus. Scand. J. Lab. Anim. Sci. 2007 ; 34 (1),21-29.
17. Reitman S, Frankel S AA. colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol.1957;28(1), 56–63,
18. King EJ, Armstrong AR.A convenient method for determining serum and bile phosphatase activity. Canad. Med. Ass .1934;31, 376-381
19. Henry JB. Clinical Diagnosis and Management by Laboratory Method". Sixteenth edtion.; Philadelphia, PA: WB Saunders and Company; 1974.
20. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsome. Steroids.1994; 159,383
21. Jagota SK, Dani HMA. New colorimetric technique for the estimation of Vitamin C using Folin phenol reagent. Anal Biochem.1982; 15, 178.
22. Beutle E, Duran O, Kelly BM Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61, 882.
23. Habig WH, Pabst MJ, Jacoby WB, G. Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. J Biol Chem .1974; 249, 7130–9.
24. Zhichao Z, Xichun P, Saoting L, Ning Z, Yong W, Hua W. Isolation and identification of Quercetin degrading bacteria from human fecal Microbes.PLOS one 2014; 9: e90531.
25. Tremaroli V, Bäckhed F.Functional interactions between the gut microbiota and host metabolism. Nature .2012; 489, 242–249
26. Guentzel MN. Escherichia, Klebsiella, Enterobacter, Serratia Citrobacter, and Proteus. In: Barron's Medical Microbiology Univ of Texas Medical Branch (Barron's et al., eds.) (4th ed.) ;1996.
27. Knothe H, Wiedemann B .Die Wirkung von Ampicillin auf die Darmflora des gesunden Menschen. Zentralbl Bakteriologie, Mikrobiologieund Hygiene. A AlW, 1965; 234-243.
28. Jason T. Wells, Catherine R. Lewis, Omar K. Danner, Kenneth L. Wilson, and L. Ray Matthews, Klebsiella pneumoniae Liver Abscess and Metastatic Endophthalmitis. J Investig Med High Impact Case Rep 2016 Jan-Mar; 4(1): 2324709615624125.
29. Devkota S., Wang Y., Musch M.W., Leone V., Fehlner-Peach H., Nadimpalli A., Antonopoulos D.A., Jabri B., Chang E.B. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice. Nature. 2012;487:104–108
30. G. Hoarau P. K. Mukherjee C. Gower-Rousseau C. HagerJ. Chandra M. A. Retuerto C. Neut S. Vermeire J. Clemente J. F. Colombel H. Fujioka D. Poulain B. Sendid and M. A. Ghannoum Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn's DiseasemBio. 2016 Sep-Oct; 7(5): e01250-16.
31. Bauer TM, Schwacha H, Steinbrückner B, Brinkmann FE, Ditzen AK, Aponte JJ et al Small intestinal bacterial overgrowth in human cirrhosis is associated with systemic endotoxemia. Am J Gastroenterol .2002;(97)9, 2364–2370.
32. Cundiff J, Joe S .Amoxicillin-clavulanic acid-induced hepatitis. Am. J. Otolaryngol. 2007;28: 28-30.
33. Cundiff JG Joe S .Augmentin-induced hepatitis in the treatment of chronic rhinosinusitis. Otolaryngol. Head Neck Surg.2004; 131, 266-267.
34. Kalghatgi S, Spina CS, Costello JC Liesa M, Morones-Ramirez JR, Slomovic S et al Bactericidal Antibiotics Induce Mitochondrial Dysfunction and Oxidative Damage in Mammalian Cells. Sci Transl Med.2013; 5 192, 192ra85.
35. Abu-Shanab A, Quigley EM The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol .2010; 7, 691 – 701.
36. Iacono A, Raso GM, Canani RB. Calignano A, Meli R .Probiotics as an emerging therapeutic strategy to treat NAFLD: Focus on molecular and biochemical mechanisms J. Nutr. Biochem .2011;22, 699–711.