Жамият ва инновациялар –
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Antibiotic-induced rat gut microbiota dysbiosis and salmonella resistance

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ARTICLE INFO

Article history:
Received March 2021
Received in revised form
20 March 2021
Accepted 15 April 2021
Available online
20 May 2021

Keywords:
antibiotics,
gut microbiota,
pathogens,
animal experiment.

ABSTRACT

Antibiotics are the most common medicines used to treat human infectious diseases. Based on their chemical structure, antibiotics mainly include the following categories: quinolones, β-lactams, macrolides, and aminoglycosides among others. The mechanism of different antibiotics varies, and there are four main mechanisms: inhibition of bacterial cell wall synthesis, interaction with cell membranes, interference with protein synthesis, and inhibition of nucleic acid replication and transcription. Antibiotics can act on pathogenic bacteria. Accordingly, antibiotics can also affect normal bacteria that colonize the human body. The size, structure, and function of the microbiota may change in response to antibiotic treatment. Significant changes in the human gut microbiota may be associated with repeated use of antibiotics [3]; in the following days, these changes were restored. However, little is known about comparing the response of the gut microbiota to antibiotic treatment.

Probiotics are beneficial to the host when administered in adequate amounts. Lactobacillus rhamnosus was one of the most common probiotics studied by scientists regarding its culture, function, and metabolism [10]. However, the effect of L. rhamnosus present in the gut microbiota on the host's susceptibility to pathogenic bacteria after taking antibiotics has rarely been discussed.

In our current study, rats were given two types of antibiotics, namely vancomycin and ampicillin, and their oral and intestinal microbiota was observed at 3 time points. The rats were treated with antibiotics or L. rhamnosus, and then infected with Salmonella entericaserovar Typhimurium (S. Typhimurium) via a gastric tube. Fecal samples were then collected to determine the pathogenic load.

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Ampicillin and vancomycin act in different antimicrobial spectra and have different absorption in the digestive tract. In addition, the concentration of these antibiotics entering the digestive tract varies; these factors can affect the host microbiota. Thus, this study aimed to compare the effects of these antibiotics on the gut microbiota at normal doses, as well as to evaluate the differences in the results.

The gut microbiota underwent dramatic changes during the administration period. Changes in the gut microbiota affected the host's susceptibility to pathogens when infected with bacteria due to changes in resistance to colonization.

Антибиотиклар таъсирида юзага келтирилган каламуш ичаги микробиотининг дисбактериоз ҳолати ва унинг салмонеллаларга резистентлиги

Калит сўзлар:
антибиотиклар,
ичак микробиоти,
қўзғатувчилар,
ҳайвонларда ўтказилган тажрибалар.

АННОТАЦИЯ

Антибиотиклар инсон юқумли касалликларини даволаш учун ишлатиладиган енг кенг тарқалган дори-дармонлардир. Уларнинг кимёвий тузилишига асосланиб антибиотиклар асосан қуйидаги туркумларни ўз ичига олади: хинолонлар, β-лактамлар, макролидлар ва аминогликозидлар. Турли антибиотикларнинг таъсир механизми турлича бўлиб, тўртта асосий шакли мавжуд: бактериал ҳужайра девори синтезини блоклаш, ҳужайра мембраналари билан ўзаро таъсир, оқсил синтезида қатнашиш ва нуклеин кислота репликацияси ва транскрипсиясини бекор қилиш. Антибиотиклар патоген бактерияларга таъсир қилади. Шунингдек, антибиотиклар ҳам инсон танасида колония ҳосил қилувчи normal бактерияларга ҳам таъсир қилиши мумкин. Микробиотнинг ҳажми, тузилиши ва функцияси антибиотикларни даволашга жавобан ўзгариши мумкин. Инсон ичак микробиотидаги сезиларли ўзгаришлар антибиотиклардан такрорий фойдаланиш билан боғлиқ бўлиши мумкин [3]; кейинги кунларда бу ўзгаришлар тикланди. Бироқ, ичак микробиотининг антибиотикларни даволашга таъсирини таққослаш ҳақида жуда кам маълумот маълум.

Пробиотиклар инсон организми учун етарли миқдорда киритилганидагина фойдалидир. Lactobacillus rhamnosuso-лимлар томонидан ўрганилган метаболизм, унинг функциялари ва экма мухитлари ичида енг кенг тарқалган пробиотиклардан биридир [10]. Бироқ, ичак микробиетида мавжуд бўлган L. rhamnosusнинг антибиотикларни қабул қилгандан кейин патоген бактерияларга хос таъсирчан-лигига таъсири камдан-кам ҳолларда муҳокама қилинди.

Ҳозирги тадқиқотимизда каламушларга антибиотик-ларнинг икки тури, яъни ванкомицин ва ампициллин берилган бўлиб, уларнинг оғиз ва ичак микробиоти 3 вақт нуқтасида кузатилган. Каламуш антибиотиклар ёки L. rhamnosus билан даволанди ва кейин ошқозон найчаси орқали Salmonella ентериса серовар typhimurium (C. typhimurium) билан зарарланган. Кейинчалик патоген флорани аниқлаш учун фекал намуналар йиғилди.

Ампициллин ва ванкомицин турли антимикроб спектрларда ҳаракат қилади ва овқат ҳазм қилиш трактида ҳар хил сўрилишга ега. Бундан ташқари, овқат ҳазм қилиш трактига тушгандан сўнг, бу антибиотиклар концентра-цияси ўзгаради; бу омиллар организм микробиётига таъсир қилиши мумкин. Шундай қилиб, ушбу тадқиқот юқоридаги антибиотикларнинг ичак микробиотига таъсирини normal дозаларда таққослаш, шунингдек, натижалардаги фарқларни баҳолашга қаратилган.

Ичак микробиоти антибиотикларни юбориш даврида кескин ўзгаришларга учради. Ичак микро-биотининг ўзгариши туфайли организмнинг бактериялар билан зарарлангандаги қаршилиги уларнинг колония ҳосил қилишдаги хусусиятларининг ўзгариши туфайли пасайди.

Антибиотик-индуцированный дисбиоз микробиоты кишечника крыс и резистентность к сальмонеллам

Ключевые слова:
антибиотики,
микробиота кишечника,
патогены,
эксперимент на животных.

АННОТАЦИЯ

Антибиотики являются наиболее распространенными лекарствами для лечения инфекционных заболеваний человека. Основываясь на своей химической структуре, антибиотики в основном включают следующие категории: хинолоны, β-лактамы, макролиды и аминогликозиды среди других. Механизм различных антибиотиков различается, и существует четыре основных механизма: ингибирование синтеза клеточной стенки бактерий, взаимодействие с клеточными мембранами, вмешательство в синтез белка и ингибирование репликации и транскрипции нуклеиновых кислот. Антибиотики могут действовать на болезнетворной бактерии. Соответственно, антибиотики также могут влиять на нормальные бактерии, колонизирующие человеческое тело. Численность, структура и функции микробиоты могут измениться в ответ на лечение антибиотиками. Значительные изменения в микробиоте кишечника человека могут быть связаны с повторным использованием антибиотиков [3]; в последующие дни эти изменения были восстановлены. Тем не менее, мало что известно о сравнении реакции микробиоты кишечника на лечение антибиотиками.

Пробиотики полезны для хозяина при введении в адекватном количестве. Lactobacillus rhamnosus был одним из наиболее распространенных пробиотиков, исследованных учеными в отношении его культуры, функций и метаболизма [10]. Однако влияние L. rhamnosus, присутствующего в микробиоте кишечника, на восприимчивость хозяина к патогенным бактериям после приема антибиотиков редко обсуждалось.

В нашем текущем исследовании крысам вводили два вида антибиотиков, а именно ванкомицин и ампициллин, и их микробиоту полости рта и кишечника наблюдали в 3 временных точках.

Крыс лечили антибиотиками или L. rhamnosus, а затем инфицировали Salmonella enterica серовар Typhimurium (S. Typhimurium) через желудочный зонд. Затем были собраны образцы фекалий для определения патогенной нагрузки.

Ампициллин и ванкомицин действуют в разных антимикробных спектрах и имеют разную абсорбцию в пищеварительном тракте. Кроме того, концентрация этих антибиотиков, попадающих в пищеварительный тракт, варьируется; эти факторы могут повлиять на микробиоту хозяина. Таким образом, это исследование было направлено на сравнение эффектов этих антибиотиков на микробиоту кишечника в нормальных дозах, а также на оценку различий в результатах.

Микробиота кишечника претерпела резкие изменения в течение периода введения. Изменения микробиоты кишечника повлияли на восприимчивость хозяина к патогенам при заражении бактериями из-за изменений устойчивости к колонизации.

GOALS

Antibiotics play a large role in the treatment of infectious diseases, but at the same time they cause serious disorders in the host microbiota. Studies of various changes in the host microbiota caused by antibiotics are relatively few. This study was aimed at examining changes in the gut and possible changes in gut resistance to salmonella caused by taking antibiotics.

THE RESULTS OBTAINED

The reaction of the gut microbiota to different antibiotics

All rats in each group (n = 4) were included in the analysis. The gut microbiota has undergone drastic changes due to the use of antibiotics, and ampicillin and vancomycin have led to a reduction in the diversity and diversity of taxa in the gut. The alpha diversity of the gut microbiota decreased markedly during treatment, and then increased after treatment. Alpha diversity was not completely the same between the pre-treatment period, the post-treatment period, and the treatment period. This phenomenon was observed in both the ampicillin and vancomycin groups, but not in the control group, indicating that this change was due to drug-induced temporal fluctuations in the gut microbiota in rats. In addition, the composition of the gut microbiota was changed. During treatment, Lactobacilli and Escherichia Shigella showed high numbers in both

treatment groups. In particular, Escherichia Shigella dominated the ampicillin group, while Lactobacilli dominated the vancomycin group.

The gut microbiota does not fully recover from the disorder caused by taking antibiotics.

The results showed that in the ampicillin and vancomycin groups, there was a significant difference in the gut microbiota after treatment and before treatment. Therefore, it can be concluded that the gut microbiota cannot completely return to the level that was before the introduction of the antibiotic.

During ampicillin treatment, the gut microbial community was separated from the before and after time points. Four weeks after the treatment, the gut microbiota recovered. But the status of the microbiota was not completely identical before and after ampicillin treatment.

The gut microbiota responses to antibiotics showed the same trend in the vancomycin group. During treatment, the microbiota changed dramatically, but recovered after stopping taking antibiotics. However, the original abundance was not restored.

Genera that did not recover from the use of antibiotics were identified as follows: Lactobacillus, Eubacterium, and Allobaculum in the ampicillin group and Lactobacillus, Lachnospiraceae, Ruminococcus, Ruminiclostridium, Anaeroplasma, and Anaerotruncus in the vancomycin group. We found that in both groups of rats, Lactobacillus did not fully recover to the baseline level. According to a previous study, many species of the genus Lactobacillus are potential probiotics that can improve intestinal function and treat intestinal resistance to disorders.

Colonizing resistance of the intestinal microbiota to bacterial pathogens can be damaged by antibiotics.

To determine whether antibiotic treatment changed the resistance of the intestinal microbiota to colonization, rats (n = 10) were injected with a probe of S. Typhimurium four weeks after treatment with an antibiotic or probiotic L. rhamnosus and the number of pathogens after infection was determined. The results show that in the first four days after infection, the number of S. Typhimurium in the groups of rats treated with ampicillin and L. rhamnosus was higher than in the control group. In the following days, there was a gradual decrease in concentration, and the levels fell lower than those observed in the control. These results suggest that the use of ampicillin may have affected the normal intestinal resistance of rats and increased the severity of infection. However, this phenomenon only lasted until the bowel resistance recovered and eventually reached normal levels. The situation in the vancomycin group was similar. The concentration of S. typhimurium was initially higher in the vancomycin group than in the control on days 1, 3, and 4, after which a gradual decrease in the concentration was observed.

It was particularly interesting that short-term consumption of L. rhamnosus did not cause any changes in the resistance of the rat gut microbiota to bacterial pathogens.

DISCUSSION

It is well known that microbiomes in different niches can act differently under environmental stress.

The results showed that the gut microbiota changed dramatically during the administration of ampicillin and vancomycin, and this change was not eliminated after discontinuation.

We found that ampicillin and vancomycin significantly altered the gut microbiome. Changes in the microbiota caused by antibiotics do not directly cause diseases. However, the risk of disease may be increased with the use of antibiotics, which may be the result of reduced resistance of the microbiota to pathogenic pressure. Thus, S. typhimurium was used to simulate pathogenic effects in animal studies. Typhimurium is a gram-positive bacterium that causes diarrhea and is commonly

used in the gut as a bacterial pathogen in animal experiments [11, 13]. In our study, rats were fed water with an antibiotic or regular water followed by infection with S. typhimurium., and the concentration of bacteria in the feces of treated rats was higher than in control rats. A possible reason for this is that the loss of some bacteria led to incomplete restoration of the gut microbiota, which weakened resistance to pathogens compared to their normal counterparts. Moreover, the two antibiotics used in this study gave different results. The rats treated with vancomycin had fewer pathogens than the rats treated with ampicillin between 2 and 4 days after infection. We assumed that different bacteria remained alive after the administration of different antibiotics, given the different antimicrobial spectra of vancomycin and ampicillin.

It has been demonstrated that antibiotics can break down the gut microbiome barrier to pathogen pressure. However, some studies have reported that probiotics can alleviate the disruption of the gut microbiota and help the host develop homeostasis after the disruption [7]. In the present animal experiment, several positive effects of the probiotic L. rhamnosus were observed in rats that did not receive antibiotics. Intestinal resistance to pathogens did not change between the group receiving L. rhamnosus and the control group.

In addition, we observed that 4 days after infection, the concentration of the pathogen in the treated group decreased to the level observed in the control group. In our experiment, no mortality was reported in mice, probably due to the fact that the rats were healthy before treatment. However, a negative prognosis can be observed in patients with severe illnesses or poor health.

CONCLUSION

Both ampicillin and vancomycin can cause significant changes in the level of the intestinal microbiota that cannot be completely restored.

In addition, susceptibility to exogenous pathogens increased after the use of antibiotics for a short period of time. The beneficial effect of probiotics in healthy rats was not observed.

MATERIALS AND METHODS

Animals

Free from specific pathogens, ten-week-old non-parodic female rats were bred and kept under SPF conditions at room temperature in an animal room. No more than five rats were kept in one cage, and the animals were given food and water.

Treatment of animals with antibiotics and collection of samples

In the first part of the experiment, 12 rats were randomly divided into three groups (n = 4), which were administered vancomycin (0.2 mg / ml; the group receiving vancomycin), ampicillin (1 mg / ml; the group receiving ampicillin), or plain water (control group) in drinking water for 3 days. The doses were selected based on previously published data [14]. Rat feces were collected at three time points: the day before the antibiotic was administered, the first day after the antibiotic was administered, and four weeks after the antibiotic was administered. All fecal samples were collected in sterile tubes.

Infection of the rat pathogen

In the second part of the experiment, 40 rats were randomly divided into four groups (n = 10). Rats in each group received ampicillin, vancomycin, L. rhamnosus, or plain water for three days prior to infection. In the group treated with L. rhamnosus, L. Rhamnosus was resuspended in 1 ml of PBS containing only 1 × 10 9 L. Rhamnosus. The probiotic was administered to rats via a gastric tube (0.1 ml / mouse) once a day for three days. Rats that were given regular drinking water were used as controls. After this treatment, all the rats were fed regular water for four weeks. Subsequently, all groups of

rats were infected with S. Typhimurium. S. Typhimurium was grown at 37 ° C with shaking (200 rpm) overnight in Luria-Bertani broth (LB). Rats were infected via a gastric tube with 0.2 ml of PBS containing approximately 5 × 10 7 CFU of S. Typhimurium. Rat feces were collected 1, 2, 3, 4, 7, and 10 days after infection (additional Figure 3). The health status of the rats was monitored for 10 days after infection. To determine the pathogenic load, fecal sediments were homogenized in sterile PBS and seeded in serial dilutions on SS agar cups, and the amount of CFU was determined after incubation overnight at 37 ° C. Ten days after infection, all the rats were killed by CO 2. Suffocation after fecal collection.

THE RESULTS OBTAINED

The gut microbiota underwent dramatic changes after treatment with vancomycin and ampicillin. The gut microbiota recovered within four weeks of stopping the antibiotics, although this recovery was incomplete. The number of pathogens in the intestine in the control group was significantly lower than in the group receiving antibiotics, but remained only for the first 4 days after infection.

CONCLUSIONS

Antibiotics cause dramatic changes in the amount and diversity of the gut microbiota. These changes in the gut microbiota may not fully recover after four weeks. When infected with pathogens after the introduction of an antibiotic, rats show a decrease in resistance to colonization in the intestine during the first four days after infection.

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