

Synbiotic Supplementation And Oxalate Homeostasis In Rats: Focus On Microbiota Oxalate-Degrading Activity

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Abstract

Background The present study aimed (i) to evaluate whether ceftriaxone treatment could affect not only intestinal oxalate-degrading bacteria number but their total activity to degrade oxalate and influence oxalate homeostasis in rats, (ii) to test the effect of commercially available probiotics and a synbiotic on total fecal oxalate-degrading activity, (iii) and to estimate the ability of synbiotic to restore fecal oxalate-degrading activity and ceftriaxone-induced disruption of oxalate homeostasis in rats.

Methods Twenty-eight female Wistar rats (200-300 g) were randomly divided into 4 groups (n = 7). Group 1 was treated with vehicle sterile water (0.1 ml, i.m., 14 days); Group 2 received synbiotic (30 mg/kg, per os, 14 days); Group 3 was treated with ceftriaxone (300 mg/kg, i.m., 7 days); Group 4 was supplemented with ceftriaxone and synbiotic. Oxalate-degrading bacteria number and their total activity, urinary and plasma oxalate concentrations were measured on days 1 and 57 after the treatment withdrawal.

Results Ceftriaxone treatment reduced total fecal oxalate-degrading activity independently on oxalate-degrading bacteria number and increased urinary and plasma oxalate concentrations. The synbiotic had a high oxalate-degrading activity vs probiotics and was able to restore fecal oxalate-degrading activity and significantly decrease urinary oxalate excretion in antibiotic-treated rats.

Conclusion Total fecal oxalate-degrading activity but not oxalate-degrading bacteria number should be thoroughly examined in the future to develop predictive diagnostics methods, targeted prevention and personalized treatment in kidney stone disease. Synbiotic supplementation had a beneficial effect on the total oxalate-degrading activity of gut microbiota, which resulted in decreased UOx excretion in rats.

Introduction

The role of the gut microbiota in the development and recurrence of kidney stone disease (KSD) is now being actively discussed [1–4]. The emerging data highlight the gut microbiota dysbiosis as an important risk factor for calcium oxalate (CaOx) lithiasis formation, the most common form of KSD [1, 4]. The ability of *Oxalobacter formigenes* and other intestine oxalate-degrading bacteria (ODB) (e.g. *Enterococcus spp.*, *Lactobacillus spp.*, *Bifidobacterium spp.*, *Bacillus spp.*) has been shown to degrade oxalate and stimulate its endogenous secretion [1, 2, 4]. These data have found a clinical application, and a strong association between the low abundance of ODB in the gut microbiota community and hyperoxaluria has been demonstrated confirming the potential role of ODB in the maintenance of oxalate homeostasis [5–7]. However, the majority of the existing studies have largely focused on the quantitative determination of ODB in feces [1, 6–8] but not on their total oxalate-degrading activity (ODA).

The term of total ODA in fecal microbiota could be defined as the general ability of different strains of ODB to metabolize oxalate [9]. Several in vitro studies have been devoted to oxalate degradation abilities of *Oxalobacter formigenes*, *Bifidobacterium* and *Lactobacillus* strains in which the efficiency degrade oxalate from 15–98% has been reported [10–12]. Therefore, the presence of ODB in the intestine might not mean their sufficient functional capabilities. It should be noted, that to the best of our knowledge,

there is a general lack of experimental or clinical studies on ODA in fecal microbiota. There is only the early study where the authors have measured total ODA directly in human fecal samples in anaerobic dilution solution with [14C]-oxalate [13].

It is little wonder that antibiotics would affect gut microbiota and reduce the number of ODB [14, 15]. The evidence of antibiotics' effect on ODB was generally based on quantification before and after antibacterial treatment [14, 15]. It has been demonstrated that the administration of different antibiotic classes reduces ODB in the gut for a long-time period after antibiotic exposure [14–16]. However, the effect of antibiotics on total ODA in fecal microbiota has never been evaluated before. Consequently, it is still unclear whether the use of antibiotics could affect not only the number of ODB but their ODA and thus influence the blood and urine oxalate concentrations.

Various pro-, pre- and synbiotic supplements have been proposed for restoring gut microbiota after antibiotic treatment and preventing KSD [17, 18]. The overwhelming majority of the published experimental and in vitro studies have demonstrated a promising effect of probiotics on increasing ODB number and reducing urinary oxalate (UOx) excretion [17, 18]. However, although synbiotics have more prominent probiotic functions than probiotics and prebiotics alone due to their synergetic effect [19], little is known about the ODA of synbiotics vs probiotics. We hypothesized that the ODA of a specific probiotic or synbiotic could explain the lack of its clinical efficacy and provide a personalized approach to their choice to reduce hyperoxaluria and prevent KSD.

Therefore, the present study aimed (*i*) to evaluate whether ceftriaxone treatment could affect not only intestinal ODB number but their total ODA and influence oxalate homeostasis in rats, (*ii*) to test some commercially available three probiotics and a synbiotic on total fecal ODA, (*iii*) and to estimate the ability of synbiotic to restore ODA and ceftriaxone-induced disruption of oxalate homeostasis in rats.

Materials And Methods

Animals

Twenty-eight female Wistar rats (200-300 g) were bred and housed in the conventional animal facility of the ESC “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv (Kyiv, Ukraine) under standard environmental conditions (12-h light/dark cycle at a constant temperature of 22°C). All the rats were kept in a standard cage and had free access to a standard stock diet and tap water provided *ad libitum*. To ensure standardized gut microbiota, rats from all groups were kept in the same room and maintained by the same person. The “Guide for the Care and Use of Laboratory Animals” (National Research Council 2011) was followed. The procedures used and the care of animals were approved by the animal committee of the ESC “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv (Protocol # 18/01/2020).

Sample size

Sample size consideration was based on previous reports on a similar topic [20, 21] and calculated using G*Power Software, version 3.1.9.4. The authors have reported the effect sizes from 0.93 to 2.56 with sample sizes ranged between 6 and 8 animals in each group. A minimum sample size of 7 animals in each group would be required to achieve a power of 0.80 and an alpha of 0.05 detecting differences between the groups using non-parametric tests. Similarly, we would need a minimum sample size of 25 rats to achieve a power of 0.80 and an alpha of 0.05 in the correlation analysis.

Study design

Animals were numbered and randomly divided into 4 groups of 7 rats each: Group 1 received sterile water (Vehicle group), Group 2 was supplied with synbiotic (Synbiotic group), Group 3 was intramuscularly injected with ceftriaxone (Ceftriaxone group) and Group 4 was given ceftriaxone and synbiotic simultaneously (Ceftriaxone + Synbiotic group) (Table 1). Bodyweight and lethargy were controlled throughout the experimental period.

Table 1
Experimental Groups and Interventions

Groups (n = 28)	Interventions
Vehicle (VEH, n = 7)	Sterile water 0.1 ml, intramuscularly for 14 days
Synbiotic (SYN, n = 7)	Synbiotic 30 mg/kg, <i>per os</i> for 14 days
Ceftriaxone (CEF, n = 7)	Ceftriaxone 300 mg/kg, intramuscularly for 7 days
Ceftriaxone + Synbiotic (CEF+SYN, n = 7)	Ceftriaxone (300 mg/kg, intramuscularly for 7 days) and synbiotic (30 mg/kg, <i>per os</i> for 14 days) simultaneously

On day 1 after treatment withdrawal, the amount of ODB and their total ODA in fecal microbiota, as well as urinary oxalate (UOx) excretion were measured in each group of rats. Then we repeated the same tests in 8 weeks (day 57) after treatment withdrawal.

On day 57 of the experimental period, all rats were anesthetized with urethane (1.1 g/kg, i.p. Sigma-Aldrich, Germany). Blood samples were collected by cardiac puncture immediately after death, and in addition to the aforementioned markers in the rats' feces and urine, plasma oxalic acid (POx) was determined. The experiment design and outline are depicted in Fig. 1.

Experimental substances

Antibiotic. The rat equivalent dose of ceftriaxone (Ind. Stock Company Darnytsya, Ukraine) was calculated based on body surface area by multiplying the human dose (50 mg/kg) by the K_m value (6 for rats) [22].

Probiotics. The ODA was tested for three commercially available probiotics: 1) manufactured by LLC "Biopharma Plasma", Ukraine, and consisted of 2 strains of *Lactobacillus spp.* (*L. fermentum* 2×10^9

CFU/g, *L. plantarum* 2×10^9); 2) manufactured by LLC "Biopharma Plasma", Ukraine, and consisted of *Bacillus subtilis* «UKMV-5020» 1×10^{10} CFU/g; 3) manufactured by Sanofi Aventis, Italy, and consisted of *Bacillus clausii* 2×10^9 CFU/g.

Synbiotic. The synbiotic was manufactured by LLC "Element of Health", Ukraine, and consisted of 4 strains of *Lactobacillus spp.* (*L. acidophilus* 5×10^8 CFU/g, *L. rhamnosus* 9×10^8 CFU/g, *L. plantarum* 2×10^7 CFU/g, *L. casei* 4×10^8 CFU/g), 2 strains of *Bifidobacterium spp.* (*B. bifidum* 5×10^8 CFU/g and *B. longum* 8×10^8 CFU/g), *Saccharomyces boulardii* 3×10^7 CFU/g, selenium 0.05 mg, oligofructose 40 mg and inulin 450 mg.

Processing of fecal specimens for ODB and total ODA analysis

Rats' fecal samples (1g) were collected and immediately dispersed in 9 ml highly selective media Oxalate Medium (g/L): K_2HPO_4 – 0.25, KH_2PO_4 – 0.25, $(NH_4)_2SO_4$ – 0.5, $MgSO_4 \cdot 7H_2O$ – 0.025, CH_3COON – 0.82, yeast extract – 1.0, rezazurin – 0,001, Na_2CO_3 – 4, L-cystein-HCl – 0.5, Trace element solution SL-10 – 1 ml (mix/L: HCl (25%; 7.7 M) – 10.00 ml, $FeCl_2 \times 4H_2O$ – 1.50 g, $ZnCl_2$ – 70.0 mg, $MnCl_2 \times 4H_2O$ – 100.0 mg, H_3BO_3 – 6.0 mg, $CoCl_2 \times 6H_2O$ – 190.0 mg, $CuCl_2 \times 2H_2O$ – 2.0 mg, $NiCl_2 \times 6H_2O$ – 24.0 mg, $Na_2MoO_4 \times 2H_2O$ – 36.0 mg; $Na_2C_2O_4$ – 5 mg) [23].

For determination of ODB number, serial dilutions (10^{-3} , 10^{-5} , 10^{-7}) of each dispersed sample were prepared. One aliquot of 0.1 ml of each dilution was seeded by a pure plate technique and cultured anaerobically (BD BBL™ CO₂ gas generators, BioMerieux, France) at 37°C for 5 days on a solid sterile Oxalate Medium. Finally, we determined the quantitative composition of microorganisms, which grew as single colonies. The number of fecal ODB was calculated as lg of colony-forming unit per 1 g of feces (lg CFU/g).

For determination of the ODA in feces, we transferred 5 ml of dispersed feces samples to 45 ml Oxalate Medium and anaerobically incubated for 48 hours at 37°C in tightly closed 50 ml bickers (test solution).

For determination of the probiotics and synbiotic ODA rate, the one dose of each substance was diluted in 20 ml of Oxalate Medium and anaerobically incubated for 48 hours at 37°C in tightly closed 50 ml bickers (test solution).

Determination of ODA

The redoximetric titration with $KMnO_4$ was adopted to evaluate the total ODA of bacteria in culture media, as described previously [9, 24]. In brief, an aliquot of 10 ml test solution and 10 ml Oxalate Medium (control solution) was centrifuged at 3000 g for 15 min at room temperature. Supernatant 10 ml was precipitated with 10 ml of 0.4 M $Ca(NO_3)_2$. The filtered precipitated calcium oxalate was acidified with H_2SO_4 (1:4), mixed with 20 ml of deionized water and heated to 80°C. Immediately, 10 ml of H_2SO_4 (1:4) solution was added before titration. The solution was titrated with $KMnO_4$ (0.02 N) until a pink color

persisted for 30 seconds. The results were expressed in: % degradation of sodium oxalate per dose of probiotics and synbiotic; and % degradation of sodium oxalate per 0.01 g of feces for rat fecal specimens.

Plasma and urine oxalate measurements

After sample collection, the blood tubes were delivered to the Laboratory and centrifugated at 2000 X g for 15 minutes at room temperature. After centrifugation, the plasma was separated into 1.5 ml Eppendorf tubes labeled with the animals' number and analyzed immediately. POx concentration was measured spectrophotometrically using a commercially available kit (MAK315, Sigma, Barcelona, Spain) according to the manufacturer's protocols. Rats' 24-h urine samples were collected in individual metabolic cages and delivered to the Laboratory immediately. Daily UOx excretion was determined using an oxalate oxidase/oxidase reagent (BioSystems, Barcelona, Spain).

Statistical analysis

Data analysis and all graphs were performed using the MedCalc Statistica Software version 20.011 (Ostend, Belgium). Since most of the data were not normally distributed, the median (Me) and interquartile ranges (Q25; Q75) were calculated. The normality of the data distribution was tested using the Shapiro-Wilk test. Differences between the groups were evaluated using the nonparametric Kruskal-Wallis test with the Conover test for post-hoc comparisons. The Spearman's test was used for the correlation analysis. Two-factor analysis with repeated measures ANOVA was performed followed by Turkey post-hoc test for multiple comparisons (main treatment effect).

Results

Seven days of ceftriaxone treatment induced a shift in oxalate homeostasis in rats

Contrarily to our expectation, on day 1 after treatment withdrawal, rats in ceftriaxone-treated groups (CEF) demonstrated a significantly higher ODB amount compared to the vehicle group ($p < 0.001$) (**Supplementary Table 1**, Fig. 2A). However, ODA rate did not increase proportionally to the ODB level and tended to decrease compared to the vehicle group (Fig. 2B). These changes were associated with a significant increase in the UOx excretion in the ceftriaxone-treated group vs vehicle group ($p < 0.001$) (Fig. 2C). On day 57 after ceftriaxone withdrawal, the number of ODB and UOx excretion did not differ between groups while ODA was statistically lower in the ceftriaxone-treated group compared to the vehicle-treated group ($p = 0.03$) (Fig. 2A-C). Moreover, POx concentration in the ceftriaxone-treated rats was significantly high compared to the vehicle-treated group ($p = 0.02$) (**Supplementary Table 1**).

Oxalate-degrading activity of the commercially available probiotics and the synbiotic

To further assess the ability of commercially available biotic products to degrade oxalate, we tested the ODA rate among 3 widely used probiotics vs the synbiotic. We found, that both monospecies probiotics consisted of *Bacillus spp.* showed the lowest ODA rate compared to the probiotic with *Lactobacillus spp.*

Bacillus subtilis degraded only 13% oxalate in Oxalate Medium, while *Bacillus clausii* degraded 23% oxalate. Combination of 2 strains of *Lactobacillus spp.* (*L. fermentum*, *L. plantarum*) was able to degrade 26% oxalate in Oxalate Medium. The synbiotic consisted of *Lactobacillus spp.* (*L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. casei*), *Bifidobacterium spp.* (*B. bifidum*, *B. longum*), *Saccharomyces boulardii*, selenium, oligofructose, and inulin, degraded 69% oxalate in Oxalate Medium and was chosen for further investigation *in vivo* to restore ODA rate and oxalate homeostasis in ceftriaxone-treated rats.

Synbiotic supplementation restored ODA and decreased the level of UOx excretion in rats following ceftriaxone treatment

The two-factor repeated ANOVA demonstrated that both antibiotic and synbiotic administration as well as time after treatment withdrawal influence oxalate homeostasis in the rats. In particular, the ceftriaxone use in both CEF and CEF+SYN groups resulted in a compensatory increase of ODB number on day 1 following the treatment compared to the other groups while the use of the synbiotic did not increase the number of ODB compared to Vehicle Group immediately after the treatment (Fig. 3A). However, synbiotic supplementation led to statistically increased ODB number on day 57 after treatment withdrawal while the ODB number in ceftriaxone-treated groups (CEF and CEF+SYN) decreased to those level in the Vehicle Groupe by this time (group interaction $F = 42.6$, $p < 0.0001$; group and time interaction $F = 63.3$, $p < 0.0001$).

That is, by the end of the experimental period (in 8 weeks following the treatment), the number of ODB in rats' fecal microbiota did not differ between the groups, regardless of the treatment performed. However, despite the identical ODB number in all experimental groups, the total ODA in fecal microbiota was significantly higher in the synbiotic-treated groups (CEF+SYN and SYN), compared with Vehicle and Ceftriaxone Groups (group interaction $F = 4.91$, $p = 0.008$; group and time interaction $F = 7.51$, $p = 0.001$). It should be noted, that despite ceftriaxone treatment, total fecal ODA in rats of the CEF+SYN Group was significantly higher compared with those in the Ceftriaxone Group (**Supplementary Table 1**, Fig. 3B).

UOx excretion in the rats of the Synbiotic group was significantly lower compared to all other groups at both times of point (Fig. 3C). There was no difference in POx concentration between the Vehicle and symbiotic-treated groups. Nonetheless, as it was mentioned above, the POx concentration of the Ceftriaxone Group was significantly higher compared to the Vehicle Group (Fig. 3D).

Total ODA in rats' feces was inversely associated with plasma and urine oxalate concentrations

To further confirm the significance of total ODA *v.* ODB number in supporting overall oxalate homeostasis we next performed correlation analysis through all studied groups independently of the treatment protocol (see Table 1). Interestingly, the ODB number was not associated with their total ODA in rats' fecal microbiota on the 1st and the 57th experimental days ($r = -0.13$, $p = 0.36$ and $r = 0.07$, $p = 0.72$, respectively). However, both the ODB number and total ODA in fecal microbiota were associated with UOx excretion on day 1 after the treatment withdrawal ($r = 0.54$, $p = 0.003$ and $r = -0.44$, $p = 0.002$, respectively). On day 57, the rats' UOx excretion was not associated with the ODB number ($r = -0.03$, $p =$

0.88) but had a strong inverse correlation with total ODA in fecal microbiota (Fig. 4A). A similar result was observed in correlation analysis between ODB, total fecal ODA and POx concentration on day 57 after the treatment withdrawal. POx concentration was not associated with ODB number in rats' fecal microbiota ($r = 0.02$, $p = 0.89$), but showed a significant correlation with total fecal ODA (Fig. 4B).

Discussion

In the present study, we tested the hypothesis that oxalate homeostasis in rats might be influenced not so much by the quantity of ODB in the intestine microbiota as by the total ability of ODB to degrade oxalate (ODA rate). We assumed that due to its synergistic effect (probiotic and prebiotic), the synbiotic can restore antibiotic-induced disturbance of oxalate homeostasis in rats. To this end, we separately evaluated the changes in the ODB number and their total ODA in fecal microbiota at two-time points after ceftriaxone and synbiotic exposure. In addition, we assessed the interaction between ODB, total fecal ODA, and plasma and urine oxalate concentrations in rats.

There are several new and unexpected findings in the study. First, treatment with ceftriaxone resulted in significant growth in the ODB number on day 1 after the treatment withdrawal, and despite the increase in the ODB quantity, the ceftriaxone exposure substantially reduced total fecal ODA compared to synbiotic-treated and vehicle-treated groups. Moreover, total fecal ODA in ceftriaxone-treated rats remained the lowest in 8 weeks (on day 57) following the treatment, although the ODB number was similar in all the experimental groups. Second, the use of synbiotic did not increase the ODB number as much as enhancing their ability to degrade oxalates even when used simultaneously with ceftriaxone, which led to a significant decrease in UOx excretion. Third, ODB number was associated neither with their total ODA in fecal microbiota nor UOx excretion and POx concentration in rats. According to our results, only total fecal ODA was associated with urine and plasma oxalate levels.

The direct link between antibiotics exposure and KSD formation has been previously postulated, mainly in the context of the loss of *O. formigenes* in the gut microbial community [1, 5, 14, 15], the sensitivity of *O. formigenes* strains to commonly prescribed antibiotics [25], or the effect of probiotic interventions [19]. However, in addition to *O. formigenes*, to date, ODA has been identified in many other representatives of the intestinal microbiota (*Enterococcus spp.*, *Lactobacillus spp.*, *Bifidobacterium spp.*, *Bacillus spp.*) [6–9, 24]. Nevertheless, the majority of published studies have been focused not on the ODB profile but differences in the general gut microbial composition between KSD patients and healthy control [2, 18].

To the best of our knowledge, this report is the first to evaluate the total ODB number and their ODA in rats' fecal microbiota in response to ceftriaxone and synbiotic exposure. Surprisingly, according to our findings, the total ODB number was significantly increased after ceftriaxone exposure compared to vehicle- and synbiotic-treated groups. This result is in line with the recent work conducted by R. Chakraborty et al., in which the authors demonstrated a ceftriaxone-induced transient increase in the abundance and extraintestinal dissemination of *Enterococcus spp.* and *Lactobacillus spp.* in a mouse model [26]. In another recent study, the authors have observed a substantially increased relative

abundance of *Enterococcus spp.*, *Lactobacillus spp.* and *Bifidobacterium spp.* after the use of a combination of four antibiotics (bacitracin, meropenem, neomycin and vancomycin) in mice [27]. From our point of view, these observations might be a consequence of the resistance of certain species of ODB to ceftriaxone, or it could be associated with the growth of some commensals due to ceftriaxone-induced loss of others. In this context, it is logical to assume a compensatory increase in ODB with a lesser ability to degrade oxalate, which could explain a significant decrease in total fecal ODA simultaneously with a transient increase in the ODB number in the ceftriaxone-treated rats. However simultaneous studies on the ODB number and their total functional ability to degrade oxalate have never been conducted before, hence the obtained results cannot be directly compared with the results of previous reports. Accordingly, the phenomena of ceftriaxone-induced increasing ODB number and a simultaneous decrease in their total ODA in fecal microbiota raises many questions that require further investigations.

Numerous *in vitro* studies have addressed the beneficial effect of probiotics on the oxalate-degrading capacity of gut microbiota and reducing hyperoxaluria [5, 11]. Clinical results are not as encouraging and need further large-scale studies [18, 28]. It should be noted that only a few studies were conducted to investigate the synbiotics effect on human health [18, 19] and the only one addressed the effects of prebiotic and synbiotic on oxalate degradation *in vitro* [17]. Moreover, there are limited data concerning the interaction between antibiotics and synbiotics [27]. Thus, it is not well understood how synbiotics alter antibiotic-induced oxalate homeostasis imbalance and whether the synbiotic supplementation changes the short-term or long-term effects of antibiotics.

The commercially available synbiotic selected for our study has never been studied before for restoration of oxalate homeostasis. However, classically, in addition to 7 strains of live probiotic microorganisms, it consisted of selenium, oligofructose and inulin as a prebiotic supplement which justified our choice. Moreover, this synbiotic was able to degrade 69% oxalate after 48 h incubation in highly selective Oxalate Medium vs other tested three probiotic supplements. It is somewhat surprising that the synbiotic exposure did not affect the ODB number but more than doubled the total fecal ODA in 2 weeks of its administration. Even with antibiotic therapy, the use of the synbiotic led to an increase in total fecal ODA, which was reflected in a significant decrease in oxaluria on day 1 after treatment withdrawal. At the end of the experiment, the number of ODB increased compared to the first measurement only in the synbiotic group. Interestingly, the synbiotic administration preserved total ODA in the fecal microbiota of ceftriaxone-treated rats during the all post-antibiotic period independently of the ODB number. In fact, on the 57th day following the treatment, the beneficial effect of enhancing total fecal ODA in the group treated simultaneously with ceftriaxone and synbiotic was almost the same as in the isolated synbiotic-treated group. In parallel with our results, A. Jačan et al have demonstrated that the synbiotic *per se* did not influence the gut microbiota but was able to modulate the antibiotic-induced dysbiosis in a time-dependent manner [27]. Our data are also consistent with the findings obtained by Ö. Darilmaz et al in their *in vitro* study. The authors have shown that the probiotic in a combination with inulin enhanced the degradation of oxalates, thereby highlighting inulin's key role [17]. Moreover, in line with our results, they have not found an association between oxalate degradation rate and bacterial growth after synbiotic exposure [17]. In our opinion, the growth of ODB in our synbiotic-treated group reflects intestinal

colonization of the microorganisms present in the synbiotic. In the case of simultaneous use of ceftriaxone, the changes in the ODB number resulted from the growth of enterobacteria and other pathogens, while lacto- and bifidobacteria, stimulated by prebiotic additives, modulated the activity and viability of other intestinal microbiota. These results could explain why not the abundance of ODB but rather their total ODA in fecal microbiota was associated with UOx excretion and POx concentration in the rats. Therefore, the synbiotic administration should not be considered as substitution therapy in KSD patients but as a way of providing conditions for the restoration of the intestine biocenosis and stimulation of ODB activity.

Conclusions

Taken together, this study for the first time demonstrated that ceftriaxone treatment decreased total fecal ODA independently of the ODB number and increased urine and plasma oxalate concentrations in the experimental rats. Total fecal ODA but not the ODB number should be thoroughly examined in the future to develop predictive diagnostics methods, targeted prevention and personalized treatment in KSD. Synbiotic supplementation had a beneficial effect on the total ODA of gut microbiota, which resulted in significantly decreased UOx excretion in antibiotic-treated rats. However, it should be noted that the experimental design of the present study precludes an unambiguous conclusion concerning the use of synbiotics for the treatment of hyperoxaluria or prevention of KSD recurrences in humans.

Abbreviations

CaOx, calcium oxalate; CEF, ceftriaxone; CEF+SYN, ceftriaxone plus synbiotic; KSD, kidney stone disease; ODA, oxalate-degrading activity, ODB, oxalate-degrading bacteria; POx, plasma oxalic acid; UOx, urinary oxalate; SYN, synbiotic; VEH, vehicle.

Statements And Declarations

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Conflicts of interest statement The authors have no conflicts of interest to disclose.

Availability of data and material The current study dataset is fully available as the Supplementary File.

Ethics approval. The study was reviewed and approved by the animal committee of the ESC “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv (Protocol #18/01/2020)

Authors' contributions Conceptualization and design: Natalia Stepanova*, Gamna Tolstanova; Experiment and Methodology: Iryna Akylenko*, Tetyana Serhiichuk, Taisa Dovbynychuk, and Svitlana Savchenko; Funding acquisition: Natalia Stepanova and Gamna Tolstanova; Original draft preparation:

Natalia Stepanova; Review and editing: Natalia Stepanova and Gamna Tolstanova. *These authors made equal contributions to the work.

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Consent to participate All authors have agreed to this submission.

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Figures

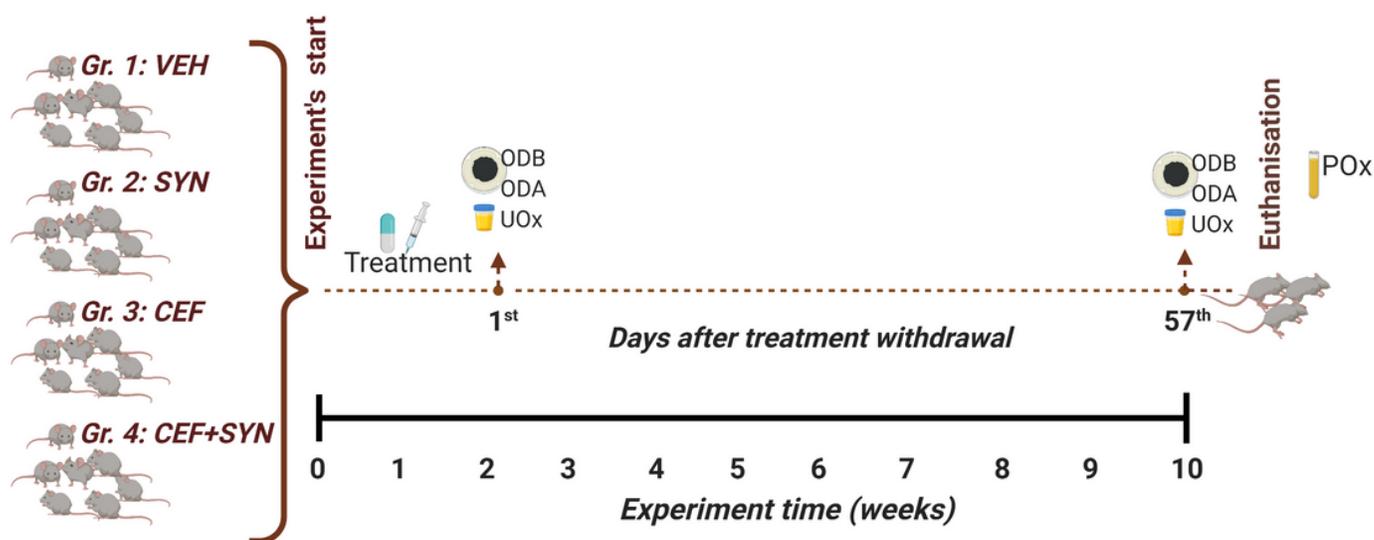


Figure 1

Experiment design and outline (created with BioRender.com). Abbreviation: CEF, ceftriaxone; CEF+SYN, ceftriaxone plus symbiotic; Gr, group; ODA, oxalate-degrading activity, ODB, oxalate-degrading bacteria; POx, plasma oxalic acid, SYN, symbiotic; UOx, urinary oxalate; VEH, vehicle.

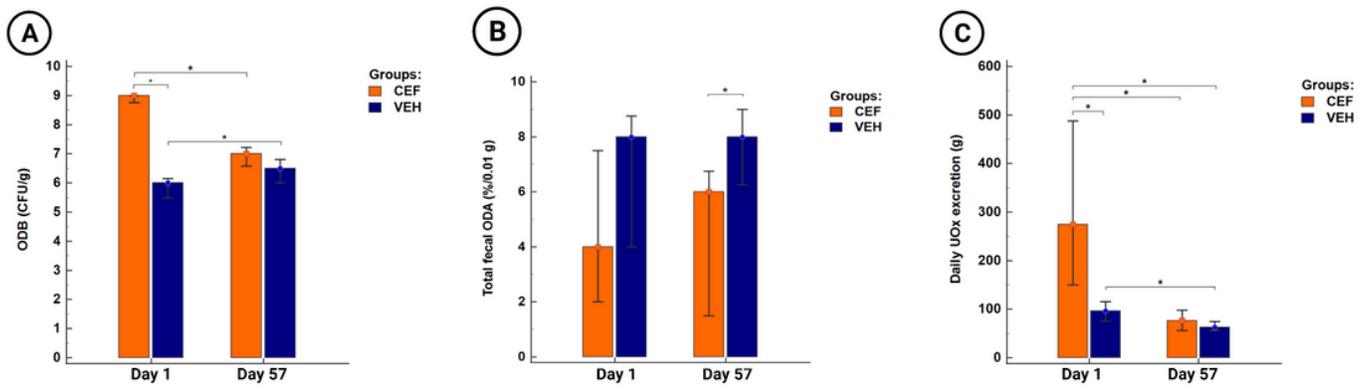


Figure 2

Ceftriaxone administration (300 mg/kg, i.m., for 7 days) induced changes in the ODB number and their activity in rats' feces and UOx excretion. The data are presented as Me (Q25-Q75) and compared using the two-factor repeated ANOVA analysis. (A): Changes in ODB number in the rats' fecal microbiota during the experimental period; (*): $P < 0.001$. (B): Changes in total fecal ODA in rats during the experimental period. (C): Changes in UOx excretion during the experimental period; (*): $P < 0.001$. Abbreviation: CEF, ceftriaxone; CEF+SYN, ceftriaxone plus symbiotic; ODA, oxalate-degrading activity, ODB, oxalate-degrading bacteria; SYN, symbiotic; UOx, urinary oxalate; VEH, vehicle.

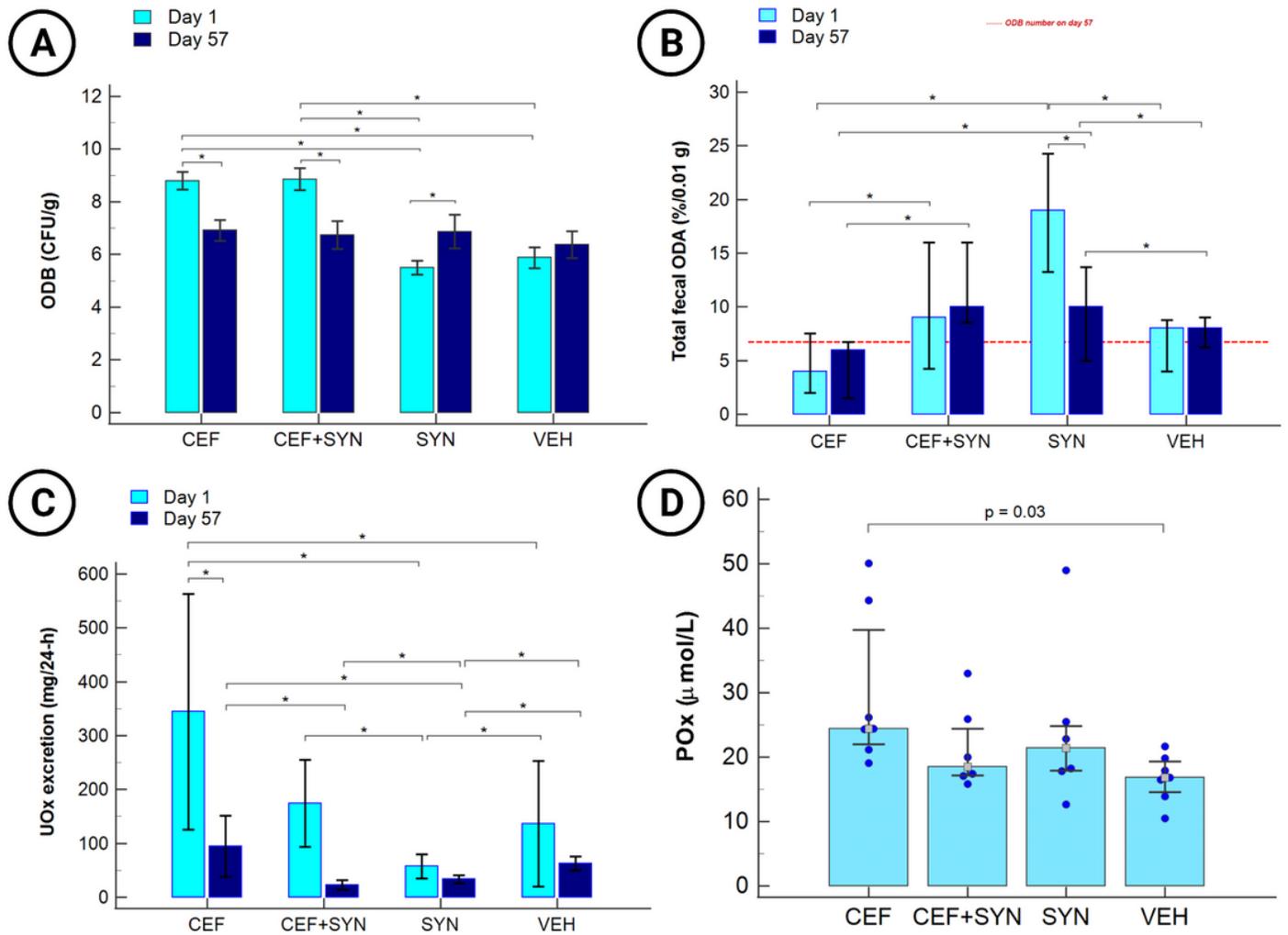


Figure 3

Synbiotic treatment (30 mg/kg, per os for 14 days) restored total fecal ODA and decreased UOx excretion in rats on days 1 and 57 following ceftriaxone treatment (300 mg/kg, i.m., 7 days). The data are presented as Me (Q25-Q75) and compared using the two-factor repeated ANOVA analysis or the Kruskal-Wallis test with Conover post-hoc. (A): Changes in ODB number in the rats' fecal microbiota during the experimental period; (*): $p < 0.0001$. (B): Changes in total fecal ODA in the rats during the experimental period; (*): $p < 0.001$. The reference line demonstrates the average ODB number (CFU/g) on day 57 following ceftriaxone treatment. (C): Changes in UOx excretion during the experimental period; (*): $P < 0.001$. (D): Changes in POx concentration on day 57 of the experimental period. Abbreviation: CEF, ceftriaxone; CEF+SYN, ceftriaxone plus synbiotic; ODA, oxalate-degrading activity, ODB, oxalate-degrading bacteria; POx, plasma oxalic acid; SYN, synbiotic; UOx, urinary oxalate; VEH, vehicle.

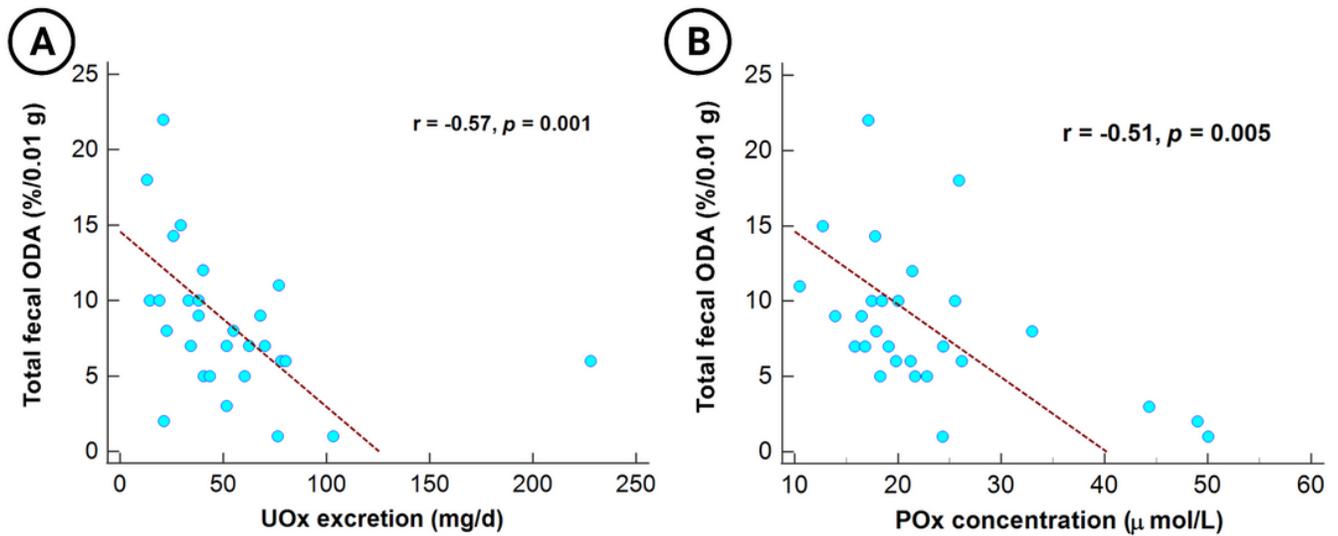


Figure 4

The association between total fecal ODA and (A) UOx excretion and (B) POx concentration in all experimental rats in 8 weeks after the treatment withdrawal. Abbreviation: ODA, oxalate-degrading activity; POx, plasma oxalic acid; UOx, urinary oxalate.

Supplementary Files

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