The effect of an antibiotic on the production of uremic toxins.
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Purpose of the Study and Background

Purpose of the Study

In a prior study we observed that the perturbation of the microbiome by one dose of oral vancomycin significantly lowered plasma concentrations of two gut-derived uremic retention solutes, indoxyl sulfate and p-cresyl sulfate [1]. The aim of this study is four-fold. First, to determine if serial doses of oral vancomycin produce a sustained decrease in the plasma concentrations of indoxyl sulfate, p-cresyl sulfate, and other putative uremic retention solutes.. Second, we aim to provide data regarding the stability of levels of uremic solutes and of the composition of the gut microbiome over prolonged periods (three months). We expect that vancomycin will produce a sustained perturbation of the microbiome, with decreased number of OTUs and, likely, sustained reduction in indoxyl sulfate and p- cresyl sulfate. Third, we hope to determine the duration of any effect of vancomycin on the plasma concentration of uremic solutes and the gut microbiome. Variability of the microbiome and uremic solutes over time has not been previously examined. Finally, the data derived from this study will provide data concerning the possible long-term effects of oral vancomycin administration.

Background

Observational studies have suggested a relationship between the plasma concentrations of two gut-derived uremic solutes, indoxyl sulfate (IS) and p-cresyl sulfate (PCS), and cardiovascular disease in patients with chronic kidney disease [2-4]. Both IS and PCS are formed from the metabolism of amino acids (tryptophan and tyrosine) by gut flora [5]. Our prior study (Nephrology Dialysis and Transplantation, 32, 11 2017, 1809-1817) showed that altering the microbiome with a single oral dose of of vancomycin significantly lowered plasma concentrations of IS and PCS. In this study, 10 patients with chronic, stable end-stage renal disease were given a single 250 mg dose of oral vancomycin on day 0. Plasma concentrations of IS and PCS were found to decrease significantly across all 10 subjects, using a mixed models analysis of the plasma levels of solute on days 0, 2, and 5 (X^2=11.57, df=2, p=0.003). By day 28, plasma solute concentrations were back to baseline [1]. The gut microbiome had not fully recovered at Day 28.

Vancomycin is an antibiotic that interferes with bacterial cell wall synthesis. It has poor oral absorption, and thus oral vancomycin is only FDA approved to treat gastrointestinal infections including C. *difficile*-associated diarrhea and enterocolitis caused by S. *aureus*. In this study it will be used to perturb the gut microbiome to lower the production of uremic solutes by intestinal bacteria. Dosage selection is based on the prior study which demonstrated a significant decrease in IS and PCS with a single dose of 250mg over the course of one week, with uremic solute concentrations returning to baseline within 28 days. This dose is well below the dose usually prescribed for the treatment of C. difficile infection.

In the prior study the plasma concentrations of IS and PCS returned to baseline after one month. With this study we hope to learn whether serial doses of oral vancomycin produce a sustained decrease in the concentrations of these two solutes. Our pilot study showed a 40% decrease in plasma concentrations of IS and PCS. In this study we hope to learn whether the suppression of toxin production in sustained, increases, or decreases over time. It is unclear whether this decrease in IS and PCS would produce clinically significant slowing of cardiovascular disease in patients with ESRD. Weekly dosing was chosen because the plasma uremic solute concentrations remained significantly decreased at 7 days after 250mg of vancomycin, but had returned to baseline by 28 days. Three months was chosen without data-driven rationale, as there are no prior studies of this sort.

Vancomycin was selected for our earlier study (on the advice of Dr. Martin Blaser) because it was possible to give the medication by mouth, limiting its effect to the GI tract. We found, as predicted that vancomycin had profound effects on the gut microbiome. We have chosen to administer vancomycin for the present study because it has proven to affect the gut microbiome and to reduce the production of at least two uremic toxins (Nephrology Dialysis Transplantation 32, 2017, 1809-1817). There are no comparable data for any other orally administered antibiotic.

It remains to be shown if reduction in any uremic solute concentration will improve clinical outcomes for dialysis patients. Nevertheless, any strategy for doing so by reducing uremic toxin production by fecal flora through administration of oral non-absorbable antibiotics will require their chronic administration.

Experience with longer term administration of oral vancomycin is reported from systematic study of the treatment of C. difficile colitis. In one trial (Clinical Infectious Diseases 2017; 64(3):265–71) comparing a 6-week course of oral vancomycin with fecal transplants none of 4 serious adverse events were deemed related to study procedures. Experience with less serious events is summarized in the table below. All of these patients received, for their 6 week course, dosing of vancomycin higher than proposed in our study.

	Early Events (0-7 days)		Late Events (7-14 Days)	
Adverse Event	FT (n = 16)	VT (n = 12)	FT (n = 11)	VT (n = 12)
Fever	1 (6.2)	0 (0.0)	2 (18.2)	1 (8.3)
Nausea or vomiting	4 (25.0)	3 (25.0)	0 (0.0)	3 (25.0)
Abdominal pain/ tenderness	6 (37.5)	5 (41.7)	4 (36.4)	9 (75.0)
Abdominal distension	6 (37.5)	4 (33.3)	3 (27.3)	4 (33.3)
Abdominal bloating	6 (37.5)	6 (50.0)	3 (27.3)	7 (58.3)
Feeling generally unwell	6 (37.5)	3 (25.0)	3 (27.3)	4 (33.3)
Mucoid stools	6 (37.5)	2 (16.7)	4 (36.4)	6 (50.0)
Bloody stools	1 (6.2)	0 (0.0)	2 (18.2)	2 (16.7)
Smelly stools	6 (37.5)	2 (16.7)	3 (27.3)	6 (50.0)
Fecal incontinence	4 (25.0)	3 (25.0)	3 (27.3)	4 (33.3)
Anorexia	4 (25.0)	2 (16.7)	2 (18.2)	3 (25.0)

Fatigue	5 (31.2)	5 (41.7)	4 (36.4)	8 (66.7)
Skin rash	0 (0.0)	1 (8.3)	0 (0.0)	2 (16.7)

Abbreviations: FT, fecal transplantation; VT, vancomycin taper.

[a] Expressed as No. of reported events with percentage in brackets; early events were from days 0–7 and late events were days 7–14.

[b] Five of the 16 patients originally randomized to FT recurred within 7 days of intervention and therefore are not included in the measurement of late adverse events.

Longer term administration of oral vancomycin has also been used to prevent colitis in stem cell transplant recipients (Blood 2016 128:2225). In this brief report of a retrospective analysis, 50 members of a cohort of 105 patients received 126 mg of vancomycin po bid from day of admission to discharge (median length of stay = 33.5 days). Adverse event details are not provided in this report, however the authors state that there were no cases of vancomycin-resistant enterococcal bactermia among the vancomycin recipients.

Tarao et al (Gut, 1990,31, 702-706) administered 8 weeks of vancomycin, 1000mg bid to 13 men and 1 woman, as part of a crossover study comparing vancomycin and lactulose. They did not comment on adverse effects in this paper.

More recently Isaac et al (Journal of Antimicrobial Chemotherapy, Volume 72, Issue 1, 1 January 2017, Pages 128–136) administered vancomycin, 250 mg qid, for 2 weeks to examine the effect of vancomycin on the human microbiome of 9 patients with newly diagnosed with rheumatoid arthritis. This study was conducted at NYU and approved by the NYU IRB. The authors comment "Altogether, our results demonstrate the negative long-term consequences of oral vancomycin administration, which should be taken into account in the decision-making prior to prescribing this antibiotic", suggesting that adverse effects were absent or minor.
It is important to understand the epidemiologic relationship between vancomycin use and the risk of vancomycin resistant enterococci (VRE) acquisition. As stated in ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2008, p. 2403–2406: "Because multiple genes are necessary to generate vancomycin resistance in enterococci, acquisition of VRE colonization does not occur through mutations in susceptible enterococci in the intestinal tract" (4, 11, 18, 19). Rather than inducing mutation the emergence of VRE reflects either the overgrowth of already present VRE, their acquisition via diet or from contact transmission.

Estimates of the risk of such acquisition therefore have varied widely, depending on the local community or institutional prevalence. We identified 3 studies that exemplify this. In one study (Journal of Infectious Diseases 1996; 173:1129-36) conducted in an environment where VRE were present in the food stream, 14/22 (64%, 95%CI 40.7,82) of those given vancomycin for treatment of C. difficile developed colonization. In contrast, in an institution were local prevalence was low (Infec Control Hosp Epi 25:413; 2004), 0% of 22 (95%ic 0,14%) individuals so treated acquired VRE while in a third study 1 of 12 patients treated acquired VRE (8.3%, 95%CI=0.2,38.5). From this perspective pre-existing colonization or acquisition of VRE would not be unexpected because ESRD patients are likely to receive vancomycin as treatment and may already be colonized. We propose to look for this in our study and quantify the risk, but we

don't feel that the experimental treatment adds meaningfully to the risk of harm to the patients or a negative effect that would necessarily preclude the future use of the agent.

While systematically collected data are limited, there is extensive clinical experience with long term use of IV vancomycin (for example for treatment of endocarditis or osteomyelitis) and oral vancomycin for the indications given above at doses substantially higher than we propose. We believe the totality of the evidence suggest that major adverse effects are unlikely to be seen and revised our protocol to incorporate studies for foreseeable ones.

Multiple observational data clearly relate higher concentrations of both of these uremic solutes with greater incidence of cardiovascular disease, including

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2. Melamed ML, Plantinga L, Shafi T, et al. Retained organic solutes, patient characteristics and all-cause and cardiovascular mortality in hemodialysis: results from the retained organic solutes and clinical outcomes (ROSCO) investigators. BMC Nephrol 2013; 14: 134
3. Liabeuf S, Barreto DV, Barreto FC et al Free p-cresyl sulfate is a precursor of mortality at different stages of chronic kidney disease, Nephrol Dial and Transplant 2010;25:1:1183-1191.
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There are no reported studies of the effect of treatments that reduce the plasma concentration or production of indoxyl sulfate or p-cresyl sulfate as no long term treatment trials have been undertaken. Indeed these studies might be considered a proof of concept to justify one approach to the design of such studies.

Study Design

This is a randomized double-blinded placebo-controlled crossover study in which 250mg of oral vancomycin will be administered to half of the subjects weekly for 3 months, and the other half of subjects will receive a placebo. After three months, the control group will be crossed over to weekly oral vancomycin (250mg) and the initial experimental group will be switched to placebo for 3 months. Plasma concentrations of IS and PCS will be monitored at first weekly for one month, then monthly during the 6-month duration of the study. Stool samples will be collected at baseline, then monthly, cultured to determine the presence of vancomycin-resisitant organisms and analyzed for microbiome composition.

Oral vancomycin 250mg capsules, packaged inside individual foil-sealed compartments, will be stored in the Reseach Pharmacy in a box labeled "vancomycin hydrochloride capsules". Placebo pills will be stored in a box labeled "placebo". Both will be purchased by the PI, shipped to his office in Bellevue Hospital, and stored in a locked cabinetin the Research Pharmacy. . The co-

investigator will deliver the drug or placebo to each subject.This will maintain blinding, as the co-investigator will not know the identity of the medication or placebo delivered.

Characteristics of the Research Population

Number of Subjects: We will enter 16 subjects into this study. We estimate that this number, based on an expected drop-out rate attributable to development of an infection or patients who are able to undergo renal trasnsplantation, will leave us with at least 13 subjects who complete the 6-month protocol. National Healthcare Safety Network data published in July 2017 (6) reported a combined infection rate of 0.293% per 100 patient months in patients with a fistula or A-V graft. Based on this data, we anticipate that no more than 3 patients will drop out of the study. Thus we feel that 16 is an appropriate number to insure that we will have at least 13 final study subjects. Subjects will be identified through patient records at the dialysis centers, both River Renal and Lower Manhattan Dialysis. Both of these centers are supervised by Albert Matalon MD, and he is the only study team member who is affiliated with these centers. Recruitment of subjects will be done by Leland Soiefer and Michelle Chang, and administration of vancomycin or placebo will be done under the supervision of the Research Pharmacy and Dr. Lowenstein. Recruitment will occur in person at both locations in order to allow for a greater pool of potential subjects. There will be no functional difference between the two sites for the purposes of this study.

Gender of Subjects: The intended gender distribution is equal between men and women.

Age of Subjects: All adults, over age 18.

Racial and Ethnic Origin: There will be no enrollment restrictions based on race or ethnicity.

Inclusion Criteria: End-stage renal disease receiving thrice-weekly hemodialysis via arteriovenous fistula or A-V graft.

Exclusion Criteria: History of prior adverse reactions to vancomycin; history of current or prior diarrheal disease; receipt of any antibiotic during the three months that precede the study; history of *C. difficile* infection; elevation of white blood cell count or fever within one week of enrollment.

Vulnerable Subjects: Vulnerable subjects will not be included in this study.

Methods and Procedures

Methods and Procedures

16 patients with chronic, stable end-stage renal disease will be recruited while at River Renal Dialysis or Lower Manhattan Dialysis for their regular hemodialysis session. Subjects will be randomized into two equal groups via computer assignment using the website www.randomizer.org. At day -2, subjects' blood (5mL) will be collected for measurement of IS and PCS pre- and post-dialysis. Pre- and post-dialysis samples (5mL) will again be collected 2 days later (day 0). Each subject in the antibiotic group will ingest a 250mg capsule of vancomycin weekly while subjects in the control group will take a placebo. Subsequent pre-dialysis blood samples (5mL) will be obtained weekly

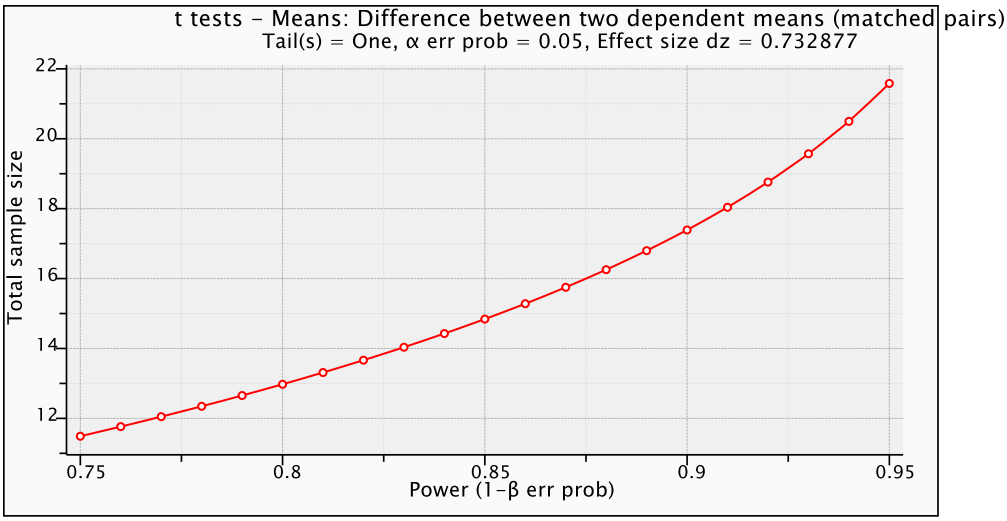
for one month, and then monthly. Initial stool samples on day -2 will be collected from all subjects, and monthly samples will be obtained after that. After three months, subjects will switch groups with blood and stool sample collection continuing in the same fashion. Blood levels of vancomycin will not be monitored in this study due to the poor oral bioavailability of the drug. We will exclude patients that have gastrointestinal problems that might lead to absorption of oral vancomycin. During the study, subjects will be interviewed regularly about development of new symptoms including diarrhea or fever, changes in diet, sick home contacts, and changes in medication use. The investigators will fill out a monthly clinical questionnaire (attached to IRB submission) assessing changes in their clinical status, including changes in GI habits, diet, and development of new symptoms. The total duration of participation for subjects will be 6 months.

In our prior acute administration study, we obtained the following results for two putative uremic toxins.

Solute	Mean Concentration (μM) on day 0	Mean Concentration (μM) on day 5	Mean difference	SD of paired differences
p-Cresyl Sulphate	39.9	18.5	21.4	29.2
Indoxyl Sulphate	29.9	20.8	9.1	21.3

To minimize any risk of harm we desire to minimize the sample size that we require. Since we will be examining the changes within each patient a T test of the paired patient differences is an appropriate statistical test. Based on the data from the acute study a sustained difference of the magnitude seen above would provide the proof of concept that we seek. Based on these considerations we consider a sustained 54% decrease in the concentration of PCS at 1, 2, or 3 months, compared to the initial level, to be a difference worth detecting. We chose the decrease in PCS concentration as the primary outcome because of the greater magnitude of the decrease that was found acutely, suggesting that this solute may be more susceptible to antibiotic-induced inhibition of synthesis.

Applying the computer program G*power v3.1.2 (http://www.gpower.hhu.de/en.html) using the data in the table above, a test of the 1-tailed hypothesis that vancomycin administration will reduce the concentration of PCS by the amount seen in the acute study after 1, 2, or 3 months of administration with alpha= 0.05 and power = 0.80 would require 13 patients. The figure below, from the program, shows the relation between sample size and power for this test. Thus, a sample size of 16 accounts for the necessary 13 subjects for a power of 0.8, and an additional 3 to ensure 13 will complete the protocol after accounting for the expected drop-out rate.



Here is the schedule for collection of samples based on "Day 0" being the first day that either oral vancomycin or placebo is administered.

Visit	Procedures
Day -2	Blood, stool
Day 0	Blood
1 week	Blood
2 weeks	Blood
3 weeks	Blood
1 month	Blood, stool
2 months	Blood, stool
3 months	Blood, stool
3 months, 1 week	Blood
3 months, 2 weeks	Blood
3 months, 3 weeks	Blood
4 months	Blood, stool
5 months	Blood, stool
6 months	Blood, stool

The blood and stool samples will be stored for future research in uremic retention solutes. This is mandatory for subject participation, as this is a dynamic field of research and it is possible that new uremic retention solutes will be identified after the completion of this study. Samples will be banked in the freezer of Dr. Skolnik. Samples will be banked for up to 10 years. They will be labled using an alphanumeric code. and only the PI will

have access to them. Only the PI will have the linking key between the code and subjects' identity.

Data Analysis

We plan to examine the difference in mean solute levels between the two groups in a manner similar to the prior study. In the previous study we performed a linear mixed effects model analysis using the R statistical program. The model included the number of days from vancomycin administration as a categorical fixed factor, the type of solute (IS or PCS) as a categorical fixed factor and subject as a random factor. If there was a significant overall test for a fixed factor, least square means were obtained and differences among the least square means were tested using Tukey's *post hoc* 'Honestly Significant Difference' method, calculated using error terms based on the model and Satterthwaite approximate degrees of freedom. The primary endpoint is plasma concentration of IS and PCS, and the secondary endpoint is perturbation of the gut microbiome.

Data Safety Monitoring Plan

The PI, Jerome Lowenstein, MD, has established a Data Safety Monitoring Committee composed of the following members: 1.Fred Valentine MD 2.Michael Simberkoff MD 3. Lada Beara-Lasic MD.

This committee will meet monthly and review accumulating data to determine if either group (Drug-first or Placebo-first) is experiencing unexpected toxicity. The major data that will be reviewed will include weekly clinical assessments by the investigator that include stool frequency, intercurrent infections including C. difficile colitis, as well as monthly blood chemistries and blood counts as routinely collected by the dialysis units, and results of monthly stool screening for vancomycin resistant enterococci. A summary form will be available to the DSMB.

Given the small size of the study and the expectation of rapid recruitment, the first interim analysis will be conducted when 10 patients have completed 1 month of experience. The expectation is that there will be 5 patients in each arm of the crossover. P-values will be adjusted for the planned repeated examinations of the data. Given the study size it is likely that interim analyses of toxicity will not be powerful, however it may be that the study's endpoints will be statistically significant and early termination can be contemplated.

Once each month the PI will examine the blood and stool samples to ensure each sample is in its proper location in Dr. Skolnik's freezer. At the three-month mark plasma samples will be sent to the lab of Dr. Bjorn Meijers for analysis of plasma IS and PCS concentrations.

Adverse events include development of C. difficile diarrhea, a drug-resistant bacterial infection, and the potential for rash. If a serious adverse event occurs, the PI will be notified, and the subject will be taken to the emergency room by the co-investigator. Any unexpected adverse events will be reported to the IRB within one week. There is abundant evidence that oral vancomycin is a safe drug, so we do not anticipate major adverse events. However, the study

will be stopped if 6 or more subjects develop an adverse event. Summary reports from data safety monitoring meetings will be submitted via the IRB's online portal.

Data Storage and Confidentiality
The data will be stored in a locked cabinet in the office of the PI. At the start of the study, subjects will be assigned an alphanumeric code following randomization. This will allow us to keep deidentified data records. Each subject will be assigned a letter, and each plasma sample and stool sample will receive a number and letter indicating the subject and the chronology of the samples. Only the PI and co-investigators will have access to the data; only the PI will have the linking key between the subject ID and the subjects' identity.

Risk/Benefit Assessment
Risk

There are no reports of long-term adverse effects of oral vancomycin in the modern era of vancomycin production and no reports of adverse effects of a dose as low as 250 mg once weekly.

Despite its effectiveness against Clostridium difficile infection (cure rate of ~90%), a subset of cured patients (14%–21%) develop recurrent infections, which are thought to be enhanced by microbiota changes promoted by vancomycin. In addition, microbiota alterations induced by vancomycin may promote intestinal colonization by other pathogens, including VRE, Klebsiella pneumoniae or Escherichia coli. If this happens, the subject will be treated appropriately to resolve the infection, including possible admission to the hospital. The other major risk is related to administration of antibiotics when subjects do not have an active infection, namely the possibility of developing resistance to the antibiotic, and possibly development of an infection with a drug-resistant organism. Oral vancomycin achieves high concentrations in the intestinal tract, resulting in suppression of anaerobic organisms, including Bacteroides spp. Such disruption of the indigenous microbiota may predispose patients to recurrent CDI due to regrowth of the original infecting C. difficile strains or acquisition of new strains (7). There is also the potential for rash. These risks are legitimate considerations, and should be balanced against the potential benefit of significantly lowering the blood concentrations of two uremic toxins that have been linked to atherosclerotic progression.

Participation in this study will involve monitoring of plasma IS and PCS concentrations and collection of stool samples, both of which present minimal risk. The adverse effect of nephrotoxicity is extremely rare with current formulation of oral vancomycin. We do not believe ototoxicity is a significant risk as this type of adverse effect only occurred with administration of high doses of IV vancomycin.

Protection Against Risks
Blood samples will be drawn at the start and end of dialysis from the dialysis access line, in order to avoid repeated venipuncture. Patients will be monitored by co-investigators Leland Soiefer, MS4 and Michelle Chang, MS4 with supervision by the PI Jerome Lowenstein, MD. The co-investigators will use the clinical questionnaire (attached to IRB submission) to monitor for development of diarrhea, infectious symptoms, or other new symptoms. Subjects will be

removed from the study if they develop significant GI distress such as new diarrhea or colitis. Exit criteria also include development of any infection that necessitates treatment with antibiotics, and receipt of renal transplant.

Potential Benefits to the patient.
Subjects will likely experience no change in their clinical status and will most likely not experience any personal benefit. Plasma concentrations of IS and PCS may be significantly reduced for several months, which could potentially slow atherosclerotic progression.

Investigator's Qualifications & Experience
Please see attached CVs.

Subject Identification, Recruitment and Consent/Assent
Identification
To identify potential subjects for this study, an investigator will go to River Renal Dialysis Unit and Lower Manhattan Dialysis and consider patients with regard to inclusion and exclusion criteria by conferring with the staff and reviewing medical records. The researchers will then approach a patient, review and confirm information with them from their record, and explain the study including answering any questions the patient may have.

Process of Consent
The co-investigators will obtain consent by explaining the nature of the study and the science behind it, presenting risks and benefits, and answering questions. Subjects will sign the consent form and receive a copy of the signed document.

Subject Capacity
All subjects will have the capacity to give consent.

Subject Comprehension
The co-investigators will ask the subject to summarize back the nature and process of the study as part of giving consent. The consent form will also be written in simple English.

Debriefing Procedures
No debriefing will be necessary in this study.

Consent Forms
See attached forms.

Documentation of Consent
The subject will sign the consent form, and the forms will be stored in a locked file cabinet in the PI's office inside Bellevue Hospital.

Costs to the Subject
Participation in the study will not cost anything to the subjects.

Payment for Participation

The subjects will not be compensated or paid for participation in the study.

References

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