

IMPLANT OF *OXALOBACTER FORMIGENES* INTO THE INTESTINAL TRACT OF LABORATORY RATS⁺

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Abstract

Two isolates of *Oxalobacter formigenes* were examined for their ability to colonize the intestinal tract of adult laboratory rats. These rats did not harbour *O. formigenes*. Both strains succeeded in colonizing the intestinal tract of adult rats fed a diet that contained 4 % sodium oxalate. Three days after rats were inoculated orally with 10⁹ viable cells of the two strains, oxalate concentration in rat's urine markedly decreased. Both isolates were not detected in the faeces of inoculated rats fed diets that contained less than 3 % sodium oxalate.

المستخلص :-

اختبرت قابلية عزلتين من *Oxalobacter formigenes* لاستيطان القناة المعوية لجردان المختبر البالغة التي لا تاوي هذه البكتريا. نجحت كلتا السلالتين في استيطان القناة المعوية لجردان بالغة تغذت على عليقة تحوي 4% اوكزالات الصوديوم. بعد ثلاثة ايام من التلقيح الفموي بحوالي 10⁹ خلية حية من هاتين السلالتين، انخفض تركيز الاوكزالات بشكل ملحوظ في ادرار هذه الجردان. لم يكشف عن وجود السلالتين كلتاهما في فضلات الحيوانات الملقحة و المتغذية على عليقة حاوية على اقل من 3% اوكزالات الصوديوم.

Introduction

Oxalate degradation by the anaerobic bacterium *Oxalobacter formigenes* is important for human health, helping to prevent hyperoxaluria and disorders such as the development of kidney stones, during the last 50 years there has been an unexplained gradual increase in the incidence of idiopathic kidney stone disease in the affluent societies [1]. A link to diet has been proposed, but there are some data that fail to support this hypothesis [2]. Others proposed that the increasing use of oral antibiotics is responsible, or at least partially responsible. According to a study done

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by Allison *et al* in 1997 [3] individuals known to be colonized by *Oxalobacter formigenes* were no longer colonized after treatment with oral antibiotics.

Collected data from a local study indicate that while approximately 69.7 % of adults sampled in Iraq as “normals” are colonized by *Oxalobacter formigenes* , the incidence of colonization (9.3 %) is much less among patients who have experienced multiple episodes of kidney stone disease [3].

Bacterial degradation of oxalate occurs in the intestine of humans and animals [4,5]. In the rumen, a selection for increased numbers of oxalate-degrading bacteria occurs with the addition of oxalate to the diet. This selection is based on oxalate availability, and the elevation of numbers of oxalate-degrading bacteria serves to limit the absorption and, thus, toxicity of high levels of dietary oxalate [6].

Laboratory rats are widely used as models for the absorption and excretion of oxalate in humans [7]; however, there is no evidence that laboratory rats are unique in that most are not colonized by oxalate-degrading bacteria [2,8]. How this fact affects conclusions from data obtained with rat is not known because the role of oxalate-degrading bacteria in the colon has yet to be defined. In a survey of commercially available rats, Daniel *et al.*, [9] discovered that rats only one of the five commercial breeders tested harboured colonic oxalate-degrading bacteria. Oxalate-degrading bacteria isolates from these rats were similar to isolates from the rumen [9], from the bowel of humans and certain nonruminant herbivores [10] and from aquatic sediments [11]. A new genus and species; *Oxalobacter formigenes*, has been proposed for this unique group of anaerobic bacteria that use oxalate as a major source of carbon and energy [10].

Reasons for the absence of *O. formigenes* from the intestinal tract of most laboratory rats are unknown. In this study, we used rats which are not harbouring *O. formigenes* to determine whether different strains of *O. formigenes* could colonize the intestinal tracts of adult laboratory rats, and studied some of the conditions (e.g. time and dietary oxalate levels) required for colonization.

Material and Methods

Thirty nine male laboratory rats (*Rattus norvegicus*) weighted 170 – 210 grams, designated *O. formigenes* free, from the animal house of Department of Biology, College of Science, University of Baghdad, indicated that *O. formigenes* was not

present in the intestinal tract of these rats. All animals were housed in plastic cages (three animals per cage).

Preparation of *Oxalobacter formigenes* inoculums

Oxalobacter formigenes strains used were isolated from human stool (isolate ox1) and stool of wild rat (isolate ox2) by a method described by Allison *et al* in 1985 [10]. This method involves use of medium A which contains (g/l: KH_2PO_4 , 0.25; K_2HPO_4 , 0.25; sodium acetate, 0.085; resazurin, 0.001; yeast extract, 0.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0; ammonium oxalate, 5.68; Na_2CO_3 , 4.0; cysteine. HCl. H_2O , 0.33; $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.33; and agar, 22). Ingredients other than the last four were mixed and pH was adjusted to 6.8. The mixture was boiled and after it had cooled, sodium carbonate was added and 7.5 ml of the mixture was placed in 18 x 150 mm tubes that contained agar. The medium was dispensed into tubes closed with rubber stoppers a solution containing cysteine and sodium sulphide was added to tubes just prior to melting the agar medium for use. Melted medium (45°C) was inoculated with faecal sample; roll tubes were immediately prepared and were subsequently incubated at 37°C. Isolates from colonies that produced clear zones on this opaque medium were considered as *Oxalobacter formigenes*.

Strains of *O. formigenes* were grown under anaerobic condition at 37°C in a rubber stoppers flasks containing the same gradients of prereduced medium A except the agar. The inoculum of the broth (1-1.5 %) was grown in culture tubes [18 x 150 mm) of the broth, and cell densities were measured at 600 nm. Cells were harvested by centrifugation [14,000 g for 10 min. at 4 C), washed once in anaerobic diluted solution (less the CaCl_2), and resuspended in anaerobic diluted solution to a final concentration of 10^9 cfu /ml in CO_2 flushed bottles.

Inoculation of rats with *Oxalobacter formigenes*

In all colonization experiments, rats were switched from normal diet to a diet containing 4.5 % sodium oxalate at least 4 days before inoculation. Groups B and C received 1ml of a cell suspension (10^9 cfu /ml) of *O. formigenes* strains ox1 and ox2 respectively, administrated orally via sterile catheter (2 mm in diameter). Group A (uninoculated rats) included as control. Stool and 24 h urine samples were collected after 1, 3, 5, and 7 days of inoculation. Stool samples were cultured in order to isolate *O. formigenes*, while urine samples were analysed for oxalate concentration.

Effect of oxalate concentration

Groups D, E, F, G, H, I, J, K, L, and M were fed diet containing 2.5, 3, 3.5, 4, and 4.5 % sodium oxalate respectively. After 4 days of feeding with oxalate diet, groups D, E, F, G, and H were inoculated orally with isolate ox1, whereas groups I, J, K, L, and M were inoculated orally with isolate ox2. Stool samples were cultured in order to isolate *Oxalobacter formigenes*.

Results and discussion

Both isolates ox1 and ox2 succeeded in colonizing gastrointestinal tract of *Oxalobacter formigenes* free laboratory rats. They were isolated from stool samples after one day post inoculation. Sylvia *et al* [1] mention that a single oral ingestion of *O. formigenes* by adult volunteers was, for the first time, shown to result in (i) reduced urinary oxalate excretion following administration of an oxalate load, (ii) the recovery of oxalate-degrading activity in feces, and (iii) prolonged retention of colonization.

Furthermore, it was noticed that the excreted oxalate concentration in urine, markedly, decreased by five folds after three days post inoculation with *O. formigenes* and 20 folds after seven days in animals which received isolate ox1 (table 1), while in those which received isolate ox2 it was 3.3 and 14.7 folds respectively. Such results could be attributed to that human, perhaps, intake oxalate containing diet more than wild rats.

However, it was not possible to isolate *O. formigenes* from animals fed on less than 3 % oxalate diet, the matter which emphasizes the importance of dietary oxalate concentration to the viability and colonizing of *O. formigenes* in the gastrointestinal tract. Since it is the unique source for carbon and energy for this bacterium, none of a wide variety of other compounds replaced oxalate as a growth substrate [10,12,13).

Table 1: Oxalate concentration (mmol/l) in 24 h urine sample of laboratory rats in respect of time course.

Animal group [*]	Day after inoculation with <i>Oxalobacter formigenes</i>				
	0	1	3	5	7
A	27	29	27.5	26	27
B	28	19	5.5	2	1.4
C	26.5	20	8	4	1.8

^{*} Group A; Uninoculated animals, B; animals received isolate ox1, C; animals received isolate ox2.

Currently, we don't know why *Oxalobacter formigenes* is absent from the intestinal tract of laboratory rats. However, even if laboratory rats are exposed to *O. formigenes*, the result of present study indicates that a laboratory diet that is low in oxalate greatly reduces the chances of *O. formigenes* becoming established in the intestinal tract. On the other hand maintaining feeding with more than 3 % oxalate containing diet enhances the establishment. An alternate possibility is that other microorganisms present in the intestinal tract of laboratory rats that were naturally colonized are important for the establishment of *O. formigenes* when dietary oxalate is limited.

More studies should be carried out for gaining a better understanding of the effect of other intestinal flora membrane on implant of *Oxalobacter formigenes* into this endogenous flora. Such animal model offers an important way to get better knowledge of factors influencing the absorption of dietary oxalate and its relation to urinary stone formation in human.

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