Lanthanum Hydroxide Protects Kidney Through Gut-Metabolite-Kidney Axis in a Rat Model of CKD

Yuan Gao

Inner Mongolia Medical College: Inner Mongolia Medical University <https://orcid.org/0000-0002-4733-365X>

Shengnan Wang

Inner Mongolia Medical College: Inner Mongolia Medical University

Lijun Sun

Inner Mongolia Medical College: Inner Mongolia Medical University

Bing Li

Inner Mongolia Medical College: Inner Mongolia Medical University

Hong Liu

Inner Mongolia Medical College: Inner Mongolia Medical University

Ren Bu

Inner Mongolia Medical College: Inner Mongolia Medical University

Changhai Su

Ordos Vocational College

Min Guo

Ordos Vocational College

Yang Liu

Ordos Vocational College

Lulu Zhao

Inner Mongolia Medical College: Inner Mongolia Medical University

Chao Gu

Inner Mongolia Medical College: Inner Mongolia Medical University

Xiaojia Li

Inner Mongolia Medical College: Inner Mongolia Medical University

Xiaorong Yuan

Inner Mongolia Medical College: Inner Mongolia Medical University

Qiwen Wang

Inner Mongolia Medical College: Inner Mongolia Medical University

Gang Li (✉ 20080268@immu.edu.cn)

Inner Mongolia Medical College: Inner Mongolia Medical University

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Abstract

Background: Recent evidence suggests alterations in the gut-kidney axis may drive chronic kidney disease (CKD).

Results: In the present study, we observed that administration of adenine to rats induced CKD, gut microbial dysbiosis, kidney pathology, and amino acid metabolism. In this model of CKD hyperphosphatemia, lanthanum hydroxide improved kidney function in CKD rats by restoring gut microbial homeostasis, thereby increasing urine ammonium metabolism. These findings demonstrated that lanthanum hydroxide improves kidney function in a CKD model in mice by restoring homeostasis of the gut-metabolite-kidney axis, which alleviated an amino acid imbalance. Lanthanum hydroxide thus shows therapeutic potential for patients with CKD, through reshaping the composition of gut microbiota.

Conclusions: Lanthanum hydroxide plays a kidney protective role through the gut-metabolite-kidney axis in a rat model of chronic kidney disease caused by adenine.

Introduction:

Chronic kidney disease (CKD) refers to chronic progressive kidney parenchymal damage caused by various reasons, which causes the kidneys to significantly shrink and cannot maintain basic functions [1, 2]. Approximately 10% of people in the world suffer from CKD, and the annual financial expenditure for kidney disease in the United States amounts to 4.8 billion U.S. dollars [3]. The current treatment methods for CKD patients include dialysis and medication. However, the therapeutic effect is not as expected. Recent studies have shown that patients with chronic kidney disease are often accompanied by neurocognitive dysfunction [4], low-grade inflammation [5], disturbance of intestinal flora [6], and damage to the intestinal barrier function [7]. The intestinal flora can participate in the body's regulation process through a variety of ways to jointly maintain the body's dynamic balance[8]. Therefore, more and more scientific experiments are conducted to study the mechanism of the intestinal flora and the brain-gut-kidney axis in CKD, hoping to delay its progress by regulating the intestinal flora [9].

A healthy adult has tens of trillions of bacteria in the intestine alone [10]. The intestinal flora has more than 3 million genes, which is 150 times the human genome [11]. According to the relationship with the host, the intestinal flora can be roughly divided into three categories: beneficial bacteria, harmful bacteria and neutral bacteria [12]. Normal intestinal flora can protect the kidneys, but in patients with CKD, the normal intestinal flora homeostasis is broken. Vaziri et al. analyzed the fecal microbial DNA of 24 patients with end-stage renal disease (ESRD) and 12 healthy people and found that compared with the healthy group, there was a significant difference in the abundance of 190 bacteria in ESRD patients [13]. Among ESRD patients, the proportions of Enterobacteriaceae, Halomonas, Moraxellaceae, Pseudomonas and Thiobacillus spp. increased significantly. Nishiyama et al. analyzed the 16S rRNA sequencing of bacteria in the intestines of 5/6 nephrectomy mice and found that in 5/6 nephrectomy mice, the types of

Bifidobacterium, other coryneform bacteria, and Zurich bacillus increased significantly, while lactic acid Bacillus, Oscillatoria, and unclassified Verrucomicrobiaceae were significantly reduced [14].

The intestinal mucosal barrier can prevent harmful microorganisms and toxic metabolites from entering the blood [15]. In patients with chronic kidney disease, the mucosal barrier is destroyed [16]. Vaziri et al. observed in vivo studies in uremic rats that the expression of tight junction proteins (such as claudin 1, occludin, and zonula occludens-1) in the colonic mucosa decreased significantly, which indicates that the renal failure of rats The intestinal barrier is obviously damaged [17]. Yang et al. found that in CKD mice, the expression of colonic HSP70 and claudin 1 decreased, while the expression of claudin-2 increased and was accompanied by increased apoptosis [18]. In addition, they also found that although there was no difference in the percentage of regulatory T cells between CKD and control mice, the ratio of cytokines and CX3CR1^{intermediate} / CX3CR1^{high} in the colon of CKD mice was significantly increased [18]. The "gut-kidney axis" theory believes that the connection between the kidney and the intestine is two-way, and if one party's function is damaged, it can affect the normal function of the other party through a variety of ways [19-21]. Intestinal flora and its metabolites play an important role in it.

Lanthanum is a rare earth element discovered after cerium and yttrium. The metal lanthanum is chemically active and easily soluble in dilute acid [22]. It is easy to oxidize in the air, and the fresh surface will quickly darken when exposed to the air, heating can burn to generate oxides and nitrides. It is heated in hydrogen to generate hydride, which reacts violently in hot water and releases hydrogen. Lanthanum exists in monazite sand and bastnasite. Moreover, the combination of lanthanum preparations with conventional binders can reduce serum phosphate levels instead of increasing serum calcium levels, and has better tolerance and no serious side effects. In recent years, lanthanum preparations have been involved in the treatment of hyperphosphatemia [23]. In this study, we found that Lanthanum hydroxide regulates the homeostasis of intestinal flora, affects amino acid metabolism, increases urinary ammonium circulation and ultimately plays a role in kidney protection in CKD rat models.

Results:

3.1 Lanthanum hydroxide has an effect on the overall structural in microbiota composition.

The goods_coverage was used to evaluate the total number of community species represented by the sequencing results. The goods_coverage of all groups was greater than 0.99, which suggested that the sequencing depth of the microbiome analysis was very deep and met the requirements (Figure 1A). The Rank-abundance curve can be used to explain two aspects of diversity, namely species abundance and species evenness. In the horizontal direction, the abundance of species is reflected by the width of the curve. The higher the abundance of the species, the larger the range of the curve on the horizontal axis. The shape (smoothness) of the curve reflects the uniformity of the species in the sample. The smoother the curve, the more even the species distribution. The W and G group have increased species abundance and more uniform species distribution compared with the M group (Figure 2A). Then, we calculated alpha diversity indices to evaluate the overall fecal microbiota richness and structural difference among these

groups. We analyzed alpha diversity (α -diversity) indexes such as observed_species, Chao 1, ACE and Simpson index values to determine changes in the composition of various bacterial species in the feces samples of different groups. The α -diversity ACE, Chao 1 and observed species indexes were higher in the W and G groups of mice compared to the M group ($P < 0.05$). The Simpson index in the W group is smaller than the M and G group, but there is no significant difference (Figure 1C-F). Next, we analyzed β -diversity indexes to identify differences in the gut microbial species among K, M and G groups of mice using Principal component analysis (PCA), Principal Coordinates Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS). The differences in the fecal microbiota among K, M and G groups were identified based on PCA (Figure 1G), PCoA (Figure 1H) and NMDS (Figure 1I) of the *weighted UniFrac* distances for the 16S rRNA genes. It can be seen from PCA, PCoA and NMDS analysis that the M group is different from WT group. Moreover, after the administration of lanthanum hydroxide, the composition of the intestinal flora tended to be the K group. The above results demonstrate that lanthanum hydroxide improves the composition of whole intestinal flora in rats with chronic kidney disease.

3.2 Composition of gut microbiota of mice in each group of phylum and major differential microbial species.

We analyzed the differences in the abundance and composition of the gut microbial phylum and genus in the fecal samples of these groups using 16S ribosomal RNA (rRNA) sequencing. From the phylum-level analysis, we found that the predominant intestinal flora in the group mice were Bacteroides and Firmicutes. The relative abundance of Firmicutes in K, M and G groups were 75.1%, 61.2% and 74.2%, respectively. The relative abundance of Firmicutes in the M group was lower than other two groups (Figure 2A). In order to verify and further determine, the LEfSe was used to identify the specific phylotypes responding to K, M and G groups. We performed linear discriminant analysis (LDA) to determine LDA effect size (LEfSe) scores followed by Kruskal-Wallis and Wilcoxon tests. The main differential gut microbial species between the K and other groups were *s_Lactobacillus_intestinalis*, *g_Enterococcus*, *f_Enterococcaceae*, *s_Enterococcus_durans*, *g_unidentified_Lachnospiraceae*, *f_Moraxellaceae*, and *g_Acinetobacter*. The main differential gut microbial species between M and other groups were *f_Bacteroidaceae*, *g_Bacteroides* and *g_Parasutterella*. The main differential gut microbial species between G and other groups were *g_Turicibacter*, *c_unidentified_Actinobacteria*, *f_Bifidobacteriaceae*, *g_Bifidobacterium*, *s_Bifidobacterium_animalis*, *o_Bifidobacteriales*, *g_Faecalibaculum*, *p_unidentified_Bacteria*, *g_unidentified_Bacteria*, *o_unidentified_Bacteria*, *g_Candidatus_Saccharimonas*, *f_unidentified_Bacteria*, *c_unidentified_Bacteria* and *g_Lachnospiraceae*. According to the dominant flora in the three groups, we conducted a PICURES functional analysis and found that the dominant flora is mainly related to metabolism (Figure 2D). At the same time, after comparison, it was found that the metabolic process of the M group was significantly different from that of the K and G groups (Figure 2E). Further analysis showed that the differential metabolism of the dominant intestinal flora was mainly concentrated in amino acid metabolism (Figure 2F). The above results indicate that differences in the dominant intestinal flora in each group will affect amino acid metabolism.

3.3 Effect of Lanthanum hydroxide on intestinal mucosa of CKD rats.

First, we evaluated the effect of Lanthanum hydroxide on the jejunum of CKD model rats (Figure 3A). Jejunum is the most important place for nutrient absorption in the digestion system, because its structural changes directly affect the digestion and absorption of rats. In the K group, the villi of the jejunum of the mice were tightly arranged, without breaks or missing, and showed finger-like protrusions. There were a large number of tightly arranged absorption cells and a small amount of goblet cells scattered among the absorption cells. The lamina propria were arranged tightly and orderly without inflammatory cells. Compared with K group, the small intestinal villi of the jejunum tissue of the M group became shorter, a large number of inflammatory cells were infiltrated in the interstitium, the lamina propria edema was obvious, and the arrangement was sparse. Compared with the M group, the inflammatory symptoms in the jejunum tissue of the L, Z and G group were significantly improved. Inflammatory cell infiltration was rare in the central chyle duct, the small intestinal villi restored their integrity, and the lamina propria was arranged very tightly and orderly. Compared with the K group, the villi of the jejunum tissue in group LC and CC are loosely arranged with breakage or loss, showing finger-like protrusions. A few number of tightly arranged absorption cells and a large amount of goblet cells scattered among the absorption cells can be seen.

Second, we analyzed the effect of Lanthanum hydroxide on the ileum of model rats. As an important part of small intestine tissue, the ileum has very important digestion and absorption functions. In the K group, the ileum villi were intact and short-tapered. There was no significant increase in the number of goblet cells and no infiltration of inflammatory cells. Compared with the K group, a large number of inflammatory cell infiltrations were existed in the ileum tissue of the M group. At the same time, inflammatory cells tend to migrate to the intestinal cavity. The above results indicate that constipation causes inflammation of the small intestine of mice. Compared with the M group, the inflammation in the ileum tissue of the L, Z and G group mice was significantly improved. No inflammatory cell infiltration or small intestinal villus shedding was existed. The lamina propria was arranged quite tightly and orderly and closely attached to the mucosal layer. The above results show that Lanthanum hydroxide effectively reduce the inflammation of the small intestine, thereby restoring its digestion and absorption function. Compared with the K group, the intestinal villi in the LC and CC group were intact and there were inflammatory cell infiltration.

Third, we detected the effect of Lanthanum hydroxide on the cecum of the model group. The cecum is the main component of the first mucus layer of the innate immunity of the intestinal tract, and is conducive to the smooth passage of grain. The goblet cells in the cecum secrete mucus proteins, which have lubricating and protective effects. There were many absorption cells and goblet cells in the cecum of mice in the K group, and lamina propria were loosely arranged without inflammatory cell infiltration. There was a small amount of inflammatory cell infiltration in the cecum of the M group, the goblet cells were significantly reduced, and the crypts became shallow. These results indicated that constipation causes inflammation of the intestinal tract and a significant decrease in goblet cells.

The inflammatory symptoms of the cecum tissue of the L, Z and G groups were significantly improved, there was no inflammatory cell infiltration, and the goblet cells were significantly increased compared with the M group. Compared with the K group, the goblet cells in the cecum tissue of LC and CC group mice were significantly decreased, and the crypts were significantly shallower. These results demonstrated that Lanthanum hydroxide restore the injured intestinal mucosa.

3.4 Metabolomics analysis reveals that lanthanum hydroxide increases the metabolism of urinary ammonium.

The imbalance of the gut microbiota homeostasis will lead to the disorder of the amino acid metabolism of small molecules. Therefore, we used untargeted metabolomics to explore the impact of changes in gut microbiota on metabolites. Using HPLC-MS/MS, we found 4370 variables in positive ion mode and 732 variables in negative ion model. The total ion current diagram, K, M and treated groups in positive and negative ion mode, indicated that the contours of each group were roughly similar, but the level of metabolites is different. First, we performed mean normalization and logarithmic transformation on all data. Then, based on the QC sample, the small molecule compounds whose relative abundance is lower than 25% of the QC sample are eliminated (Figure 4A). The principal component analysis (PCA) was performed on the sample data of each group. QC samples had a high degree of aggregation, demonstrating high repeatability and stability (Figure 4B-D). The partial least square discriminant analysis (PLS-DA) was further applied to the samples of each group. The groups were clearly separated. WT and M group were clustered on the left and right sides respectively. There were obvious differences in the metabolites. These results shown that the model was successfully. Treated and K group were significantly separated and approached M group. It was consistent with the results of biochemical indicators and pathological changes. It was demonstrated that the model was reliable and didn't over-fitting (Figure 4E, F).

Based on the above analysis, we screened differential metabolites in the K, M (Figure 5A) and M, administration groups (Figure 5B) according to $P < 0.05$ and $P(\text{corr}) > 0.06$ (Table 1). There are 47 kinds of differential metabolites in positive and negative ion mode, among which 25 kinds of differential metabolites have been identified and confirmed (Figure 5C). The significant metabolites screened out were imported into MetaboAnalyst 5.0 for metabolic pathway analysis, and 8 related metabolic pathways were found (Figure 5D, E). The main metabolic pathways included urea cycle and arginine biosynthesis (Figure 8a, b). Above results demonstrated that lanthanum hydroxide increase urine ammonium metabolism.

3.5 Lanthanum hydroxide delays the progression of kidney disease and improves kidney function.

In order to evaluate the protective effect of lanthanum hydroxide on the kidneys of CKD rats, we tested the serum phosphorus, creatinine, and urea nitrogen levels 12 weeks after administration. Compared with the control group, the serum phosphorus, creatinine, and urea nitrogen in the M group was increased, and compared with the normal control group, there were significant differences ($P < 0.01$), suggesting adenine The joint 1.2% high-phosphorus diet was successfully modeled (Figure 6A-C). The results of HE staining

of rat kidneys showed that compared with K group, the degeneration and necrosis of the proximal convoluted tubule epithelial cells in the renal cortex and the disappearance of the nucleus were observed in the other groups (Figure 6D). The mesenchyme is accompanied by a large number of mononuclear cell infiltration, glomerular necrosis and disappearance, and visible protein casts and obvious expansion of the renal tubules in the renal tubules. Among them, the pathological changes in M group were more significant. Chronic granulomatous inflammation was observed. Purine deposits were seen in some renal tubules. At the same time, there were white blood cell casts and renal mesenchymal fibrous tissue focal hyperplasia lesions in the renal tubules. The pathological results of the lanthanum hydroxide (0.4g/kg/d, 0.2g/kg/d, 0.1g/kg/d) group showed that compared with the M group, cell deformation and infiltration were lighter, and the degree of renal tubule dilatation was significantly improved. Kidney interstitial hyperplasia is also effectively controlled, and the glomerular structure is relatively complete.

In summary, lanthanum hydroxide significantly reduces serum phosphorus levels, protects the kidneys, and slows down the development of kidney disease.

Discussion:

With the increasing incidence of chronic kidney disease (CKD), kidney disease has become one of the world's major public health problems [24]. According to the latest global kidney disease health report released by the World Kidney Conference in 2021, 1 out of 10 people in the world suffers from kidney disease [25]. In recent years, more and more evidence has shown that there are disorders of intestinal flora and impaired intestinal barrier function in patients with chronic kidney disease [26-30]. Therefore, more and more scientific experiments are investigated the mechanism of the intestinal-renal axis in CKD, hoping to delay its progress by regulating the intestinal flora.

Lanthanum is a rare earth element with high phosphorus binding capacity and low oral bioavailability [31]. It forms a highly insoluble compound in the gastrointestinal tract by combining with the phosphorus in the food to form a highly insoluble complex that is excreted from the body and effectively reduces the absorption of phosphorus. A small part of the absorbed lanthanum is mainly excreted through bile, ensuring that the pharmacokinetics of lanthanum in CKD patients and healthy people are similar. Lanthanum carbonate is a new generation of phosphorus binder without aluminum and calcium developed by British Shire Pharmaceutical Company [32]. In 2004, the US FDA approved lanthanum carbonate for the treatment of hyperphosphatemia, which does not cause hypercalcemia. It can be seen that lanthanum plays an important role in reducing phosphate in the blood, so the development of other compounds of lanthanum is one of the directions for the development of innovative drugs for phosphate binders [33-36]. This article mainly studies the effect of lanthanum hydroxide on adenine-induced CKD and hyperphosphatemia rats.

In this study, we demonstrated that lanthanum hydroxide improved kidney function by promoting urea metabolism through restoration of gut microbial homeostasis in the model mice. Firstly, using 16S ribosomal RNA (rRNA) sequence, we found that lanthanum hydroxide has an effect on the overall

structural in microbiota composition. PICTURES functional analysis indicates that lanthanum hydroxide affected the amino acid metabolism. In order to explore the effect of lanthanum hydroxide on amino acid metabolism, we conducted a untargeted metabolomics study. Moreover, lanthanum hydroxide increases the metabolism of urinary ammonium by increasing carbamoyl phosphate, aspartate, L-Citrulline, L-Arginine and reducing glutamate, nitrogen, N-Acetylornithine, N-Acetyl-L-citrulline. Finally, lanthanum hydroxide plays a role in renal protection (Figure 7).

There are several limitations in this study. Firstly, the relationship between amino acid metabolism and composition of the gut microbiota is not well known. Secondly, intestinal epithelial integrity testing is not comprehensive enough. Therefore, future studies are required to further explore the regulatory role of lanthanum hydroxide in alleviating constipation and metabolites.

Materials And Methods:

Animals and ethics statement

Six-week old male Wistar rats, the Specific pathogen free (SPF), were purchased from Beijing Weitong Lihua Biotechnology Co., Ltd., housed in a light and temperature-controlled room, and fed with food and water. All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of Inner Mongolia Medical University. The experiments were carried out according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Experimental Protocol

For 1-2 weeks, model rats were given a 2% adenine suspension at 200mg/kg per day. For 3-4 weeks, the same concentration and dose will be given by gavage every other day. After the model is successful, the model rats are divided into lanthanum hydroxide group (0.4g/kg/d, 0.2g/kg/d, 0.1g/kg/d), lanthanum carbonate group (0.3g/Kg/d), calcium carbonate Group (0.4g / kg / d) (n = 11) and model group, each with 12 animals. Another 12 Wistar rats with similar coat color and body shape were selected as the blank control group, and no drugs were given. All animals were sacrificed on the last day of 12 weeks. On the day before execution, all animals were forbidden to eat, they were allowed to drink freely, and feces were collected in a metabolic cage for 12 hours. Rats were anesthetized with 50mg/kg pentobarbital, blood was collected from the abdominal aorta, and serum was collected by centrifugation. The kidney of each animal was fixed in 10% neutral formaldehyde for subsequent histological examination.

LC/MS analysis of serum metabolites

Serum samples were incubated for 10 minutes with pre-chilled methanol in a ratio of 1: 3 to precipitate the proteins. The samples were centrifuged at 12000r/min for 15 minutes at 4°C. The supernatants were analyzed by Thermo Scientific Dionex UltiMate3000 Rapid Resolution Liquid Chromatography and QExactive mass spectrum. The chromatographic conditions are shown in Table1. The analytes were separated in a XBridge BEH Amide chromatographic column (2.1×100 mm, Waters Co., Milford, MA, USA)

using 0.1% formic acid and acetonitrile as mobile phases A and B, respectively. The flow rate was set at 0.4 ml/min, injection volume was 5 µl, and column temperature were set at 25°C. The mass spectrum signals were obtained using the positive and negative ion scanning mode. The ion spray voltage and other specific MS parameters were shown in Table 2.

Gut microbiota composition

Fecal samples were collected from all mice and immediately stored at -80°C. The V3+V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq (Beijing Novogene Co. Ltd., Beijing, China) and analyzed using the QIIME open platform to determine the gut microbiota profiles.

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA). The data plots were generated using GraphPad Prism 8.0.1 (GraphPad Software, La Jolla, California, USA). Partial least squares discriminant analysis (OPLS-DA) of SIMCA-P+13.0 (Umetrics, AB, Umeå, Sweden) and Principal Components Analysis (PCA) were used to assess normalized LC-MS spectral data. Variable Influence on Projection (VIP) values were used to identify significant variables with VIP values >1.0 and $p < 0.05$. These significant variables were used to identify the spectral peaks. Student's t-test was used to analyze differences between two groups of data. The taxonomic rank differential between groups was determined using Student's test (v3.1.2, R programming language). The correlation between genera abundance and mouse behavior was calculated using Spearman correlation coefficients (R language). $p < 0.05$ was considered statistically significant. The data are presented as means $\pm$ SD.

Declarations

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Authors'contributions

GL designed the experiments and drafted part of the manuscript, HL, RB and BL performed biochemical analyses and behavioral tests, drafted part of the manuscript, and revised the manuscript. The final manuscript was approved by all the authors.

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Availability of data and materials

Not applicable

Ethics approval and consent to participate

Not applicable

Competing interests

The authors declare that there are no conflicts of interests.

Author details

1 Inner Mongolia Medical College, Hohhot, China. 2 Ordos Central Hospital, Ordos, China.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

Figure 1

Overall structural changes in gut microbiota composition. (A) The goods_coverage, (B) relative abundance, (C) Ace, (D) Chao 1, (E) observed_species and (F) simpson index of these groups; (G) Principal component analysis(PCA); (H) PCoA plots of Bray-Curtis-computed distances between saliva and tonsils; (I) non-metric multidimensional scaling method (NMDS). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by one-way ANOVA analysis followed by Dunnett's post hoc test. All values are mean $\pm$ SD. $n = 5$.

Figure 2

Composition of gut microbiota and the major differential microbial species. (A) The relative abundance of the top 5 dominant gut microbiota phylum in each group. (B-C) LDA scores of the significant species and LefSe cladogram. Only taxaon with LDA scores of more than 3.5 and a significant value less than 0.05 was presented. (D-F) PICURES functional analysis of each group's dominant intestinal flora. All values are mean $\pm$ SD. n=4.

Figure 3

Effect of Lanthanum hydroxide on intestinal mucosa of CKD rats. (A) Morphologic analysis on gut barrier among experimental groups, including histopathological assay for ileum and cecum by H&E staining.

Figure 4

Screening of differential metabolites in each group. (A) Perform mean normalization and logarithmic conversion on each group of data. (B, C) PCA analysis of differential metabolites in each group. (D) Cluster analysis of different metabolites in each group. (E-F) PLS-DA analysis of different metabolites in each group. All values are mean $\pm$ SD. n=8.

Figure 5

Functional enrichment analysis of differential metabolites. (A) S-plot of the difference metabolites between K and M group. (B) S-plot of the difference metabolites between M and G group. (C) Heat map of relative content of different metabolites. (D) Differential metabolite GO enrichment analysis. (E) Differential metabolite KEGG enrichment analysis. All values are mean $\pm$ SD. n=8.

Figure 6

Lanthanum hydroxide delays the progression of kidney disease and improves kidney function. (A) Serum phosphorus, (B) serum creatinine and (C) serum urea nitrogen levels were tested after 12 weeks of administration. (D) HE staining of kidney tissue. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, versus the K group rats, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, versus the M group rats, by one-way ANOVA analysis followed by Dunnett's post hoc test. All values are mean $\pm$ SD. $n = 8$.

Figure 7

Lanthanum hydroxide plays a kidney protective role through the gut-metabolite-kidney axis in a rat model of chronic kidney disease caused by adenine.

Supplementary Files

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