Rubidium chloride modulated the intestinal microbiota community in mice

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Abstract

Background

The intestinal microbiota plays an important role in host health. Although rubidium (Rb) has been used to study for depression and cancers, the interaction between intestinal microbial commensals and Rb is still unexplored. To gain the knowledge of the relationship between Rb and intestinal microbes, 51 mice receiving RbCl-based treatment and 13 untreated mice were evaluated of their characteristics and bacterial microbiome changes.

Results

The 16S ribosomal RNA gene sequencing of feces showed RbCl generally maintained the microbial community diversity, while the shifts in gut microbial composition were apparent after RbCl exposure for the first time. RbCl significantly enhanced the abundances of Rikenellaceae, Alistipes, Clostridium XIVa and sulfate-reducing bacteria including Deltaproteobacteria, Desulfovibrionales, Desulfovibrionaceae and Desulfovibrio. While, RbCl significantly inhibited the abundances of Tenericutes, Mollicutes, Anaeroplasmatales, Anaeroplasmataceae and Anaeroplasma lineages. Besides, with regarding to the composition of archaea, RbCl significantly enhanced the abundances of Crenarchaeota, Thermoprotei, Sulfolobales, Sulfolobaceae and Sulfolobus lineages.

Conclusions

These results revealed that enrichments of Clostridium XIVa, Alistipes and sulfate-reducing bacteria could act on brain-gut-microbiota axis by affecting serotonergic system and immune system. Therefore, it was likely that RbCl would have beneficial anti-effects on depression and cancers by modifying brain-gut-microbiota axis.

1 Background

Rubidium (Rb) was found in air, soil, water and organisms, which is a less studied alkali metal element and can efficiently transfer to the human body through the food chain (soil-plant-human) [1]. Since the first report on its correlation with phenylketonuria and maple-syrup-urine disease [2], some studies have suggested its effects on tumor [3, 4], depression [5–7] and cardiovascular system [8].

Researchers reported that many cancers were caused by the changes of Rb\(^+\) levels in the body [3, 9]. Some other studies have shown that Rb was a candidate anticancer drug [4, 10]. Rb has been used as an anti-depressant drug for 40 years due to its low toxicity and high drug resistance [5, 6, 11]. While, up to now, it is still unclear what are the inhibition mechanisms of Rb on tumors and depression.
The microbiome is a dynamic ecological community which mainly include bacteria, archaea, fungi and viruses [12]. During the recent years, the potential role of gut microbiome in various human diseases has attracted the attention of researchers [12, 13]. Many factors can fluctuate the composition of gut microbiome such as host genomes, antibiotic use, lifestyle, hygiene and diet [14]. The dynamic relationship between gut microbiome and cancer is complex. It can inhibit the occurrence of cancer in normal condition when intestinal microenvironment changes. It will promote the occurrence and development of cancer conversely [15]. Moreover, some researchers reported that intestinal microorganisms may be involved in the development of depression through the brain-gut-microbiota axis [16, 17]. The brain-gut-microbiota axis consists of bidirectional communication between the gut and brain [18, 19]. The intestinal microorganisms produce and secrete substances consisting of neurotransmitters, which could affect intestinal microbial function in turn [16]. There is growing evidence proving that the gut microbiome plays key roles in the cancer and neurological disease [20–23].

To our best knowledge, no work till now has studied on the effect of chemical element Rb on the gut microbiome, and whether Rb inhibits tumor and depression thorough changing the community composition of gut microbiome is still not clarified. So, the present study was to investigate the relationship between the addition of rubidium chloride (RbCl) and composition of gut microbiome in order to further understand the mechanism of Rb against cancer and neurological disease from the perspective of microbial diversity.

2. Results

2.1 Effect of RbCl on animal characteristics

To better understand the effects of RbCl on mice, we conducted a follow-up study of 64 mice and recorded body weights and multiple organ weights of each mouse. Changes of the body weights in all the groups were shown in Fig. 1a. The weights of mice in the drug groups decreased when compared with mice in control group and were negatively correlated with dosage whereas these differences were not statistically significant. Additionally, multiple organ coefficients were observed (Fig. 1b). Interestingly, as RbCl concentration increased, the organ coefficient of stomach gradually decreased. However, there was an increase of organ coefficients among other organs such as pancreatic, spleen, kidneys, lungs and heart. Changes of gastric organ coefficient during drug administration could be explained by route of administration. These data indicated that RbCl had little effect on animal characteristics.

2.2 Effect of RbCl on the microbial communities

From the results of 16S rRNA gene sequencing, we obtained a total of 1481388 high-quality reads for 64 fecal samples of four groups, which could be clustered into 486 OTUs. Figure 3a indicates the rarefaction curves of all samples. The curves tended to be flat as the number of extracted sequences increased, indicating that the sequencing depths included most of the microbes in samples. As shown in Fig. 2, the indices reflecting community richness include Sobs, Chao and Ace. The indices reflecting community
diversity are Shannon and Simpson. The richness and diversity indexes demonstrated no statistically differences in control, low-dose (Chao, P=0.9401; Sobs, P=0.5239; Ace, P=0.7497; Shannon, P=0.5082; Simpson, P=0.5401), middle-dose (Chao, P=0.6578; Sobs, P=0.7346; Ace, P=0.7640; Shannon, P=0.8858; Simpson, P=0.5176) and high-dose groups (Chao, P=0.7105; Sobs, P=0.3809; Ace, P=0.7243; Shannon, P=0.8954; Simpson, P=0.4176). In addition, to assess the effect of the different treatments on the assembly of bacterial communities, we compared the β-diversity (between-samples diversity) using Bray Curtis distances and performed constrained principal coordinate analysis (CPCoA). This analysis revealed a clear differentiation of samples belonging to the control, low-dose, middle-dose, and high-dose groups that explained as much as 6.62% of the overall variance of the data (Fig. 3b; P<0.001). Thus, the above results showed that RbCl did not affect the diversity and richness of the microbial community in general. However, it altered the structure of bacterial community, reflected in changes in microbial composition.

2.3 Effect of RbCl on intestinal bacterial composition

All OTUs were clustered into 12 phyla, 19 classes, 27 orders, 44 families, 92 genera. The venn diagram (Fig. S1) showed 352 shared OTUs among all the fecal samples, and samples in control, low-dose, middle-dose and high-dose groups had 7, 14, 8 and 10 unique OTUs, respectively. Results indicated that although the proportion of shared microbial communities was very high, distinct microbial communities still existed in different treatment groups. Compositions of intestinal bacteria in all stool samples were determined using 16S rRNA gene sequencing. The microbial compositions at phylum level were seen in Fig. 4a. In all samples, Firmicutes was the dominant phylum with average abundances of 51.03%, 50.18%, 47.15% and 43.73% in control, low-dose, middle-dose and high-dose groups, respectively (Fig. 4b). Bacteroidetes, the second dominant phylum, was no signicant differences among the four groups (Fig. 4c). Moreover, low proportions of Tenericutes (the average abundances were 0.86%, 0.23%, 0.05% and 0.08%) and Actinobacteria (the average abundances were 0.03%, 0.03%, 0.04% and 0.07%) were observed in control, low-dose, middle-dose and high-dose groups, respectively (Fig. 4a). As shown in Fig. 4d and e, enrichment of Actinobacteria and depletion of Tenericutes (p<0.01) were correlated with high doses of RbCl.

The gut microbiota from 4 groups were separated into 3 known dominant classes including Bacteroidia, Clostridia and Epsilonproteobacteria, and other 16 classes with relatively low proportions (Fig. 6a). Statistically significant differences between the experimental groups and control group were also performed in our study. The bacterial class Deltaproteobacteria was significantly higher in three experimental groups (p<0.05) compared with control group (Fig. 6b). In addition, Differences in the relative abundances of Mollicutes were significant in control, low-dose (p=0.0175), middle-dose (p=0.0014) and high-dose groups (p=0.0022) (Fig. 6c).

The relative abundances of gut microbiota in control, low-dose, middle-dose and high-dose groups at the level of order were shown in Fig. 7a. A total of 27 orders were observed in all samples. The relative proportion of Anaeroplasmatales was significantly increased in control group (p<0.05) (Fig. 7b), while the
Desulfovibrionales was significantly higher in low-dose (p = 0.0176), middle-dose (p = 0.0219) and high-dose groups (p = 0.0033) than control group (Fig. 7c).

At the family level, gut microbiota with relative abundance above 1% were shown in Fig. 8a. Among these families, the abundance of Anaeroplasmataceae was found significantly higher in control group (p\(\leq 0.05\)) (Fig. 8b), while the abundances of Desulfovibrionaceae were significantly increased in three experimental groups (p\(\leq 0.05\)) (Fig. 8c). Besides, compared with the control group, the abundances of Rikenellaceae significantly increased in low-dose (p = 0.0006), middle-dose (p = 0.0054) and high-dose groups (p = 0.0033) (Fig. 8d).

Figure 9a showed the microbial compositions with relative abundance above 1% at the genus level. The microbial community compositions were similar but relative abundances of genera varied. OTUs unclassified at the genus level were the most abundant and there were no statistical differences among all fecal samples. The following genera were Bacteroides and Helicobacter (the average abundances were 13.96–20.80% and 6.87–13.46%, respectively). Figure 9b showed that the proportions of Bacteroides did not change among the four groups. Additionally, Helicobacter showed an increasing trend in relative abundances while there were no significant differences (Fig. 9c). We could also get this information from heatmap (Fig. S2). Low proportions of Anaeroplasma (Fig. 9d; P < 0.001) and Desulfovibrio (Fig. 9e; P < 0.001) were significantly different in various treatment groups. We observed an increase in the proportion of Desulfovibrio in RbCl treatment mice. Besides, the abundances of Alistipes (Fig. 10a; P < 0.01) and Clostridium XIVa (Fig. 10b; P < 0.05) were significantly higher in low-dose, middle-dose and high-dose groups.

The LEfSe with default parameters was used to identify significant differences in relative abundances of gut microbiota between the RbCl groups and control group. LEfSe analysis further confirmed enrichment microbes in different groups (Fig. 11a and b). The RbCl groups were significantly enriched for Deltaproteobacteria, Desulfovibrionales, Desulfovibrionaceae, Desulfovibrio, Rikenellaceae, Alistipes and Clostridium XIVa. The control group was enriched for Tenericutes, Mollicutes, Anaeroplasmatales, Anaeroplasmataceae and Anaeroplasma.

### 2.4 Effect of RbCl on intestinal archaea composition

We also analyzed the abundance of various archaea in fecal samples from each treatment group. At the phylum level, the intestinal archaea from 4 groups were separated into Crenarchaeota and Euryarchaeota (Fig. 4a). The abundance of Crenarchaeota was significantly higher in middle-dose group (p = 0.0238) than control group (Fig. 5a), while the abundances of Euryarchaeota did not change among the four groups (Fig. 5b).

The intestinal archaea were separated into Thermoprotei and Thermoplasmata at the class level (Fig. 6a). The abundance of Thermoprotei was significantly higher in middle-dose group (p\(\leq 0.05\)) (Fig. S3a). Additionally, the abundances of Thermoplasmata were not significantly different among the four groups (Fig. S3b).
The relative abundances of intestinal archaea in control, low-dose, middle-dose and high-dose groups at the order level were shown in Fig. 7a. We observed an increase in the proportion of *Sulfolobales* in middle-dose group (p < 0.05) (**Fig. S4a**). In addition, the archaea *Thermoplasmatales* did not change among four groups (**Fig. S4b**).

At the family level, less abundances of *Sulfolobaceae* (the average abundances were 0-0.01%) and *Ferroplasmaceae* (the average abundances were 0-0.01%) were observed. Difference in the relative abundance of *Sulfolobaceae* was significant in middle-dose (p < 0.05) (**Fig. S5a**), while the abundances of *Ferroplasmaceae* demonstrated no statistically differences in control, low-dose, middle-dose and high-dose groups (**Fig. S5b**).

The less abundances of *Sulfolobus* and *Acidiplasma* at the genus level were observed. The abundance of *Sulfolobus* significantly increased in middle-dose group (p < 0.05) (**Fig. S6a**). Moreover, the abundance of *Acidiplasma* did not change among four groups (**Fig. S6b**). Thus, with regarding to the composition of archaea, RbCl significantly enhanced the abundances of *Crenarchaeota, Thermoprotei, Sulfolobales, Sulfolobaceae* and *Sulfolobus* lineages.

### 3. Discussion

This study found no differences in the alpha-richness and diversity indexes, which were consistent with some reports. Naseribafrouei et al. [24] reported no significant differences in microbiota diversity between depressed and non-depressed individuals. Furthermore, study by Wang et al. had compared the gut microbiota of healthy volunteers and Colorectal cancer patients, with no significant differences reported in diversity indexes [25]. It should be noted that the diversity of gut bacteria was affected by several factors, including health status, age, diet, medication and so on [26]. No difference in the alpha-richness and diversity may be explained in part by the consistency of age and diet among all samples. Part of the reasons may be that RbCl did not affect the diversity of the microbial communities. In addition, we found that RbCl altered the structure of bacterial community, reflected in changes in microbial composition. Wei et al. [27] observed that gut microbiota were significantly different between healthy rats and chronic diseased rats. Moreover, Zhang et al. [28] revealed that the gut microbiota structure changed significantly in response to high fat diet (HFD) feeding and berberine administration. Shifts of intestinal microbiota structure were also thought to occur in Crohn's disease patients [29]. Thus, changes in gut microbial composition have played an important role in the progression of human diseases.

In our study, we observed that RbCl maintained the abundances of *Firmicutes, Bacteroidetes, Actinobacteria, Bacteroides* and *Helicobacter*. Chen et al. reported that *Firmicutes* significantly reduced in intestinal lumen of patients with colorectal cancer (CRC) [30]. In Crohn's disease, the abundance of *Firmicutes* was also significantly decreased [31, 32]. In addition, most works showed that *Firmicutes* was the higher abundant phylum in breast tissue [33–35]. In depression patients, it was also found that the relative abundance of *Firmicutes* significantly changed [36, 37], which was related to depression through inflammation [38]. Therefore, these findings indicated that changes of *Firmicutes* were closely associated
with diseases. Anticancer and anti-depressant effects of RbCl might be mediated by maintaining the abundance of *Firmicutes* in the gut. *Bacteroidetes* was non-endospore-forming anaerobes with bile resistance, accounting for more than 25% of gastrointestinal microbiota [39–41]. Proportions of *Bacteroidetes* were significantly lower in CRC rats than in healthy rats [25, 42]. Although the exact physiological implications of *Bacteroidetes* in CRC were not fully understood, it was likely that inflammatory bowel diseases were known risk factors for CRC, and a significant reduction of the phylum *Bacteroidetes* occurred in inflammatory bowel diseases [29, 43]. In addition, Jiang et al. [37] reported that *Bacteroidetes* were significantly more abundant in active-major depressive disorder subjects. The increase in *Bacteroidetes* was mainly promoted by *Alistipes*. Naseribafrouei et al. [24] reported increased abundance of *Alistipes* in the depressed subjects. Therefore, it can be inferred that changes of *Bacteroidetes* were closely associated with diseases. The *Actinobacteria*, which is comprised of gram-positive bacteria, includes 5 subclasses and 14 suborders [44]. Major depressive disorder (MDD) patients characterized by significant increase in the relative abundance of *Actinobacteria* [36]. Yang et al. [45] reported that the abundance of *Actinobacteria* was significantly higher in the depression mice. It was possible that enrichment of *Actinobacteria* was closely related to the development of depression. Exactly, RbCl did not significantly increase the abundances of *Actinobacteria*. *Bacteroides* is anaerobic, bile-resistant, non-spore-forming, gram-negative rods [46]. Changes of *Bacteroides* were assumed to be associated with metabolic diseases such as obesity and diabetes [47, 48]. In Type I diabetes mellitus patients, *Bacteroides* was significantly increased [49]. The *Bacteroides*, which was known to be associated with increased gut permeability and inflammation, was positively associated with β-cell autoimmunity. Moreover, Zhu et al. [42] reported greater genera *Bacteroides* abundance in colon cancer patients compared with controls. It was likely that *Bacteroides* produced a metalloprotease known as fragilysin, which might favor carcinogenesis. Taken together, these findings indicated that variations of *Bacteroides* were closely associated with diseases. It should be noted that RbCl did not change the proportion of *Bacteroides*. Lower abundance of *Helicobacter* was observed in gut microbiota of overall gastric cancer (GC) patients as compared to healthy controls [50]. It was possible that low proportion of *Helicobacter* contributed to the pathogenesis of GC. Exactly, RbCl did not change proportion of *Helicobacter*.

We also found RbCl significantly inhibited the abundances of *Tenericutes, Mollicutes, Anaeroplasmatales, Anaeroplasmataceae* and *Anaeroplasma* lineages. Yang et al. [45] reported that the abundance of *Tenericutes* was significantly lower in the depression mice. RbCl did not improve reduction of *Tenericutes*, which was consistent with reports. A previous animal study demonstrated that antidepressant drug (R)-ketamine and (S)-ketamine also did not improve the reduced proportion of *Tenericutes* [45]. Additionally, Ketamine, known to induce antidepressant effects, also significantly reduced abundances of *Tenericutes* [51]. Tully et al. [52] reported that some species of *Mollicutes* were significant pathogens in human disease. A study also found that some *Mollicutes* were associated with diseases [53]. It was worth noting that the abundances of *Mollicutes* were significantly lower after treatment with RbCl. The reduction of *Mollicutes* could decrease the pathogenesis of depression and cancers. However, one study has reported a significant reduction in the relative abundance of *Mollicutes* in MDD patients [45]. As the physiological
mechanism of *Mollicutes* in depression was unclear, further studies on the relationship between depression and *Mollicutes* are needed. *Anaeroplasmatales* is an order of *Mollicutes* bacteria which do not have cell wall [54]. Song et al. [55] found *Anaeroplasmatales* significantly increased in depression group. In addition, ketamine, known to induce antidepressant effects, significantly reduced the abundance of *Anaeroplasmatales* [51]. Exactly, the abundances of *Anaeroplasmatales* were significantly lower in RbCl groups. *Anaeroplasmataceae*, which belongs to Class *Mollicutes* and Order *Anaeroplasmatales*, is strictly anaerobic wall-less bacteria [56]. The abundance of *Anaeroplasmataceae* was significantly higher in depression group [55]. Moreover, *Anaeroplasmataceae* significantly increased in patients with Crohn's disease localized in the colon (CCD), but significantly decreased in patients with ulcerative colitis (UC) [57]. Interestingly, we observed that RbCl inhibited the proportion of *Anaeroplasmataceae*. The reduction of *Anaeroplasmataceae* could decrease the pathogenesis of depression. In the study of colon cancer, Zeng et al. [58] found that the abundance of *Anaeroplasma* increased in the HFD-azoxymethane (AOM) group. The *Anaeroplasma* bacteria is negative by Gram stain, which belongs to *Mollicutes* class, Tenericutes phylum. *Anaeroplasma* was opportunistic pathogens which elicited various host immune responses in numerous human diseases including colon cancer [59, 60]. Interestingly, the results of RbCl inhibited the proportion of the bacteria.

Expressions of sulfate-reducing bacteria (SRB) including *Deltaproteobacteria, Desulfovibrionales, Desulfovibrionaceae* and *Desulfovibrio* were significantly higher in RbCl groups. *Deltaproteobacteria* belonging to *Proteobacteria* is sulfate-reducing bacteria [61]. Hydrogen sulfide (H$_2$S) produced by SRB was a process of sulfate reduction [62]. H$_2$S could lead to chronic inflammation and imbalance between cellular proliferation, apoptosis and differentiation by damaging the intestinal epithelium [63]. Reports showed that *Deltaproteobacteria* was possibly associated with CRC [64, 65]. Jin et al. [66] reported that *Deltaproteobacteria* was commonly pathogenic bacteria in the intestine. *Desulfovibrionales*, belonging to *Deltaproteobacteria*, is also a sulfate-reducing bacteria that can reduce sulfur to produce hydrogen sulfide (H$_2$S) [61]. *Desulfovibrionaceae*, which was the main biological source of hydrogen sulfate (H$_2$S), involved in a wide range of physiological processes by influencing cellular signaling pathways and sulfhydration of target proteins [67, 68]. Zhang et al. [69] reported that the proportion of *Desulfovibrionaceae* was higher in animal models of metabolic syndrome. *Desulfovibrio* could also produce hydrogen sulfide (H$_2$S) by reducing sulfate [70]. H$_2$S derived from *Desulfovibrio* was associated with gastrointestinal disorders, such as UC, Crohn's disease, and irritable bowel syndrome [67]. Besides, Hale et al. [71] also reported that *Desulfovibrio* produced metabolites such as secondary bile acids, which may catalyze the formation of colorectal cancer. However, it should be noted that the proportions of sulfate-reducing bacteria were promoted by RbCl. RbCl led to the enrichment of sulfate-reducing bacteria which could cause inflammation directly or indirectly in mice. It was likely that RbCl used as antigen in healthy mice which could elicit immune responses.

In addition, RbCl significantly increased the abundances of *Rikenellaceae, Alistipes* and *Clostridium XIVA*. Wu et al. [72] found that the abundance *Rikenellaceae* decreased in the colitis-associated colorectal cancer (CAC) group compared with control group. Alkadhi et al. [73] also reported that the proportion of
Rikenellaceae reduced in CAC mice. In addition, the report found that Rikenellaceae was overrepresented in healthy control subjects [36]. Following RbCl treatment, the abundance of Rikenellaceae increased in the present study. Therefore, the increase in Rikenellaceae abundance could accelerate the antitumor efficacy of RbCl. Alistipes, which belongs to Bacteroidetes, is present in the human intestinal tract [74]. Alistipes was indole-positive and may thus influence tryptophan availability [75]. In our results, RbCl promoted the abundance of Alistipes. As tryptophan was also the precursor of serotonin, enrichment of Alistipes might affect serotonergic system by interfering with tryptophan metabolism. Clostridium XIVa, belonging to Firmicutes phylum, produces short-chain fatty acids (SCFAs) [76]. The SCFAs produced in the gut are mainly acetate, butyrate and propionate [77]. SCFAs could modulate cell functions either by inhibiting histone deacetylase activity, or through the activation of ‘metabolite-sensing’ G-protein coupled receptors (GPCRs) such as GPR43 and protect the integrity of epithelial barrier [78–80]. RbCl promoted the abundance of Clostridium XIVa. The increase in abundance Clostridium XIVa could alleviate the pathogenesis of depression and cancers. Clostridium XIVa was significantly lower in CRC patients [76]. Clostridium XIVa was overrepresented in healthy control subjects [36, 81].

Regarding the composition of archaea, the abundances of Crenarchaeota, Thermoprotei, Sulfolobales, Sulfolobaceae and Sulfolobus lineages significantly increased in RbCl groups. Crenarchaeota was originally considered to grow in habitats characterized by high temperature, high salinity, or an extreme pH. Later studies found that Crenarchaeota also seem to occur ubiquitously in temperate or cold aquatic [82] and terrestrial environments [83]. The presence of Crenarchaeota in intestinal tracts was reported by Friedrich et al [84]. In addition, Rieu-Lesme et al. [85] suggested that Crenarchaeota was present in the microbiota of the human digestive ecosystem. Thermoprotei, the crenarchaeal class, consists solely of obligate thermophiles. Thermophiles were well-known for participating in rampant lateral gene transfer (LGT) [86, 87]. It was likely that the nature of their extreme environments encouraged the exchange of genetic material. Thermoprotei mostly occurred in the marine environment [88]. However, report showed that Thermoprotei was observed to have an appreciably higher representation in healthy child [89]. Interestingly, the proportion of Thermoprotei was promoted by RbCl in this study. Sulfolobales, a monophyletic group within the Crenarchaeota, is thermophilic sulfur-metabolizing archaea [90]. The report found that Sulfolobales was present in human feces sample [85]. The family Sulfolobaceae is composed of extreme thermoacidophiles that are found in terrestrial environments [91]. The Sulfolobaceae could produce bacteriocin, which played an important role in microbial interaction or microbe-environment interactions, and therefore improved their adaptation in extreme environments [92]. Enrichment of Sulfolobaceae promoted by RbCl may be beneficial in combating disease-related adverse environments. The genera Sulfolobus, which belongs to Sulfolobaceae, grows at low pH (2–3) and high temperature(70–85°C) [93, 94]. The acidophilic and thermophilic properties of Sulfolobus offered many obvious advantages for industrial applications [95, 96]. In addition, Sulfolobus was able to reduce ferric iron when growing on elemental sulfur as an energy source [97].

Furthermore, RbCl maintained the abundances of archaea Euryarchaeota, Thermoplasma, Thermoplasmatales, Ferroplasmaceae, Acidiplasma lineages. Euryarchaeota, one of the four major divisions of archaea, contributed substantially to global energy cycling [98]. Euryarchaeota was detected
in marine picoplankton [99, 100] and in coastal salt marsh and continental shelf sediments [101]. *Methanobrevibacter smithii*, which belonged to *Euryarchaeota* phylum, was major archaeal player in human gut system [102]. A few studies confirmed that *M. smithii* was probably involved in inflammatory bowel disease (or Crohn’s disease), irritable bowel syndrome, colorectal cancer, and obesity [103, 104]. *Methanobrevibacter oralis*, belonging to *Euryarchaeota* phylum, was the predominating methanogenic species in the oral cavity [102]. *M. oralis* was identified in apical periodontitis [105]. Therefore, these findings proved that *Euryarchaeota* might play key roles for human health and disease. However, the proportions of *Euryarchaeota* did not change after RbCl treatment. *Thermoplasmata* was affiliated with *Euryarchaeota* phylum. Auguet et al. [106] showed that *Thermoplasmata* represented important component of soil microbial communities. In human body, Li et al. found that *Thermoplasmata* was not the predominant archaeons in the subgingival dental plaque and *Thermoplasmata* was closely correlated with chronic periodontitis [107]. Following RbCl treatment, the abundance of *Thermoplasmata* did not change. Horz et al. found that *Thermoplasmatales* existed in the human oral cavity [108]. He et al. reported that *Thermoplasmatales* was also observed in healthy subjects, but the abundance of *Thermoplasmatales* increased in individuals with periodontitis [109]. It was possible that enrichment of *Thermoplasmatales* contributed to the pathogenesis of periodontitis. Exactly, RbCl inhibited the enrichment of *Thermoplasmatales*. The *Ferroplasmaceae* is represented by cell wall-deficient, acidophilic, facultatively anaerobic and iron-oxidizing archaea [110]. As iron oxidizers, the family *Ferroplasmaceae* may contribute to the cycle of iron and sulfur [111]. It was likely that *Ferroplasmaceae* was involved in the pathogenesis of diseases through oxidizing iron. Thus, further studies on the relationships between diseases and *Ferroplasmaceae* are needed. Interestingly, RbCl did not change the abundance of *Ferroplasmaceae*. *Acidiplasma*, which belongs to the family *Ferroplasmaceae*, order *Thermoplasmatales*, phylum *Euryarchaeota*, is a novel acidophilic, cell-wall-less archaeon [112]. The genera *Acidiplasma* included two species, namely *Acidiplasma aeolicum* and *Acidiplasma cupricumulans* [111]. *Acidiplasma aeolicum* and *Acidiplasma cupricumulans* were isolated from the hydrothermal pool located on Vulcano Island (Italy) and chalcocite/copper-containing heaps (Myanmar), respectively [112]. It should also be noted that there were no reports on the relationships between *Acidiplasma* and diseases. In our results, *Acidiplasma* was observed in stool samples and its abundances were not affected by RbCl. These data showed that intestinal archaea were profoundly altered by RbCl. which might provide direct evidence of the relationship between Rb and diseases.

Some reports found Rb could be used as anticancer and anti-depressant drugs. The mechanisms of Rb against cancer and neurological disease remain unclear. Gut microbiota may participate in the pathogenesis of depression through the brain-gut-microbiota axis [113]. Serotonin (5-HT) is a critical signaling molecule in the brain-gut-microbiota axis [114]. The accumulation of 5-HT and the rate of synthesis of 5-HT in the brain were enhanced by intraperitoneal administration of RbCl [115]. In the present study, *Clostridium XlVa*, SCFAs producing bacteria, was significantly promoted by RbCl. SCFAs could promote colonic 5-HT production [116, 117]. Enrichment of *Alistipes* promoted by RbCl might disrupt the intestinal serotonergic system by affecting tryptophan metabolism. Therefore, it was likely
that RbCl would have beneficial effects on depression and cancers by modifying brain-gut-microbiota axis.

4. Conclusions

In summary, our results revealed RbCl significantly altered gut microbial composition. RbCl maintained the abundances of dominant bacteria. However, RbCl significantly altered the abundances of less richness microbes. Enrichment of *Clostridium Xle*Va, *Alistipes* and SRB could act on brain-gut-microbiota axis by affecting serotonergic system and immune system. It was likely that RbCl would have beneficial effects on depression and cancers by modifying brain-gut-microbiota axis. The shifts of gut microbial composition in this work may facilitate a better understanding of interaction between microbes and RbCl and provide theoretical basis for their roles in cancers and neurological diseases.

5. Methods

5.1 Experimental animals and experimental design

Three-week old male Swiss mice used as experimental animals (license number SCXK (Xiang) 2016-0002) were purchased and raised in the Laboratory Animal Science Department (LASD) of Central South University with Specific Pathogen Free (SPF) level environment. The living environment of the mice was of constant temperature (20 ± 2 °C), constant humidity (50 ± 10%), and free access to water and food. Mice were strictly controlled in normal biological rhythms and the light and dark environments were 12 h, respectively. All animal experiments in this study were approved by the Animal Breeding and Committee of the Department of Laboratory Animal Science of Central South University and were strictly evaluated in accordance with the Regulations on Animal Management of Central South University. The mice were kept in the LASD for a week without any treatment to adapt to the environment. Sixty-four mice were randomly assigned into four groups: one was blank control group which was intervened with normal saline (n = 13), and the other three groups were divided into low-dose (n = 17), middle-dose (n = 17), and high-dose group (n = 17) according to the different RbCl dosage (20, 50 and 100 mg/L, respectively). Five or four mice were randomly placed in each mouse cage. The mice of the above experimental groups were intragastrically administered of RbCl in 0.2 mL twice per day for 6 consecutive weeks. During this period, the mice were weighed weekly.

5.2 Fecal samples collection and properties analysis

After 6 weeks of drug treatments, the mice to be sampled were placed on a clean ultra-clean bench with sterile filter papers for taking stool samples. The fecal samples were collected into the sterile tubes immediately after defecation. The tubes were marked and frozen in a liquid nitrogen tank quickly. All mice were sacrificed by pentobarbital overdose in the ultra-clean workbench. The kidneys, heart, lungs, pancreatic, spleen, stomach and liver were rapidly excised from mouse and weighed.

5.3 Fecal DNA extraction and sequencing
Each fecal sample (approximately 0.2 g) was used for total gut microbiome DNA extraction with QIAGEN QIAamp kit. Extractions were performed according to specific operating instructions. The extracted total genomic DNA was detected by agarose gel electrophoresis and qualified DNA samples were used in subsequent experiments. PCR amplification and library preparation were performed using 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers to target the V4 region of the 16S rRNA gene. The PCR products for each sample were subjected to electrophoresis at a voltage of 100 V for about 1 h using a 2% agarose gel. The target band was recovered by tapping under UV light, and E.Z.N.A.TM Gel Purification Kit (OMEGA Bio-Tek Inc, USA) was used for product purification. The purified product was quantified using a Nanodrop spectrophotometer (ND-1000 spectrophotometer, Wilmington, USA). Illumina MiSeq (Illumina, San Diego, CA) sequencing required the library constructed from the mixture of 200 ng of each purified product.

5.4 Data processing and sequence analysis

The MiSeq sequencing data were analyzed using the Galaxy pipeline developed by Prof. Zhou's lab (http://zhoulab5.rccc.ou.edu/) at University of Oklahoma. The resulting sequences were further filtered based on quality score and sequence length. To merge the paired-end reads into full-length amplicon sequence, the FLASH software tool was used based on overlapping bases. The sequences were clustered into operational taxonomic units (OTU) at or above 97% identity. According to previous reports, OTUs reaching 97% similarity were used to analyze alpha diversity (Shannon and Simpson), and richness (Ace, Chao and Sobs) [118, 119].

5.5 Statistical analysis

IBM SPSS Statistics 19.0 software was used for statistical analysis. Since comparison was performed between two groups (saline and low, middle, high, respectively), Student T-test was applied for detecting significant differences in specific measured parameters. All values were expressed as the mean ± standard deviation (SD). Probability values of less than 0.05 were considered to show a statistical significance. Microbiota community diversity and richness were analyzed using vegan package and R software (version 3.5.1). LEFSe (Linear discriminant analysis effect size), CPCoA (constrained principal coordinate analysis) and Heatmap plot were performed on ehbio BioPharm platform (http://www.ehbio.com).

Abbreviations

Rb: rubidium; RbCl: rubidium chloride; CPCoA: constrained principal coordinate analysis; HFD: high fat diet; CRC: colorectal cancer; MDD: major depressive disorder; GC: gastric cancer; CCD: crohn's disease localized in the colon; UC: ulcerative colitis; AOM: azoxymethane; SRB: sulfate-reducing bacteria; H₂S: Hydrogen sulfide; CAC: colitis-associated colorectal cancer; SCFAs: short-chain fatty acids; GPCRs: G-protein coupled receptors; LGT: lateral gene transfer; 5-HT: serotonin; LASD: Laboratory Animal Science Department; SPF: Specific Pathogen Free; OUT: operational taxonomic units; LEFSe: Linear discriminant analysis effect size; ANOVA: one-way analyses of variance.
Declarations

Ethics approval and consent to participate

All experiments were approved by the Animal Breeding and Committee of the Department of Laboratory Animal Science of Central South University and were strictly evaluated in accordance with the Regulations on Animal Management of Central South University.

Consent for publication

Not applicable.

Availability of data and material

All sequence data were deposited into the NCBI Sequence Read Archive database (accession No. PRJNA630020). All the 16S rDNA sequences of 64 samples have been upload to NCBI database (SRA accession No. SRR11671062 - SRR11671125).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, H.Z. and Z.H.; methodology, Q.C. and Y.Z.; formal analysis, Q.C. and S.L.; investigation, Q.C.; resources, Z.H. and H.Z.; data curation, Q.C.; writing—original draft preparation, Q.C. and W.Y.; writing—review and editing, L.H. and H.Z.. All authors have read and approved the manuscript.

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References


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**Supplementary File**

Supplementary file 1 Venn analysis of shared and unique OTUs. (TIFF 2.77 MB)

Supplementary file 2 Heat map of gut bacteria at the genus level. (TIFF 4.69 MB)

Supplementary file 3 Significance analysis of intestinal archaea (class). (TIFF 6.13 MB)

Supplementary file 4 Significance analysis of intestinal archaea (order). (TIFF 6.13 MB)

Supplementary file 5 Significance analysis of intestinal archaea (family). (TIFF 6.13 MB)

Supplementary file 6 Significance analysis of intestinal archaea (genus). (TIFF 6.13 MB)

**Figures**
Figure 1

Effects of rubidium chloride on the mice characteristics. (a) Body weight. (b) Organ coefficients. Values are means ± SD; Control, n = 13; Low, n = 17; Medium, n = 17; High, n = 17. *P < 0.05, **P < 0.01, ***P < 0.001 when compared with control.
Figure 2
The diversity and richness of samples collected from 64 mice; (a) Sob's richness; (b) Ace's richness; (c) Chao's richness; (d) Shannon's diversity; (e) Simpson's diversity.

**Fig. 3**

(a) Variations of microbial communities in four groups. (a) Rarefaction curves of the samples; (b) Constrained PCoA plots of Bray-Curtis distances among the four groups.

**Figure 3**

Variations of microbial communities in four groups. (a) Rarefaction curves of the samples; (b) Constrained PCoA plots of Bray-Curtis distances among the four groups.
Figure 4

Altered composition in the gut microbes at the phylum level. (a) The relative abundances of each phylum in fecal samples of the four groups 6 weeks after treatments with different doses of RbCl or saline. (b) The abundances of Firmicutes were not significantly altered. (c) The abundances of Bacteroidetes showed no significant differences. (d) The abundances of Actinobacteria showed no significant differences. (e) The abundances of Tenericutes were significantly lower in RbCl groups (P < 0.05). Data are shown as mean±S.D. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 5

Significance analysis of intestinal archaea at the phylum level (a) The abundances of Crenarchaeota were significantly increased in medium-dose group (P < 0.05). (b) The abundances of Euryarchaeota showed no significant differences. Data are shown as mean±S.D. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 6

Altered composition in the gut microbiota at the class level. (a) The relative abundances of each class in fecal samples of the four groups 6 weeks after treatments with different doses of RbCl or saline. (b) The abundances of Deltaproteobacteria were significantly increased (P < 0.05). (c) The abundances of Mollicutes were significantly decreased (P < 0.05). Data are shown as mean±S.D.. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 7

Altered composition in the gut microbiota at the order level. (a) The relative abundances of each order in fecal samples of the four groups 6 weeks after treatments with different doses of RbCl or saline. (b) The abundances of Anaeroplasmatales were significantly decreased (P < 0.05). (c) The abundances of Desulfovibrionales were significantly increased (P < 0.05). Data are shown as mean±S.D.. *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 8

(a) Relative abundance (%)

(b) Anaeroplasmataceae

(c) Desulfovibrionaceae

(d) Rikenellaceae

Relative abundance (%)
Figure 8

Altered composition in the gut microbiota at the family level. (a) The relative abundances of each family in fecal samples of the four groups 6 weeks after treatments with different doses of RbCl or saline. (b) The abundances of Anaeroplasmataceae were significantly decreased (P < 0.05). (c) The abundances of Desulfovibrionaceae were significantly increased (P < 0.05). (d) The abundances of Rikenellaceae were significantly increased (P < 0.05). Data are shown as mean±S.D.. *P < 0.05, **P < 0.01, ***P < 0.001.
Fig. 9

(a) Relative abundance (%) of different bacteria across different conditions.

(b) Bacteroides

(c) Helicobacter

(d) Anaeroplasma

(e) Desulfovibrio

The graphs show the relative abundance of various bacteria across different conditions (Control, Low, Medium, High). The significance levels are indicated by asterisks: "*" for p < 0.05, "**" for p < 0.01, and "***" for p < 0.001.
Figure 9

Altered composition in the gut microbiota at the genus level. (a) The relative abundances of each genera in fecal samples of the four groups 6 weeks after treatments with different doses of RbCl or saline. (b) The abundances of Bacteroides were not significantly altered. (c) The abundances of Helicobacter showed no significant differences. (d) The abundances of Anaeroplasma were significantly decreased. (e) The abundances of Desulfovibrio showed significant differences. Data are shown as mean±S.D.. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 10

Significance analysis of gut bacteria at the genus level. (a) The abundances of Alistipes significantly increased. (b) The abundances of Clostridium XIVa were significantly higher in RbCl groups. Data are shown as mean±S.D.. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 11

LEfSe analysis of enriched bacterial taxa in gut microbiota between RbCl groups and control group. (a) Taxonomic representation of statistically and biologically consistent differences between RbCl and control mice. Significant differences were represented by different colors (red and green represented the enriched microbes in the RbCl and Saline treatment groups, respectively). (b) Histogram of the LDA scores for differentially abundant genera between the two treatment groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FS1VennanalysisofsharedanduniqueOTUs.tif
- FS2Heatmapofgutbacteriaatthelevel.tif
- FS3Relativeabundanceofintestinalarchaeaclass.tif
- FS4Relativeabundanceofintestinalarchaeagene.tif
- FS5Relativeabundanceofintestinalarchaeafamily.tif
- FS6Relativeabundanceofintestinalarchaeagenus.tif
- Additionalfile1ARRIVEGuidelinesChecklist.docx