N-(1-CARBAMOYL-2-PHENYLETHYL) BUTYRAMIDE (FBA), A BUTYRATE-RELEASING DERIVATIVE, IMPROVES ANTIBIOTIC-INDUCED INTESTINAL INJURY IN MICE

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Oral antibiotic therapy is commonly associated with a deep impairment of intestinal tract, since it promotes some alterations both in gut homeostasis and microbiota. Recently, short term changes in healthy gut microbiota, after a broad-spectrum antibiotics treatment, were addressed to long-lasting modifications (1). Dysbiosis in the gut composition may impair short-chain fatty acids (SCFAs) availability, mainly due to a decrease in butyrate-producing bacteria amount (1). Among SCFAs, butyrate plays a pivotal role as important energy source for colonocytes regulating inflammation, differentiation and apoptosis (2).

N-(1-carbamoyl-2-phenylethyl) butyramide (FBA), a butyrate-releasing derivative, has shown a pharmacological profile similar to that of butyrate, lacking butyrate unpleasant rancid taste. In our previous studies, we demonstrated that this butyrate-based compound reduced hepatic insulin resistance (3), colon inflammation (4) and pain perception (5).

The aim of this research was to evaluate the protective and anti-inflammatory effect of FBA in colon tissue using a mice model of antibiotic-associated intestinal injury (AIJ). The experimental approach consists in the oral administration of ceftriaxone (8 g/kg body weight) for 5 days, to induce systemic and colon inflammation (6). C57Bl/6 mice were divided into three groups 1) control group (CON) receiving vehicle (n=8); 2) AIJ group receiving ceftriaxone per os and killed at 5th or 15th day of the experimental period (n=16); 3) AIJ mice orally treated with FBA (212,5 mg/kg) for 5 or 15 days (AIJ+FBA) (n=16). Serum, colon tissue and faecal mass were collected at 5th and 15th day of the experimental period for following determinations.

The oral administration of ceftriaxone, despite its poor intestinal absorption, induced the unbalance between pro-inflammatory (TNF-α, IL-1β and IFN-γ) and anti-inflammatory mediators (IL-10) in serum. This systemic antibiotic alteration was counteracted by FBA treatment.

At intestinal level, AIJ group showed a marked colon inflammation associated to an increased TNF-α and COX-2 mRNAs compared to CON group, whereas FBA significantly reduced these parameters both at 5th and 15th day. We also analyzed the mRNA expression of the anti-inflammatory AnxA1 in the colon tissue. Interestingly, high levels of this pro-resolving factor in AIJ and AIJ+FBA were observed compared to those control animals. To assess one of the possible mechanisms by which butyrate exerts its anti-inflammatory effect, we showed FBA capability to modulate the inflammatory NFκB signaling pathway in colon tissue.

At 5th day, FBA reduced the translocation of NFκB p65 subunit in nucleus associated to a significant increase of IκB-α expression in cytoplasm. Since butyrate regulates gene expression epigenetically by inhibiting histone deacetylase (HDAC) (7), we also demonstrated that FBA was

able to significantly reduce the transcriptional levels of HDAC9, a specific member of class IIA HDAC, involved in colon inflammation, and to restore H3 histone acetylation.

Notably, FBA treatment modulated the expression of butyrate monocarboxylic acid transporter (MCT1), at colonic level. We found an increase in mRNA transcription of this carrier in FBA-treated mice, suggesting an increased butyrate uptake by colon, markedly reduced in AIJ group. Finally, we analyzed occludin gene transcription, a tight junction highly expressed in colon, whose levels were down-regulated in AIJ mice compared to CON, and restored by FBA treatment. These data suggest FBA potential in maintaining colon integrity, compromised in AIJ.

In conclusion, we showed the beneficial effect of FBA in reducing systemic and colon inflammation via the inhibition of NF-κB signaling pathway and restoring histone acetylation in a mice model on AIJ. All our findings indicate FBA as a postbiotic butyrate releasing derivative, in the therapy of AIJ.

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