# N-(1-carbamoyl-2-phenylethyl) butyramide (FBA), a butyrate-releasing derivative, reduces inflammation and restores gut microbiota alteration improving antibiotic-induced intestinal injury in mice

A. Lama[1], F. Di Guida[1], C. Pirozzi[1], M.P. Mollica[2], L. Coretti[3], F. Lembo[1], A. Calignano[1], R. Meli[1] and G. Mattace Raso[1]

[1]Dept. of Pharmacy and [2]Department of Biology, University of Naples Federico II; [3]Istituto di Endocrinologia ed Oncologia Sperimentale, IEOS, Consiglio Nazionale delle Ricerche CNR, Naples, Italy

Antibiotic-Associated Diarrhea (AAD), defined as diarrhea associated with the administration of oral antibiotics without another obvious cause, occurs in approximately 5%–25% of treated patients, varying with the class of antibiotics and patient risk factors. Only 15%–25% of AAD cases are due to the overgrowth of Clostridium difficile [1], while non–C. difficile AAD are associated with an alteration in the composition and quantity of gut microbiota [2], with a shift from eubiosis to severe dysbiosis and leading to loss of beneficial metabolic activities of the physiological colonic microbiota.
Short chain-fatty acids (SCFAs), and mainly butyrate, play a pivotal role as nutrients for the colonic epithelium, influencing indirectly the composition of gut microbiota [3-4]. The alteration of gut microbiota, and hence of butyrate-producing bacteria by antibiotic therapy might impair butyrate availability and luminal content, reducing its actions and beneficial effects.
N-(1-carbamoyl-2-phenylethyl) butyramide (FBA), a synthetic amide derivative of butyrate, has demonstrated a pharmacological profile similar to that of butyrate, with better efficacy without the typical unpleasant rancid odor and taste of butyrate. This compound constantly releases butyrate throughout the intestinal tract. In our previous studies, we demonstrated that this novel butyrate-based compound reduced hepatic inflammation, insulin resistance and pain perception [5-6].
The aim of this study was to evaluate the protective and anti-inflammatory effect of FBA in colon tissue and its capability to restore gut microbiota homeostasis in a mouse model of AAD. At the onset of the study, C57BL/6 mice were divided into three groups (n=8): 1) control group (CON); 2) AAD group receiving ceftriaxone (8 g/kg body weight via os) for 5 days; 3) AAD mice receiving FBA (212,5 mg/kg via os) for 15 days (AAD+FBA). Colon tissues and sera were collected for following analysis. Faeces of each animal were collected at 5 and 15 day of the experimental period.
The administration of antibiotic led to systemic inflammation, in fact AAD animals showed a marked increase of serum pro-inflammatory cytokines (TNF-Î±, IL-1β and IFN-γ). Moreover this group exhibited high levels of transaminases in serum, that were restored by FBA treatment. To confirm these data, we investigated inflammatory parameters in colon tissues. Consistently, AAD group presented higher levels of TNF-Î± and COX-2 mRNAs than CON, while FBA treatment significantly reduced these parameters. In addition, this compound increased anti-inflammatory cytokines, such as IL-10, confirming its beneficial effect. Moreover, in FBA-treated animals annexin A1 transcription was significantly reduced compared with AAD group, suggesting the resolution of inflammation. Notably FBA treatment was able to modulate the expression of its own transporter carrier at colonic level. Indeed, we found an increase in mRNAs transcript of monocarboxylate transporter 1, suggesting an increase in butyrate uptake by the tissue. Finally, we analyzed colon integrity through the evaluation of occludin gene transcription, that was up-regulated in FBA treated mice. Notably, FBA was also able to modulate the AAD-induced dysbiosis, leading to the repopulation of positive gut bacteria.
In conclusion, beyond the known beneficial activities of butyrate in gut, this study highlights novel pharmacological effect of FBA, modulating not only inflammation, but also the gut microbiota composition, suggesting this butyrate-based compound as potential therapy of AAD.

REFERENCES

[1] Bartlett (2002). N Engl J Med. 346, 334–339.
[2] Young et al (2004). J Clin Microbiol. 42, 1203–1206.
[3] Puertollano et al. (2014). Curr Opin Clin Nutr Metab Care. 17(2), 139-44.
[4] Berni Canani et al. (2011). World J Gastroenterol. 17(12), 1519-28.
[5] Mattace Raso et al. (2013). PLoS One. 8(7), e68626.
[6] Russo et al. (2015). Pharmacol Res. 103, 279-91.