ANTI-INFLAMMATORY PROPERTIES OF *L. CASEI* LOADED WHEY PROTEIN-ALGINATE MICROPARTICLES IN ANIMAL MODEL OF COLITIS

K. Smilkov[1], M. G. Dodov[2], J. Hadzieva[2], M. J. Pavlova[3], T. P. Ivanovska[2], T. Ristoski[4], I. Gjurovski[4], L. P. Tozi[2], K. Mladenovska[2]

[1]Faculty of Medical Sciences, University "Goce Delcev", Stip, Republic of Macedonia
[2]Faculty of Pharmacy, [3]Faculty of Medicine and [4]Faculty of Veterinary Medicine, University "Ss. Cyril and Methodius", Skopje, Republic of Macedonia

INTRODUCTION

Inflammatory bowel diseases are chronic conditions that affect large population. Regular administration of probiotics incorporated in pharmaceutical and/or functional food products may significantly prolong, delay or diminish occurrence of these diseases or serve as supplements to conventional drugs. The probiotic *L. casei* has proved its beneficial effects in improving acquired immunity, decreasing colon inflammation, serum cholesterol and increased blood pressure, improving lactose tolerance, controlling irritable bowel syndrome and decreasing risk of colon cancer [1]. However, during oral administration in the aggressive conditions of the GIT it is easily degraded. For these reason, microparticles composed of a probiotic carrier Ca-alginate and coating of whey protein were prepared to obtain particles with smaller size and high probiotic vability during processing, storage and GI transit and optimal charge for effective colonization in the lower parts of the GIT, especially in inflammatory conditions [2]. The aim of this study was to evaluate the anti-inflammatory properties of *L. casei* loaded in whey protein-alginate microparticles after oral administration to rats in which TNBS-colitis was induced.

EXPERIMENTAL

Preparation and characterization of microparticles

Emulsion technique was applied to aqueous dispersion of alginate (Protanal LF 10/60 LS, fG 35%-45%, IMCD, FMC Biopolymers, PA, USA) and *L. casei* (Chr. Hansen, Denmark), activated in MRS broth at 37°C, 24 h, under aerobic conditions, to obtain spherical particles, which were subsequently cross-linked in $CaCl_2$ solution and coated with whey protein isolate (ISO 100, Dynamatize Nutrition, TX, USA). Formed microparticles were isolated, washed and freeze-dried (-50°C, 0.07 mbar, 24h, Labconco, USA). To obtain optimal formulation, polynomial regression model at 2nd level was used, with three independent variables: concentrations of alginate, whey protein and $CaCl_2$. Optimal formulation was prepared of 2.5% *w/w* alginate, 3% *w/w* whey protein and 3% *w/w* $CaCl_2$. Spherical microparticles were obtained with d_{50} 8.65±1,02μm (Mastersizer Hydro 2000G, UK), zeta potential -28,04*mV* (Zetasizer Nano ZS, UK), Ca-content 3.76% (AES-ICP, Varian, CA) and high probiotic viability, 10.55±0.21 $\log_{10}$cfu/g after preparation of the microparticles, and 8.68±0.15 $\log_{10}$cfu/g in simulated *in vivo* GI conditions [2].

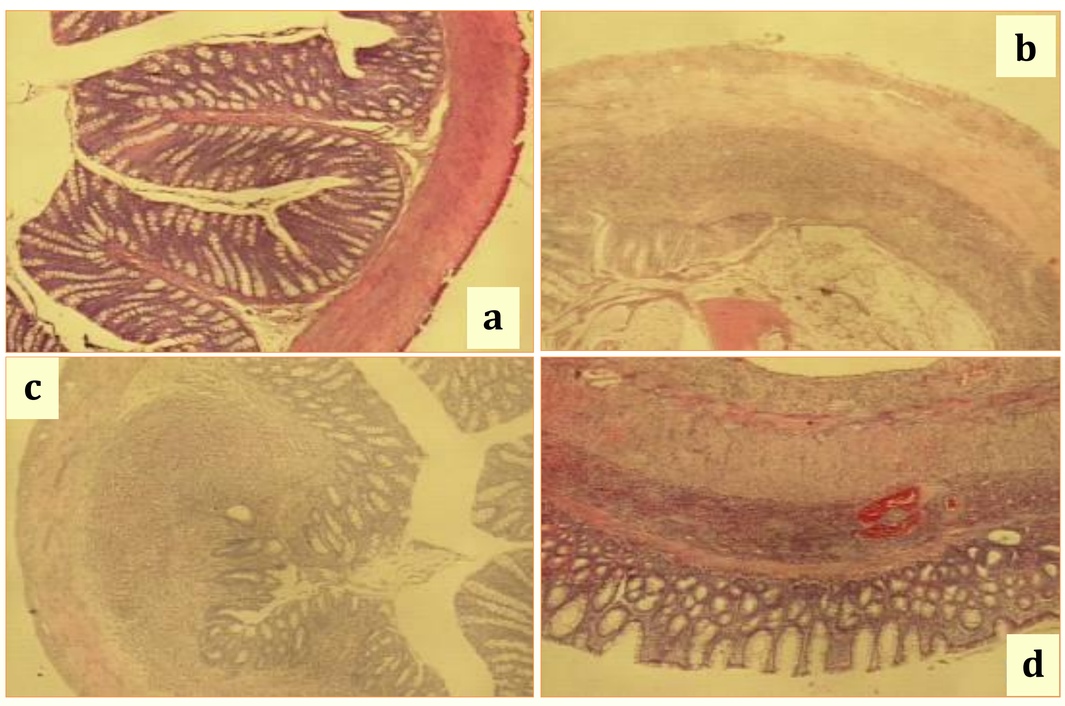

Figure 1. Histological sections of rat colons treated with a: PBS; b: TNBS in ethanol; c: non-encapsulated *L. casei*; d: microencapsulated *L. casei*.

Induction of colonic inflammation, experimental design and dosing

To two groups of Wistar rats (n=6, 200-260g, 12-15w.) in which TNBS colitis was induced, suspension of free and encapsulated probiotic respectively, was administered orally, once daily in amount of 8.7 $\log_{10}$cfu/g. To the third group, vehicle only was administered (0.25ml milk, positive control), while the forth group of rats (negative control) was treated with 0.25ml PBS, pH 6.8. The colitis was induced in the first three groups after 2 weeks of treatment, which continued for the next 6 days. After 24 hours starvation, 0.25ml TNBS in 50% ethanol (10mg/kg) were administered rectally, 8cm proximally from the anus. Rats were sacrificed after day 6 and the anti-inflammatory effect was evaluated in respect to the clinical activity/total damage score (quantified by loss on weight, consistency of faeces and rectal bleeding), macroscopic and pathohystological changes, colon weight/ body weight ratio and myeloperoxidase (MPO) activity [3].

RESULTS AND DISCUSSION

TNBS model appeared to show high correlation between the pathohystological, immunological and clinical features of the inflammation in IBDs. Compared to the positive control, the total damage score and colon weight/body weight ratio decreased when *L. casei* was administered, with non-significant difference when free and encapsulated cells were administered (23% and 31% for the total damage score, and 5% and 8% for the colon weight/body weight ratio, respectively). The activity of MPO was also decreased with the probiotic administration and the lowest value was observed when microparticulated probiotic was administered (Table 1). Macroscopic and histological evaluation confirmed the higher potential of the microencapsulated probiotic to decrease the parameters of inflammation (Fig. 1). Visible segments of ulcerations were not observed in this group, while at the histological sections subepithelial polymorph nuclear infiltration was observed with preserved epithelium. Also, dilated blood vessels in submucosal layer and dilated intestinal glands were observed (Fig. 1d).

Table 1. Anti-inflamatory effect of microencapsulated *L. casei*.

Parameter	Group			
	Negative control	Positive control	Non-encapsulated *L. casei*	Encapsulated *L. casei*
Colon weight/ total weight (mg/mg)	0.0067±0.0006	0.0076±0.0014	0.0072±0.0004	0.0070±0.0005
Activity of MPO (U/g)	8.23±3.32	48.43±8.68	38.97±6.73	18.15±5.10
Total damage score	0	2.6	2.0	1.8

CONCLUSION

In conclusion, the microparticluated *L. casei* showed higher potential to survive in the GIT and it can be used as adjuvant therapy in IBD when incorporated in pharmaceutical dosage form /functional food product.

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