Trans-10, *cis*-12 conjugated linoleic acid reverses actin polymerization of canine peripheral blood polymorphonucelar neutrophilic leukocytes attenuated by *Clostridium diffilice* toxin B exposure

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Purpose: The objective of this study was to examine whether *in vitro* treatment with *trans*-10, *cis*-12 conjugated linoleic acid (t10c12-CLA) modulates the filamentous actin (F-actin) polymerization, phagocytic capacity, and oxidative burst activity (OBA) of canine peripheral blood polymorphonuclear neutrophilic leukocytes (PMNs). We also examined the effect of *Clostridium difficile* toxin B (TcdB) on these PMN functions because TcdB inhibits Ras-homologous guanosine triphosphatas (Rho GTPases) known to play essential roles in neutrophil immune functions.

Materials and Methods: The actin polymerization was assayed by flow cytometry and confocal microscopy, an the phagocytic capacity and oxidative burst activity (OBA) were analyzed simultaneously by use of flow cytometry. The Rho GTPase Cdc42 activation level was determined by affinity precipitation using Cdc42 activation assay kit.

Results: Treatment with t10c12-CLA, but not linoleic acid, enhanced PMN F-actin polymerization, phagocytosis, and OBA, while TcdB suppressed these functions. t10c12-CLA reversed the suppressive effects of TcdB on these PMN functions. t10c12-CLA stimulated F-actin polymerization regardless of whether phagocytosis was stimulated by microspheres but only elevated OBA when microspheres were added. We asked whether the effects of t10c12-CLA were associated with changes in the activation of the Rho GTPase Cdc42. Treatment with t10c12-CLA augmented Cdc42 activity in both TcdB-treated and TcdB-naive PMNs during phagocytosis.

Conclusion: These results suggest that t10c12-CLA up-regulates PMN phagocytic responses that have been attenuated by TcdB. This effect is associated with an increase in actin polymerization and may involve the activation of Cdc42.

Key words: actin polymerization, canine, Cdc42, neutrophil, phagocytosis, Rho GTPase

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