The Effects of Carbamate Derivatives on Lipopolysaccharide-Induced Murine Macrophages

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Carbamates are known acetylcholinesterase inhibitors that have recently been found to act as anti-inflammatory agents. In vivo studies have shown that carbamate derivatives mitigate the inflammatory response in mice skin exposed to nitrogen mustard. As animal testing tends to be very costly and time-consuming, the purpose of the current study is to develop an in vitro model to determine the effects of these carbamate derivatives on lipopolysaccharide-induced RAW BlueTM macrophages. The carbamate derivatives are pro-drugs consisting of three components: an aromatic ester-carbamate linkage, a choline bioisostere, and indomethacin. RAW BlueTM macrophages are derived from RAW 264.7 murine macrophages and contain a reporter gene construct for secreted embryonic alkaline phosphatase (SEAP), which is inducible by NF-kB, a protein complex produced in the pro-inflammatory NF-kB pathway. RAW BlueTM macrophages (100,000 cells per well) were plated in triplicate in a 96 well plate either with or without lipopolysaccharide (2.5 µg/mL).

The carbamate compounds (120 µM) were added and the cells were then incubated for 24 hours at 37°C. After 24 hours, the supernatant was removed and plated with QUANTI-BlueTM, which turns blue in the presence of SEAP. The absorbance was measured at 650 nm after incubation at 37°C for 30 minutes, 1 hour, 2 hours, and 3 hours. The test compounds yielded lower absorbance values compared to the control, indicating a decreased inflammatory response. These results confirm the potential use of the carbamate pro-drugs as anti-inflammatory agents as well as the potential use of the SEAP assay as a rapid, cost-effective screening test for future compounds.