Serotonin Antagonists Inhibit Reactive Oxygen Species Generation in Polyurethane Seeded Monocyte Derived Macrophages

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Statement of Purpose: Inflammation is a normal host response to implanted biomaterials such as polyurethane (PU) and is characterized by the presence of activated platelets and macrophages. Macrophage release of reactive oxygen species damages the polyurethane surface and results in the failure of the implanted device. Serotonin (5-HT) is a well-studied neurotransmitter that is released from activated platelets at inflammatory sites. 5-HT mediated cell signaling via G-Protein coupled 5-HT receptors surface receptors can increase phagocytosis and reactive oxygen species (ROS) production in monocyte derived macrophages (MDM). Extracellular 5-HT is cleared by the serotonin transporter (SERT) whereupon it is metabolized by the mitochondrial enzyme monoamine oxidase (MAO). Hydrogen peroxide is a side-product of metabolized 5-HT. Serotonin reuptake inhibitors such as fluoxetine (Prozac) target SERT and prevent 5-HT metabolism.

Previously we reported a bulk modification of polyether polyurethanes (Tecothane TT 1074A, Thermedics, Waltham, MA) using a chemical reaction that involved bromo-alkylation of the urethane nitrogens followed by reactive binding of thiol-containing anti-oxidant di-tert-butylphenol (DBP), to the urethane nitrogen groups to hypothetically promote resistance to oxidative degradation. DBP modified PU was resistant to reactive oxygen species in an accelerated model of oxidative degradation involving exposure to $CoCl_2$ and hydrogen peroxide. In addition, recent *in vivo* testing, using an established rat subdermal implant model, showed DBP conferred resistance to oxidative degradation in a dose dependent manner. However, the antioxidant capacity of DBP is eventually expended and thus provides only a temporary barrier to oxidative degradation. Thus additional strategies are necessary to address MDM mediated oxidative degradation of polyurethanes.

We test herein the hypothesis that the 5-HT signaling pathway can be targeted to inhibit reactive oxygen species generation in polyurethane seeded MDMs.

Methods: The human MDM cell line, THP-1 (ATCC, Manassas VA), were stimulated with 1.6×10^{-7} M PMA. After 1 week, attached differentiated THP-1 cells were trypsinized, seeded (100,000 cells/well) and allowed to spread onto triplicate samples of PU films composed of the polyether polyurethane, Tecothane, inserted into the bottom of 96 well plates. Cells were incubated with 5 μM DHR-123 for 2 hours at 37°C and then washed 2X with PBS. Fresh media was added containing 10^{-7} M 5-HT and, where applicable, 10 μM of the following pharmacological inhibitors of 5-HT signaling pathway: fenfluramine (SERT inhibitor), fluoxetine (SERT inhibitor), pargyline (monoamine oxidase inhibitor), or the 5-HT2B/2A receptor antagonist SB206553. Where indicated, dose response studies of selected pharmacological agents were performed. ROS levels were determined by monitoring the fluorescence of rhodamine generated by the oxidation of DHR-123. Fluorescence was measured at 500 nm (excitation) and 536 nm (emission) using a Spectramax Gemini series spectrofluorometer (Molecular Devices, Sunnyvale CA). Background fluorescence was subtracted from all readings and data were expressed as arbitrary fluorescent units (AFUs).

Results/Discussion: At physiological concentrations of 5-HT (10^{-7}-10^{-9} M) ROS levels were 2-fold higher than at more concentrated levels of 5-HT (10^{-5} M). These data support the role of 5-HT as a scavenger of ROS at higher concentrations. Western blot analysis confirmed the presence of SERT in MDM cells. ROS production was measured as 21.8 ± 1.9 (mean ± standard error) in MDMs incubated with 5-HT. 5-HT induction of ROS production was reduced when SERT inhibitors were added. Fenfluramine treatment significantly reduced ROS production to 13.5 ± 0.6 (p=0.003) and Fluoxetine treatment reduced ROS to 5.4 ± 0.70 (p< 0.05). Interestingly the level of Fluoxetine inhibition of ROS production was significantly different from that of Fenfluramine (p=0.003). Pargyline added to PU seeded MDM cultures reduced 5-HT initiated ROS levels from 25.7 ± 3.4 AFU to 15.0 ± 4.4 AFU. The pargyline inhibition of ROS production was specific only for 5-HT treated cells and pargyline had no effect upon ROS levels in control cultures not treated with 5-HT. SB206553, an antagonist of the 5-HT receptor subtypes 2A and 2B, significantly reduced 5-HT mediated ROS production 31.6 ± 0.5 vs. 9.5 ±1.7 (p < 0.05).

Conclusions: These data suggest that there are several different mechanisms by which 5-HT can increase ROS levels in polyurethane seeded MDMs. In addition, pharmacological targeting of the 5-HT signaling pathway reduces ROS production in polyurethane seeded MDMs. These results may offer a pharmacological strategy for preventing macrophage mediated oxidative degradation of polyurethanes.