Macrophage expression of phospholipase A_2 is altered by polycarbonate-urethane surfaces.

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Background: Phospholipase A_2 (PLA_2) enzymes hydrolyze arachidonic acid (AA) from cell membranes, and are a key component of the inflammatory response with AA release being the rate limiting step in the production of inflammatory mediators. Previous studies demonstrated that polycarbonate-urethane (PCNU) surfaces activated PLA_2 enzymes (as demonstrated by ^{3}H-AA release from macrophages) as well as a role for PLA_2 enzymes in the mechanisms leading to macrophage-mediated PCNU degradation[1]. However, it has yet to be determined which PLA_2 isoforms are responsible. Inhibitor studies suggested that 2 of the 3 broad groups of PLA_2 enzymes were involved, these being secretory ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$)[1]. The current study assessed the intracellular expression of these enzymes in U937 cells in response to interaction with PCNUs.

Methods: Model PCNUs were synthesized with either 1,6-hexane diisocyanate (HDI) or methylene bisphenyl diisocyanate (MDI) with polycarbonate (PCN) and butanediol (BD) in stoichiometric ratios of HDI/PCN/BD 4:3:1 or 3:2:1 (referred to as HDI431 and HDI321 respectively) or MDI/PCN/BD 3:2:1 (MDI321). U937 cells, an established cell model for macrophage-mediated PCNU degradation[2], were differentiated to macrophage-like cells, resuspended and reseeded (2×10^6 cells/well in 24 well tissue culture plates) onto tissue culture grade polystyrene (PS – control non-degradable surface) and PCNU coated glass slips. Cell lysates were collected at 2 or 48 hrs post-reseeding, corresponding to an initial reseeding time point (2 hr) and a 48 hr time point previously established for measuring PCNU degradation[2]. SDS-PAGE gels were loaded with cell lysates and separated proteins were transferred to nitrocellulose membranes and subsequently blotted with antibodies to $cPLA_2$ and $sPLA_2$, autoradiographed and quantified for relative banding intensity.

Results and Discussion: The differences in intracellular protein expression for $cPLA_2$ and $sPLA_2$ in U937 cells was assessed for cells adherent to the different surfaces. Western blots of whole cell lysates showed that $cPLA_2$ expression increased at least 2-fold on each PCNU surface relative to PS (Figure 1). Although $cPLA_2$ expression at 48 hours demonstrated a clear increase on PCNU surfaces, quantification of Western blots revealed that only the $cPLA_2$ expression at the 2 hr time point was statistically significant on each PCNU surface ($p<0.05$)(Figure 2) relative to PS. $sPLA_2$ Western blots showed no clear differences for U937 cells adherent to PS versus PCNU surfaces at either 2 or 48 hours (Figure 1), which was also evident upon quantification of $sPLA_2$ protein bands (Figure 2). Since $cPLA_2$ expression was shown to significantly increase only at 2 hr post-adherence, further studies were pursued to detect the earliest increase of $cPLA_2$ expression. Assessing a 10–60 min post-reseeding time course, there was a small yet significant peak at 30 min post-reseeding (data not shown). This dual spike (30 min and 2 hr) in $cPLA_2$ expression coincides with previous studies that demonstrated 2 phases of AA release from stimulated cells[3]. $cPLA_2$ is the primary PLA_2 enzyme thought to be involved in AA release from phospholipid membranes[3], which possibly correlates with the dual increase in $cPLA_2$ but not $sPLA_2$ protein expression, (Figure 1) along with ^{3}H-AA release being stimulated by PCNU[1].

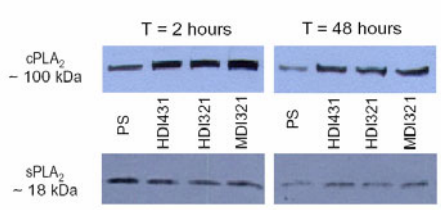

Figure 1 – Representative Western blots of intracellular $cPLA_2$ and $sPLA_2$ protein expression at 2 and 48 hours post-reseeding to PS (control) and PCNU surfaces.

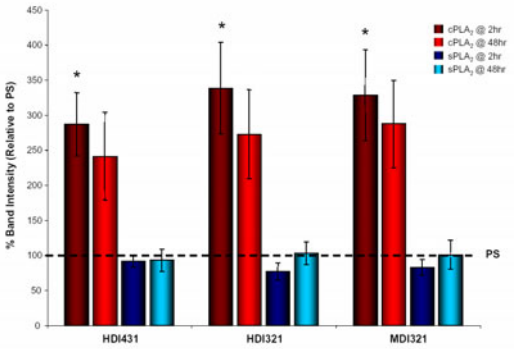

Figure 2 – Quantification of intracellular $cPLA_2$ (red) and $sPLA_2$ (blue) expression from PCNU adherent U937 cells relative to PS (---) at 2 hr (dark) and 48 hr (light). *$p<0.05$ - significant increase in banding intensity relative to PS.

Although these studies have shown increased macrophage $cPLA_2$ expression in response to PCNU surfaces, the effect of PCNU on $cPLA_2$ membrane localization (in order to elicit AA release) has not yet been established.

Conclusions: PCNU surfaces stimulate an increase in macrophage intracellular $cPLA_2$ but not $sPLA_2$ protein expression. $cPLA_2$ activation can result in the release of destructive mediators[3]. It is undetermined but likely that $cPLA_2$ can partially contribute to the mechanism of PCNU degradation, via inflammatory or signaling pathways. The current study has demonstrated that although PCNUs are relatively stable in comparison to other biomaterials, they can still elicit a strong inflammatory response that may be detrimental to the tissues surrounding the biomaterial site and the material itself.

References: 1) Dinnes DM et al (2005) Biomater. 26: 3881-9. 2) Matheson LA et al (2002) J Biomed Mater Res. 61: 505-13. 3) Shirai Y et al (2005) Biochim Biophys Acta 15:119-29.

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