EFFECT OF MATERIAL SURFACE CHEMISTRY ON THE PHOSPHOLIPASE A_2 PATHWAY IN HUMAN MACROPHAGES

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INTRODUCTION

Polyurethanes (PU)s are currently used in medical devices (such as ventricular assist devices, angioplasty balloons and pacemaker leads). Their use is widespread because of their relative biocompatibility, biostability and flexibility. Despite the widespread use of these PUs, the mechanism of action of their biodegradation *in vivo* is unknown [1]. *In vitro*, biodegradation of these PUs can be measured by incorporating a radiolabel (^{14}C) into the polymer structure.

The interaction of cells with a material surface has the potential to initiate several signaling pathways. The activation of the phospholipase A_2 (PLA_2) pathway occurs during the inflammatory response to biomaterials resulting in the release of arachidonic acid (AA). Previously, significant differences were shown in the release of AA from neutrophils, adherent to PUs and tissue culture grade polystyrene (PS) [2]. When inhibitors of PLA_2 were added to activated monocyte-derived macrophages (MDM), re-seeded on PUs, inhibition of biodegradation was observed [3]. In the present study, inhibitors of PLA_2 were assessed for their effects on biodegradation of PUs by U937s and MDM. U937s were characterized previously for use as a model cell line for MDM in the biodegradation of PUs [3]. The effect of material surface chemistry on AA release from U937s and MDM was also assessed. Determining cellular responses, what specifically in the material surface activates the cells, what signaling pathways are activated, and consequently, what is secreted by these cells is key in understanding and altering biostability and biocompatibility of polyurethanes.

MATERIALS AND METHODS

The model PU used for this study was a polycarbonate (PCN) based PU (PCNU). It was synthesized with a radiolabel incorporated into its structure with hexane diisocyanate (^{14}C-HDI), PCN and butanediol (BD) in a 4:3:1 ratio (referred to as HDI) as described in detail previously [4]. U937 cells were maintained as a promocytic cell suspension and then differentiated on tissue culture grade polystyrene (PS) with phorbol myristate acetate (PMA) for 72 hours. Monocytes were isolated from the whole blood of healthy human volunteers using a modification of Boyum as reported in detail previously [5]. The monocytes were then differentiated for 14 days on PS. In the PLA_2 inhibitor study, U937s were resuspended and re-seeded onto ^{14}C-HDI coated glass slips, MDM were trypsinized and then re-seeded. After adherence to the material surface for 1 hour in media only, the incubation was continued for 48 hours with and without the addition of PLA_2 inhibitors in media. Biodegradation was then assessed by measuring radiolabel release into the cell supernatant. The remaining adherent U937s were lysed and the DNA analyzed in order to normalize the radiolabel release to 10 μg DNA. A modification of the fluorometric assay of Labarca and Pajgen was used to determine the amount of DNA present in a 0.05% Triton X-100 cell lysate with Hoechst dye (number 33258), as described in detail previously [5]. All data were related to 10μg DNA which represented the number of cells that remained adherent to the material surface.

^{3}H-AA release assays were carried out by adding 0.10 μCi/mL of ^{3}H-AA to differentiating U937s or MDM at the last 24 hours of differentiation. Differentiation was then allowed to continue for an additional 24 hours. The cells were resuspended (U937s) or trypisinized (MDM) and washed with media to remove any unincorporated ^{3}H-AA. The cells were then re-seeded onto non-radiolabelled HDI and PS. The U937s or MDM were allowed to adhere to the different surfaces for different periods of time. At each timepoint, the medium was removed, centrifuged and ^{3}H-AA release into the supernatant measured and related to DNA as indicated above.

RESULTS

Previously, the PLA_2 pathway has been shown to be involved in the biodegradation of PUs [2,3]. One objective in this study was to find PLA_2 inhibitors that inhibit biodegradation which did not affect cell viability. Those successful were: bromophenacyl bromide (BPB), aristolochic acid (ARIST), palmityl trifluromethyl ketone (PMK) and quinacrine (QUIN) (Figure 1). Although the inhibitor with the greatest inhibition was QUIN (52.73%), it also greatly decreased cell viability. This effect on cell viability could be a contributing factor to the inhibition of radiolabel release. PMK had a minimal effect on cell viability and inhibited radiolabel release by 50.58%. ARIST and BPB had moderate effects on cell viability

with a radiolabel release inhibition of 30.29% and 45.05% respectively. The specificity of the PLA_2 inhibitors used are shown in Table 1.

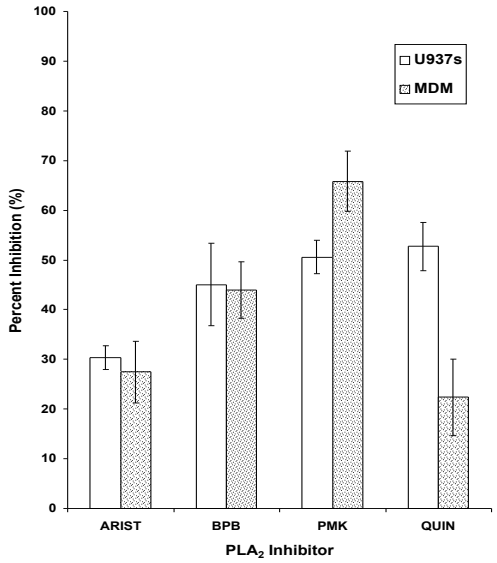

Figure 1 – Inhibition of radiolabel release by PLA_2 inhibitors in U937s and MDM. PLA_2 inhibitors aristolochic acid (ARIST), bromophenacyl bromide (BPB), palmityl triflurometh yl ketone (PMK) and quinacrine (QUIN) all significantly inhibited ($p < 0.05$) radiolabel release from ^{14}C-HDI in U937s (open bars) and MDM (grey bars) in comparison to media alone.

Table 1 – PLA_2 inhibitors used in this study with concentrations and specificity
($cPLA_2$-cytosolic, $sPLA_2$-secretory, $iPLA_2$-Ca^{2+}independent)

Inhibitor	Concentration	Specificity
Aristolochic acid	200 □M	$sPLA_2$
Bromophenacyl bromide	25 □M	$sPLA_2$
Palmityl triflurometh yl ketone	4 □M	$cPLA_2$, $iPLA_2$
Quinacrine	1 mM	$sPLA_2$, $cPLA_2$

^{3}H-AA release experiments were carried out by comparing HDI to the control surface of PS. Significantly more ^{3}H-AA was released from the U937s adherent to HDI than PS after 10 minutes of adherence to the material surfaces ($p<0.05$) (Figure 2). After the first 10 minutes, this difference in ^{3}H-AA release between the two surfaces was no longer apparent. ^{3}H-Radiolabel release on HDI peaked immediately after reseeding whereas ^{3}H-AA release from PS did not reach its maximum until 30 minutes. A similar significant increase in the release of ^{3}H-AA from HDI-adherent cells was also seen in MDM. Significantly more ^{3}H-AA was released from HDI-adherent MDM in comparison to PS-adherent MDM at 10 minutes after adherence to the material surfaces ($p< 0.05$) (Figure 3).

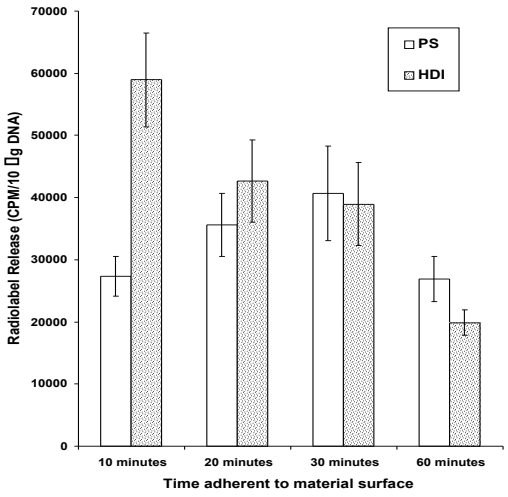

Figure 2 – Effect of material surface chemistry on ^{3}H-AA release in U937s. ^{3}H-AA release was significantly increased in HDI-adherent U937s (grey bars) as compared to PS-adherent U937s (open bars) after 10 minutes of adherence to the material surfaces. ^{3}H-AA release from PS-adherent U937s continued to increase until a peak at 30 minutes, whereas the release from HDI-adherent U937s decreased after the initial 10 minutes.

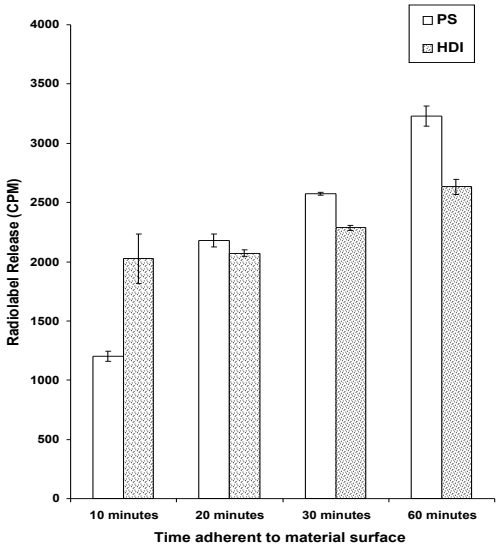

Figure 3 – Effect of material surface chemistry on ^{3}H-AA release from MDM. ^{3}H-AA release was significantly increased in HDI-adherent MDM (grey bars) as compared to PS-adherent MDM (open bars) 10 minutes after adherence to the material surfaces.

DISCUSSION

These results have confirmed previous studies that showed that PLA_2 was involved in the biodegradation of polyurethanes [2,3]. Both neutrophils and MDM were activated by the material surface chemistry, triggering the release of AA. The activation of PLA_2 pathways is one of the first cell signaling events in inflammation leading to the release of AA. AA itself or its metabolites may then go on to stimulate the secretion of the biological activities which cause biodegradation. It will be necessary to confirm that the radiolabel release measured was AA or its metabolites (Figure 2). It appears that more than one PLA_2 pathway may be activated by the material surface based on the specificities of the PLA_2 inhibitors involved (Table 1). By using the PLA_2 inhibitors, which inhibited radiolabel release, on AA release assays it may be possible to elucidate the activation pathways leading to biodegradation.

ACKNOWLEDGEMENT

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REFERENCES

1. Stokes M, *et al.*[1998] Biomater Appl, 9:321-354.
2. Labow RS, *et al.* [2001] J Cell Physiol, 186(1): 95-103.
3. Matheson LA, *et al.* [2002] J Biomed Mater Res, In Press.
4. Tang Yw, *et al.* [2001] J Biomed Mater Res, 57(4): 597-611.
5. Labow *et al.* [2000] J Biomed Mater Res, 54:189-197.