



## Novel Sphingolipid Biomarkers of Gut Injury Induced by Radiation

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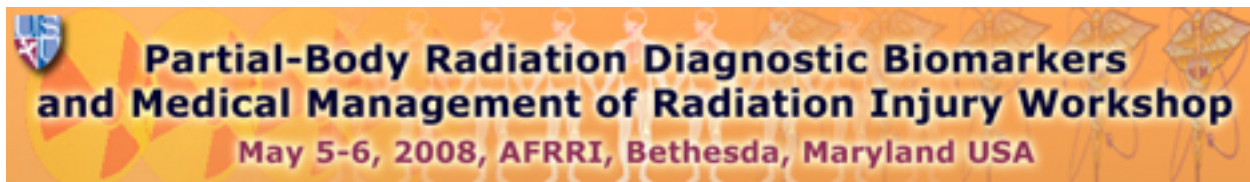
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Sphingolipid metabolites are ubiquitous regulators of the cellular response to stress<sup>1</sup>. Ceramide is a sphingolipid metabolite that accumulates in cells treated with radiation and in the serum of irradiated patients<sup>2-5</sup>. This is due in large part to the radiation-induced hydrolysis of sphingomyelin by sphingomyelinases (SMase) in the plasma membrane<sup>5-7</sup>. Ceramide clusters membrane rafts, induces apoptotic signaling cascades and contributes to epithelial and endothelial cell death in radiation-induced enteritis<sup>8,9</sup>. Reducing ceramide by blocking SMase attenuates radiation injury and mucositis in rodent models<sup>10</sup>. However, ceramide can be generated by more than one route. Further, inhibitors of SMase and enzymes of ceramide biosynthesis are not specific and have associated toxicities that may preclude their use in children, pregnant women, the elderly and in large populations where risk of radiation exposure may be uncertain. Thus, alternative or complementary approaches are warranted.

Ceramide can be further catabolized to sphingosine-1-phosphate (S1P), a bioactive lipid that acts through well-defined signal transduction pathways to promote proliferation and survival of many cell types, including endothelial cells, stem cells and enterocytes<sup>11-16</sup>. S1P signaling is also essential for angiogenesis and vascular maturation<sup>17-19</sup>. S1P antagonizes growth-inhibitory and apoptotic pathways including those induced by ceramide and radiation<sup>20-28</sup>. Importantly, S1P promotes cell survival in response to radiation and prevents radiation-induced oocyte apoptosis and sterility in mice<sup>29-34</sup>. Thus, while ceramide contributes to radiation enteritis, its metabolite S1P provides an internal fine-tuning signal limiting the intensity of radiation responses by acting as an angiogenic factor and radioprotectant.

S1P is irreversibly degraded by the enzyme S1P lyase (SPL)<sup>35</sup>. SPL is a ubiquitously expressed, intracellular enzyme, and its highest levels of expression and activity are found in intestinal villi, where it metabolizes dietary sphingolipids. It has been proposed that high SPL expression and corresponding low tissue S1P levels in intestinal epithelium facilitate the rapid cell turnover characteristic of this tissue. SPL expression is induced by DNA damage, and its increased expression and activity promote apoptosis in an S1P-dependent manner (i.e., S1P addition can reverse the effects of SPL)<sup>27</sup>. In contrast, SPL is downregulated in intestinal adenomas, leading to S1P elevation, which drives proliferation<sup>14,15</sup>. SPL can be considered to function as an anti-oncogene whose downregulation may contribute to tumorigenesis. Long-term S1P accumulation could be tumor-promoting and, thus, is undesirable. However, in the scenario of acute radiation injury, a short period of SPL inhibition and S1P accumulation could enhance the survival of endothelial cells and enterocytes, thereby promoting crypt restoration, angiogenesis and recovery.

Our current study aims to use small molecule inhibitors to block (murine) SPL and raise circulating and tissue S1P levels for radioprotection. However, we have made the serendipitous observation that gut SPL activity rises on day 4 after 15 Gy TBI and, at the corresponding time point, S1P levels in blood plasma fall in a dose-dependent manner, with a reduction of 10% at 8 Gy, 24% at 10 Gy and 50% at 15 Gy. It is likely that intestinal SPL activation may be the explanation for reduced circulating S1P after radiation exposure. However, multiple tissues and cell types including erythrocytes, platelets and endothelium are now recognized as contributors to circulating S1P levels; in the case of endothelium, changes in endothelial SPL expression in response to shear stress produce alterations in circulating S1P<sup>36-39</sup>. This finding suggests the possibility that plasma S1P levels could be an indicator of radiation-induced changes throughout the body and in different vascular beds. Based on these observations and the knowledge that



circulating S1P levels can be reliably determined in blood plasma using tandem MS, HPLC and TLC methods, we hypothesize that S1P and possibly other sphingolipid metabolites may serve as useful biomarkers for radiation exposure. To adequately assess the utility of plasma S1P as a radiation biomarker, future studies will need to address remaining gaps in our current understanding of S1P regulation, such as potential effects of fasting, stress and other factors on circulating S1P levels, degree of correlation between TBI dose and S1P levels, and effects of partial body exposures and/or partial body shielding on circulating S1P levels. Additionally, development of rapid and simple methods for quantification of S1P in blood, such as by an ELISA system using the S1P monoclonal antibody, would be advantageous<sup>40</sup>.

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