Nitrogen Mustard Targets Pathways for Tetrahydrobiopterin Biosynthesis in Human Keratinocytes

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Nitrogen mustard, mechlorethamine (bis(2-chloroethyl)methylamine; HN2), and sulfur mustard are potent vesicants that can modify cellular macromolecules and disrupt metabolism. Important in many metabolic processes including endothelial function, cell growth regulation, neuropathic pain, oxidative stress, immunity and inflammation, is tetrahydrobiopterin (BH4, 6R-L-erythro-5,6,7,8-tetrahydrobiopterin) a key cofactor for nitric-oxide synthases as well as aromatic amino acid hydroxylases important in synthesizing catecholamines, neurotransmitters, and indoleamines. We have discovered that HN2 targets the BH4 biosynthetic pathway in HaCaT cells, a human keratinocyte cell line. BH4 is generated in HaCaT cells from dihydrobiopterin (BH2) via the enzyme NADPH sepiapterin reductase using sepiapterin as a substrate. Once formed from sepiapterin, BH2 is converted to BH4 by various intracellular reductases including dihydrofolic acid reductase. Treatment of HaCaT cells with HN2 for 1-4 hr was found to cause a concentration-dependent inhibition of intracellular biosynthesis of BH2 and BH4 (IC_{50} = 28.5 μM) without affecting cell viability. Inhibition of BH4 biosynthesis was not due to inhibition of the reduction of BH2. Thus, HaCaT cells pretreated with HN2 (10-160 μM) readily synthesized BH4 when incubated with BH2. In cell lysates, incubation with sepiapterin (200 μM) readily generated BH2. Treatment of cell lysates with HN2 caused a concentration-dependent inhibition of BH2 formation (IC_{50} = 31.6 μM) indicating that the vesicant was targeting NADPH sepiapterin reductase. Using human recombinant NADPH sepiapterin reductase, we found that HN2 caused a time- and concentration-dependent inhibition of enzyme activity (IC_{50} = 74.6 μM). Taken together, our data demonstrate that HN2 targets BH2/BH4 biosynthesis in human keratinocytes by targeting NADPH sepiapterin reductase. Overcoming inhibition of this enzyme by HN2 will be important in ameliorating toxicity of HN2 associated with enzymes that are dependent on BH4. *Supported by NIH grants AR055073 and ES005022.*