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## Thymine Analogue<sup>1</sup>

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### Abstract

Eosinophils use eosinophil peroxidase, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and bromide ion (Br<sup>-</sup>) to generate hypobromous acid (HOBr), a brominating intermediate. This potent oxidant may play a

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role in host defenses against invading parasites and eosinophil-mediated tissue damage. In this study, we explore the possibility that HOBr generated by eosinophil peroxidase might oxidize nucleic acids. When we exposed uracil, uridine, or deoxyuridine to reagent HOBr, each reaction mixture yielded a single major oxidation product that comigrated on reversed-phase HPLC with the corresponding authentic brominated pyrimidine. The eosinophil peroxidase-H<sub>2</sub>O<sub>2</sub>-Br system also converted uracil into a single major oxidation product, and the yield was near-quantitative. Mass spectrometry, HPLC, UV-visible spectroscopy, and NMR spectroscopy identified the product as 5-bromouracil. Eosinophil peroxidase required H<sub>2</sub>O<sub>2</sub> and Br<sup>-</sup> to produce 5-bromouracil, implicating HOBr as an intermediate in the reaction. Primary and secondary bromamines also brominated uracil, suggesting that long-lived bromamines also might be physiologically relevant brominating intermediates. Human eosinophils used the eosinophil peroxidase-H<sub>2</sub>O<sub>2</sub>-Br system to oxidize uracil. The product was identified as 5-bromouracil by mass spectrometry, HPLC, and UV-visible spectroscopy. Collectively, these results indicate that HOBr generated by eosinophil peroxidase oxidizes uracil to 5-bromouracil. Thymidine phosphorylase, a pyrimidine salvage enzyme, transforms 5-bromouracil to 5-bromodeoxyuridine, a mutagenic analogue of thymidine. These findings raise the possibility that halogenated nucleobases generated by eosinophil peroxidase exert cytotoxic and mutagenic effects at eosinophil-rich sites of inflammation.

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