Effect of Dietary Supplements of N-Methyl-N-Nitrosourea Mediated Guanine Methylation

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Abstract

Stomach cancer is estimated to affect 27,510 patients per year with a fatality rate of over 40%. Due to these numbers, a therapeutic lead is needed to prevent the onset of stomach cancer by targeting its causes. One such cause is the consumption of nitrates. N-methyl-N-nitrosourea (NMU) is formed *in situ* in the gut after the intake of foods containing nitrate preservatives. Due to the low pH in the stomach, NMU may decompose into to a methyl diazonium salt (Scheme 2) that specifically alkylates guanine at the O-6 position (Scheme 3). The alkylation of guanine can lead to mispairing mutations linked to tumorigenesis. NMU was successfully synthesized from the reaction of N-methyl urea with various nitrating agents. Reaction conditions were developed to mimic the alkylation of guanine *in vitro*. The effect of different dietary supplements, vitamins, and flavonoids on the relative amounts of alkylated guanine and non-alkylated guanine will be determined. These data will provide a lead compound that will form the basis for the design and synthesis of novel compounds for inhibiting guanine methylation.

Research Question

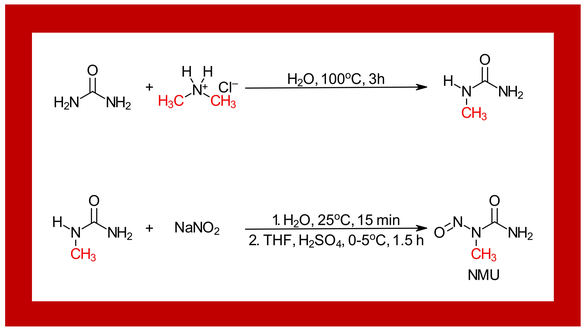
Following the results from previous studies showing the inhibition of NMU formation *in vivo* by Vitamin C and E, we are searching for lead compounds from flavonoids to prevent the alkylation of guanine.

Methods

Alkylation of Guanine: An equal molar ratio of guanine and NMU were weighed and added to 1ml of a 0.1M NaCl solution in a micro reaction kit. The reaction was conducted under reflux conditions with stirring for 24 hours.
Preparation of Guanine for HPLC: 600µl of reaction solution was added to 300µl of ethanol and 10µl of NaOAc (3M). The solution was centrifuged at max speed for 10 minutes and the supernatant was discarded. The guanine was dissolved in 100% methanol and transferred to HPLC vials.
Inhibition of alkylation using Vitamin C: Guanine, NMU, and vitamin C were all weighed at 1:1:1 molar ratio and underwent the same reaction conditions for 24 hours. The reaction sample was centrifuged and dissolved in 100% methanol for HPLC. Inhibition of alkylation was shown through the overlay of a guanine standard signal and an alkylated guanine standard signal shown in **Figure 1**.

Results

- N-methyl-N-nitrosourea (NMU) synthesized
- HPLC methods developed for analysis
- Upon exposure of guanine to NMU (Scheme 2)
  - Vitamin C methylation ↓ (**Figure 1**)
  - Vitamin E methylation ↓ (**Figure 2**)
  - Quercetin no effect
- Standard curve produced for 6-O-methylguanine signal on LC-MS (**Figure 3**)



Scheme 1. Laboratory synthesis of N-methyl-N-nitrosourea starting from urea and dimethyl amine hydrochloride

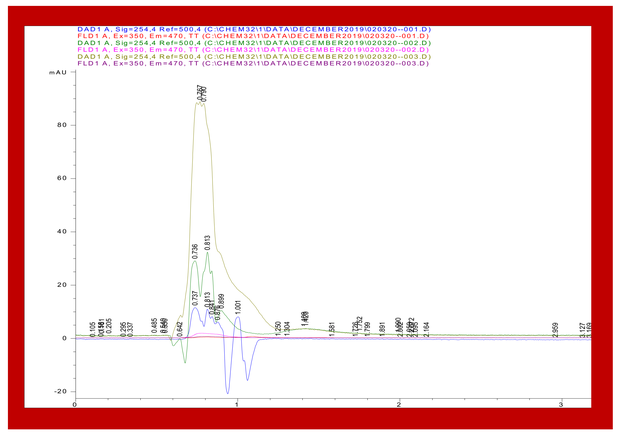


Figure 1. HPLC peaks of alkylated guanine standard (brown), guanine standard (blue), and guanine with Vitamin C (green).

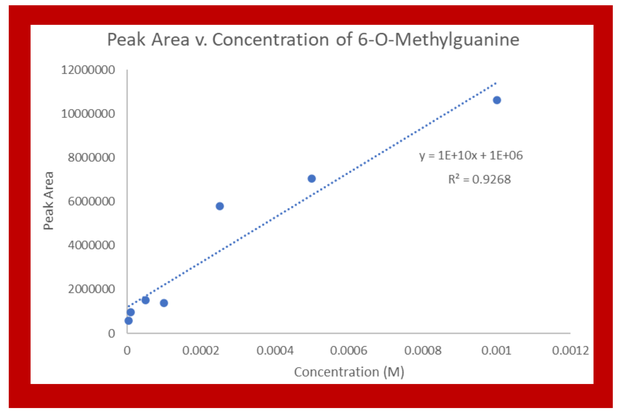
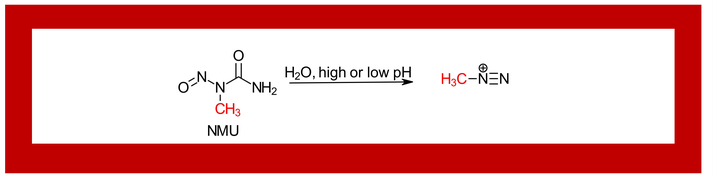
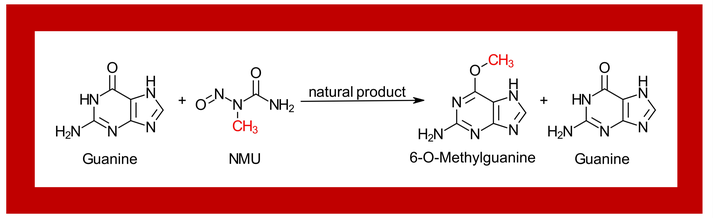


Figure 3. Standard curve of 6-O-methylguanine on HPLC-MS.



Scheme 2. Decomposition of NMU to the active alkylating agent, methyl diazonium salt.



Scheme 3. Methylation of guanine by NMU in presence of natural products to produce 6-O-Methylguanine and un-alkylated guanine

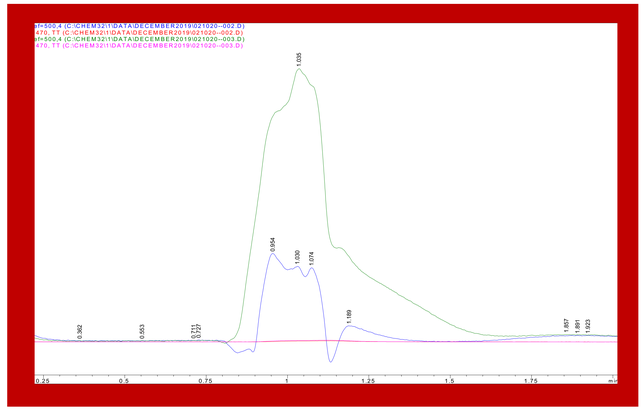
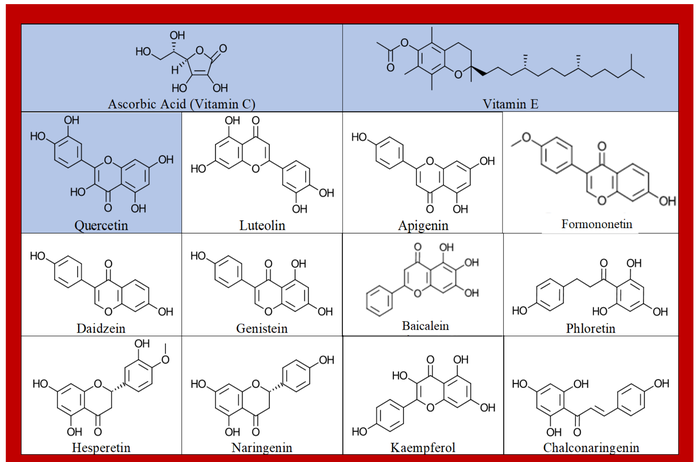


Figure 2. HPLC signals of alkylated guanine standard (brown) overlaid with vitamin E standard (blue)



Selected commercially available antioxidants and structures (already tested in blue)

Future Work

- Optimize the alkylation reaction (Scheme 3) to eliminate exotherm:
  - Concentration of reagents
  - Reaction scale
  - Temperature of reaction
- Transition HPLC method to HPLC-MS
- Produce a standard curve of non-alkylated guanine signal on HPLC-MS
- Screen all flavonoids for their effect of guanine methylation
- Produce relative amount of inhibition for each antioxidant using fraction of alkylation equation:

$$\text{FA} = \frac{6\text{-O-methylguanine}}{\text{guanine}}$$

- Determine the most effective concentration of antioxidant for inhibition of guanine alkylation
- Design lead compound for alkylation inhibition using QSAR model
- Design and perform a synthesis for the lead compound

Acknowledgments and References

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