Mapping of the DNA adductome to study the genotoxic effects of red meat consumption

L.Y. Hemeryck, C. Rombouts, L. Van Meulebroek, J. Vanden Bussche and L. Vanhaecke

Lieselot Hemeryck

Laboratory of Chemical Analysis
Dpt. of Veterinary Public Health and Food Safety
Ghent University, Belgium

http://www.vvv.ugent.be

NuGO week 2015

Barcelona, Spain
Worldwide cancer incidence

Globocan 2012 (IARC)
Colorectal cancer risk factors

≡ 10 % genetic
≡ 90 % non-genetic

– Tobacco
– Alcohol
– Unhealthy diet
– Physical inactivity
– …

Willett, Environ. Health Perspect., 1995
Globocan 2012 (IARC)

Colorectal cancer incidence rates per 100,000 men/women worldwide
Colorectal cancer risk factors

Western-type diet  80%  Unhealthy diet

Willett, Environ. Health Perspect., 1995
Epidemiological data

Increased risk of 29% with 100 g / day

Increased risk of 21% with 50 g / day

WCRF, 2007
Red meat and colorectal cancer hypothesis

Haem!

Myoglobin

Oostindjer *et al.*, Meat Science, 2014
Red meat vs. colon cancer: role of GI red meat digestion?

Meat with haem pass through GIT and stimulate formation of:

1. Lipid peroxidation products (LPOs)
2. N-Nitroso compounds (NOCs)
Red meat vs. colon cancer: role of GI red meat digestion?

NOCs and LPOs

DNA damage → DNA adducts

Mutations

K-RAS, p53
Experimental setup

1. *In vitro digestion* of red (vs. white) meat
2. Mapping of DNA adducts by means of **UHPLC-HRMS** in meat digests

**DNA adduct?**

- When a chemical covalently binds to a DNA nucleobase
- First step in chemically induced carcinogenesis
- Different types:
  - By NOCs
  - By LPOs
  - And many others...
**In vitro digestion of meat**

**Grinding and cooking**

**STATIC IN VITRO DIGESTION (37°C)**

- **Stomach (2h)**
- **Small intestine (4h)**
- **Large intestine/colon (72h)**

**Gastro-intestinal juices**

**Colonic microbiota of volunteers**
DNA adduct analysis: DNA adductome mapping

1. Sample preparation:
   a. Acid DNA hydrolysis (0.1 M HCl)
   b. Solid Phase Extraction

2. UHPLC-HRMS analysis
   a. Targeted (“profiling”)
   b. Untargeted (“fingerprinting”)

3. Data processing
   a. ToxID™
   b. SPSS®
   c. Sieve™
   d. Simca™
Results (1):

• What?
  • Digestion of beef
  • Colonic digestion by means of the colonic microbiota obtained from 5 different volunteers
  • Increase, decrease or shift during colonic digestion?

• How?
  • ToxID™ data processing based on $m/z$ of known diet-related DNA-adducts

- N.D.
- Rise in DNA adduct levels:
  DNA adduct formation
- Decrease in DNA adduct levels:
  DNA adduct degradation or dilution
- *(*)(*)(* isomers
Results (2):

• **What?**
  • Digestion of beef vs. chicken
  • + vs. with or without added calcium
  • Microbiota of 2 volunteers:
    • P1 & P2
    • 3 biological replicates
  • Increase, decrease or shift?

• **How?**
  ❖ ToxID™ data processing

- N.D.
- Beef (B) > Chicken (C) OR beef/chicken without added calcium > with added calcium (+Ca)
- Chicken (C) > Beef (B) OR beef/chicken with added calcium (+Ca) > without added calcium
- *(*)*(*)*(*) isomers
Results (3):

• What?
  • Comparison of digestion of beef vs. chicken
  • + vs. with or without added calcium
  • 2 volunteers
    • P1 & P2
    • 3 biological replicates
  • Discriminative DNA adducts?

• How?
  ❖ Sieve™:
    *spectral data processing*
  ❖ Simca™:
    *OPLS discriminant analysis*
Most prominent findings

• Colonic microbiota
  • Active production/formation of (genotoxic) molecules

• DNA adduct profiling
  • Several DNA adduct types significantly higher or lower in different meat preparations...
    • ...

• Discriminant analysis
  • ...


The International congress of Prebiotics & Probiotics in Pediatrics 2016, organized by Prof. Y. Vandenplas, will take place at "The Pand" in Ghent from April 28 until April 30, 2016. The program will cover all major topics related to prebiotics, probiotics, microbiota and gut health. Many key opinion leaders are invited and confirmed their participation.

The scientific program highlights the current advances in the research, production and use of probiotics and prebiotics in children, with particular focus on their role in maintaining health and preventing diseases. The major goal is to provide a scientific forum for stakeholders and to enable the interactive exchange of state-of-the-art knowledge. Ghent is a beautiful city, just 50 km away from Brussels, easy to reach from the national airport (direct train connection every half hour), and offers a unique mixture of medieval and modern architecture. The symposium will be held in the heart of the city, at a distance of only 30 km from Bruges.

You will hear about the novel clinically-relevant studies emphasizing the importance of pro/prebiotics for new indications. Subscribe before January 31, 2016 (end early bird registration) and join us in Ghent, Belgium.

Check out the Scientific program and other important information on the website!

Date: April 28-30, 2016

MORE INFORMATION:
Find out more about this event by visiting our website: www.PreProPed2016.be

Subscribe to this symposium before January 31, 2016 and join us in Belgium!

www.PreProPed2016.be

Thank you!
www.vvv.ugent.be