Calcium and tocopherol suppress cured-meat promotion of colon carcinogenesis in rats

A volunteer study also suggests protection in humans


Heme-Cancer, ANR Project 2006-2010

Nutrition and cancer
Hot topics from biology to public health issues

20 juin 2013

Team E9: Prevention and promotion of carcinogenesis by food
Background

- **Colorectal cancer**: A major cause of mortality in western countries
  - Environmental factors, particularly food

- **Epidemiological studies**: Association between colorectal cancer and fresh red meat & cured-meat intake

<table>
<thead>
<tr>
<th>RR (For 100 g/day)</th>
<th>Red meat</th>
<th>Processed meat (For 50 g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al., 2011</td>
<td>1.17 (1.05 - 1.31)</td>
<td>1.18 (1.05 – 1.28)</td>
</tr>
<tr>
<td>Xu et al., 2012</td>
<td>1.36 (1.17 - 1.58)</td>
<td>1.28 (1.03 - 1.60)</td>
</tr>
</tbody>
</table>

**WCRF recommendations** (2007-2010): **limit** fresh red meat & **avoid** cured-meat intake

- **Challenge** for scientists to find prevention strategies and for industries to produce safer cured-meat

Reduce colorectal cancer risk
Heme: a major factor

- Carcinogenesis studies in rats

Mecanism proposed

Heme → CATALYSIS → Lipoperoxidation → Aldehydes → Carcinogenesis steps

Pierre et al., 2003

a: significantly different from control diet without heme
Evidence of aldehydes role

- **Mechanistic studies**
  
  Bastide et al., in preparation
  
  Addition of polymer beads
  
  To bind aldehydes

- **Prevention studies**

  Pierre et al., 2003

Rats given heme

Fecal water

TBARs Lipoperoxidation biomarker

TBARs (eq. MDAµM in FW)

Before treatment

After treatment

Cytotoxicity (% of dead cells)

Before treatment

After treatment

No direct effect of calcium on colorectal carcinogenesis

Major role of aldehydes in promotion by heme
Fresh red meat studies

- **Promotion by beef**

  - Pierre et al., 2004

- **Prevention by calcium**

  - Pierre et al., 2008

**TBARs**

Lipid peroxidation biomarker

**Cytotoxicity**

\[ a, b, c \] are significantly different
Cured meat studies

- Carcinogenesis studies in rats

Model of cured meat = DCNO

High heme, Cooked, Nitrite treated and Oxidized meat

\[ \text{Carcinogenesis studies in rats} \]

\[ \text{Model of cured meat} = \text{DCNO} \]

\[ \text{High heme, Cooked, Nitrite treated and Oxidized meat} \]

\[ \text{DCNO} \]

\[ \text{Cytotoxicity} \]

\[ \text{Heme} \]

\[ \text{Lipoperoxidation} \]

\[ \text{Nitrosation} \]

\[ \text{ATNC} \]

\[ \text{Apparent total N-nitroso compounds} \]

\[ \text{TBARs} \]

\[ \text{ThioBarbituric Reactive Substances} \]

\[ \text{Mean +/- SEM} \]

\[ a: \text{significantly different from control diet without heme} \]
Objectives & studies design

**First study**
Rats, short-term study

DCNO ± 5 compounds: Calcium Carbonate, Inulin, Rutin, Carnosin, α-tocopherol

**Second study**
Rats, carcinogenesis study

**OBJECTIVE 1**
Calcium & Tocopherol = Diminution of

Mucin-depleted foci: Precancer lesions

<table>
<thead>
<tr>
<th>DCNO</th>
<th>DCNO + calcium carbonate</th>
<th>DCNO + α-tocopherol</th>
</tr>
</thead>
</table>

Fecal biomarkers

**Third study**
Healthy volunteers

**OBJECTIVE 2**
DCNO ➔ Increase of

Fecal biomarkers

**OBJECTIVE 3**
Calcium & Tocopherol = Diminution of

Fecal biomarkers

Anti-peroxidation or anti-nitrosation properties

OBJECTIVES:
- Anti-peroxidation or anti-nitrosation properties
- Diminution of Mucin-depleted foci: Precancer lesions
- Diminution of Fecal biomarkers
Carcinogenesis study in rats

**Design**

Dimethylhydrazine initiation

- 1 week
- 100 days

Adaptation

- DCNO
- DCNO + calcium carbonate
- DCNO + α-tocopherol

**Results**

- Mucin-depleted foci: Precancer lesions
- Fecal biomarkers

Mean +/- SEM, Dunnett's test

- a: significantly different from DCNO

**Graphs:**

- TBARs (μM MDA eq.) Lipoperoxidation biomarker
- ATNC (μmol/g) Nitrosation biomarker
Carcinogenesis study in rats

**Objective 1**: Done!

- **Calcium carbonate** and **α-tocopherol** suppress DCNO promotion of carcinogenesis & production of nitrosated compounds in the gut

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**Carcinogenesis steps**

- Initiation
- Promotion
- Progression

**Tissue samples**

- Normal crypt
- Aberrant crypt
- Aberrant crypt focus (ACF)
- Adenoma
- Adenocarcinoma

**Biomarkers**

- **TBARs (µM MDA eq.)** (Lipoperoxidation biomarker)
- **ATNC (µmol/g)** (Nitrosation biomarker)

**Mean ± SEM, Dunnett**

- DCNO: Precancer lesions
- DCNO+CaCO₃: Lipoperoxidation biomarker
- DCNO+Toco: Nitrosation biomarker

**Calcium carbonate** and **α-tocopherol** significantly suppress DCNO promotion of carcinogenesis.
Human volunteer study

- **Design**
  Cross-over study
  16 healthy volunteers

  - **Design**
    - **Cross-over study**
      - **16 healthy volunteers**

  - **Results**

    - **Objective 2**: Done!
      - DCNO increased lipoperoxidation and nitrosation in humans

    - **Objective 3**: Done!
      - Calcium decreased lipoperoxidation and nitrosation
      - Tocopherol only decreased lipoperoxidation

    - **Results**

      - **TBARs (µM MDA eq.)**
        - Lipoperoxidation biomarker

      - **ATNC(µmol/g)**
        - Nitrosation biomarker

      - **Mean +/- SEM, Wilcoxon**

      - a: significantly different from DCNO

      - **Fecal biomarkers**

      - **DCNO**
      - **DCNO + calcium carbonate**
      - **DCNO + α-tocopherol**
Prevention by calcium

- **Human results**

  - **TBARs (µM MDA eq.)**
    - Lipoperoxidation biomarker
  
  - **ATNC(µmol/g)**
    - Nitrosation biomarker

- **Rat results**

  - **TBARs (µM MDA eq.)**
    - Lipoperoxidation biomarker
  
  - **ATNC(µmol/g)**
    - Nitrosation biomarker
  
  - **Precancer lesions**

Mean +/- SEM,
Wilcoxon for human study
Dunnett for rat study

a : significantly different from DCNO
Conclusion

1. First demonstration that a model of cured meat increased fecal biomarkers in healthy volunteers

2. \(\alpha\)-tocopherol & calcium carbonate decreased precancer lesions in rats

3. Nitrosation is a probable cause of cured-meat associated carcinogenesis

4. Calcium carbonate suppresses cancer-associated changes in rats and in volunteers

Calcium: Protective agent \(\rightarrow\) Protection of colorectal carcinogenesis by dietary products?

But chelation of heme \(\rightarrow\) Nutritional issues (Anemia)

In search for other protective agents...
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