"Interaction of Probiotics and Mycotoxins - Benefits to Human Health"

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Probiotics

- Definition
  Living microorganisms when administered in adequate amounts confer health benefits on the host

Dr. Minoni Shirota
First developed *L. casei* Sirota
as a commercial product in 1935
Probiotics – Daily example

1. Fermented Milk Products

Milk
Lassi
Smoothie

Cheese

Frozen yogurt
Ice cream

Valio Gefilus® 20 years of probiotic dairy products in Europe
Probiotics - Daily example

2. Food & Drinks
- Candy & granola bars
- Mint candies
- Cookies
- Cereal
- Infant formula

3. Pharmaceutical products
- Probiotics supplement
  In form of: Tablets, Capsules, Granules
  Single or Cocktail of species
  Encapsulation help protect probiotics from:
  - Air & moisture (esp. anaerobic species)
  - Stomach acid

Dosage:
$10^9 - 10^{10}$ bacteria / day / adult
Probiotics have several mechanisms of action that may contribute to human health.

How about interactions with food toxins????
Hepatocellular Carcinoma (HCC)

- Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer in the world with an estimating 473,000 new cases annually, accounting for 5.4% of all human cancer cases.

- Late presentation, typically males aged 66 with chronic liver disease.

- Median survival of 6 months from time of diagnosis.

- Surgery is the only potentially curable form of treatment.
Risk factors for HCC

• Viral
  *Chronic hepatitis B
  *Chronic hepatitis C

• Preexisting liver disease
  * Cirrhosis
    - Metabolic liver disease
    - Alcohol abuse
  * Adenoma

• Environmental
  * Aflatoxin
  * Contraceptives and androgens
The aflatoxins

- Turkey “X” Disease
  - Fungal infection by *Aspergillus flavus* and *Aspergillus parasiticus*
  - Primary contamination
    - High energy content foods e.g. grain, nut and soy products
  - Secondary contamination
    - Dairy products, meat & eggs
Commodities in which aflatoxins have been detected

<table>
<thead>
<tr>
<th>Flour</th>
<th>Cocoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn meal</td>
<td>Cheese</td>
</tr>
<tr>
<td>Peanut</td>
<td>Sausage</td>
</tr>
<tr>
<td>Meat pies</td>
<td>Bread</td>
</tr>
<tr>
<td>Milk</td>
<td>Macaroni</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>Copra</td>
</tr>
<tr>
<td>Cassava</td>
<td>Cooked meat</td>
</tr>
<tr>
<td>Brazil nuts</td>
<td>Pistachio nuts</td>
</tr>
<tr>
<td>Oilseeds</td>
<td>Rice</td>
</tr>
<tr>
<td>Pumpkin seeds</td>
<td>Soy</td>
</tr>
</tbody>
</table>
TOXIC EFFECTS OF AFLATOXIN

• Human
  – Aflatoxin B$_1$ is highly mutagenic, probably through mechanisms of epoxidation resulting in covalent binding to DNA.
  
    – A specific mutation at the third base of codon 249 of the tumor-suppressor gene p53 has been described in HCC tissue and significantly associated with exposure to aflatoxin B$_1$. 
Prevalence of 249 $^{\text{Ser}}$ p53 mutation – Aflatoxin Exposure Incidence of Hepatocellular Carcinoma

(Total number of cases: ~ 1000)
Populations at risk of aflatoxin exposure

*Estimated 66% of 1.2 billion people

**Figure 3.** Areas and populations at risk of chronic exposure to uncontrolled aflatoxin. LAC, Latin America and the Caribbean.

Chronic hepatitis B together with exposure to dietary aflatoxins increases the risk of liver cancer.

Relative Risk of primary liver cancer

- HBV (HBsAg): 7.3
- Aflatoxins (urinary biomarkers): 3.4
- HBV and aflatoxins: 59.4
- No risk factors: 1

Adapted from Qian et al, 1994
Available options for solving the problem

Once food is contaminated with toxins, there are only two options if the food is to be used:

- the toxin can be removed
- the toxin can be degraded into less toxic or non-toxic compounds
Control measures

• Physical control (e.g. UV radiation, electronic sorting)
  - suitable for very limited products
• Chemical control (e.g. ammoniation)
  - health effects are not fully studied
• Monitoring AF levels and rejection of produce
  - extremely costly option
Strategies for intervention at individual level

Aflatoxins in food

Absorption (portal circulation)

Duodenum

Ileum

Jejunum

Colon

Liver

Blood

Toxic products
Non toxic products

Metabolism

Breast milk
Urine

Feces (unabsorbed)
Blocking/reducing absorption of AFB₁ from the small intestine

Aflatoxin

Portal vein

Liver

Small intestine

Unabsorbed aflatoxins

Aflatoxin + blocker

Systemic circulation

Unabsorbed aflatoxins
Requirements for dietary tools of blocking/reducing aflatoxin absorption in humans

- Part of normal human diet
- Long history of safe use
- Able to bind a range of harmful compounds including aflatoxins
- Binding takes place immediately and is stable under GIT conditions
- No effect on absorption of micro and macro nutrients
- Inexpensive and practical for food enrichments
Lactic acid bacteria (LAB)

- LAB involved in the production of fermented foods
  - one quarter of our diet
  - characterised by safe history
  - extended shelf life compared to raw materials

- LAB has some health effects
  - growth inhibition of food spoiling bacteria
  - production of antimicrobial compounds
  - probiotic effects as live organisms in food
Kinetic studies on binding and release of toxins (dose-response)

Mechanisms of binding (chemical and structural factors)

In vitro toxicity studies (to examine if the binding will detoxicate the AFs)

Selection of bacteria with GRAS status (available commercially or isolated from microflora of healthy humans)

In vitro binding assays with AF

Ex vivo ligated loop in chicks

Feeding studies in animals

Stability of complex, effect on absorption and bioavailability

Clinical trials in populations exposed to AFs (body burden and biomarkers)
Aflatoxin is bound by probiotic bacteria - *in vitro* evidence

- Certain strains of lactobacilli are capable of binding up to 80% of AFB$_1$ *in vitro* (El-Nezami *et al*, 1996, 1998a,b,c), Fusarium toxins (El-Nezami *et al*, 2002a,b, 2004), PhIP and Trp-P-1 (Haskard *et al*, 2001)

- AFB$_1$ is predominantly bound to a carbohydrate moiety on the surface of the bacteria (Haskard *et al*, 2002)

- The complex formed between the bacteria and AFB$_1$ is stable under different conditions (Haskard *et al*, 2002, Lee *et al*, 2003)

**Ex vivo** study in chicks

The concentration of AFB$_1$ ± SD extracted from

<table>
<thead>
<tr>
<th>Group</th>
<th>Duodenal tissue$^b$</th>
<th>Soluble fraction$^c$</th>
<th>Insoluble fraction of luminal fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1min</td>
<td>60 min</td>
<td>1 min</td>
</tr>
<tr>
<td>AFB$_1$ only</td>
<td>0.27 ± 0.09 ND</td>
<td>1.04 ± 0.36</td>
<td>0.05 ± 0.01 ND</td>
</tr>
<tr>
<td>LBGG+AFB$_1$</td>
<td>0.07 ± 0.05 ND</td>
<td>0.48 ± 0.15 ND</td>
<td>0.76 ± 0.04</td>
</tr>
<tr>
<td>LC705+AFB$_1$</td>
<td>0.17 ± 0.11 ND</td>
<td>0.58 ± 0.10</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>PJS+AFB1</td>
<td>0.10 ± 0.05 ND</td>
<td>0.67 ± 0.13</td>
<td>0.13 ± 0.02</td>
</tr>
</tbody>
</table>

Intestinal AFB₁ transport and toxicity

- Transport of AFB₁ through monolayer was reduced by GG
- AFB₁ induced TER (membrane integrity) reduction was attenuated
- AFB₁ induced DNA damage was attenuated

In vivo protective effects of probiotics against AFB1 toxicity

AFB$_1$ (1.5 mg/kg bw, single dose on day 0)

GG (5x10$^{10}$ CFU, daily for 6 days)
Rat results

**GG administration:**

- Increased fecal $\text{AFB}_1$ by 122%
- Increased fecal $\text{AFM}_1$ by 152%
- Decreased plasma $\text{AFB}_1$-albumin by 29%
- Decreased change in liver function (ALT) by 54%
- Prevented body weight loss

WHY CHINA?

- Primary liver cancer (PLC) is one of the most common cancers in China.
- There more than 250,000 new cases diagnosed yearly with liver cancer in China.
- The mortality rates both in rural and urban areas are 25 and 21 per 100,000, respectively, in the EU 3 per 100,000.
- The main 3 factors for the development of liver cancer are prevalent in China. Aflatoxins are consistent contaminants of the food supply in China, HBV and HCV are endemics in China.
- 500,000,000 individual infected with HBV
  (250,000,000 in China)
- 170,000,000 individuals infected with HCV
  (10,000,000 in China)
- 1,000,000 individuals dies annually because of complication associated with HBV, similar figure also expected for HCV
  (250,000 in China)
**Probiotic intervention in China**

**Recruitment**
- 300 healthy Chinese men screened for urinary AFM$_1$
- 142 subjects had detectable level of AFM$_1$ in their urine
- 90 recruited based on physician's examination and blood chemistry

**Intervention and sampling**
- The subjects were randomized in two groups receiving either 2 placebo or 2 probiotic capsules/d for 5 weeks
  - *Bioprofit®* containing $10^{10}$ cfu/capsule
- Fecal, urine and blood samples were collected at baseline (day 1), and during intervention (days 21 and 35). Additional fecal samples were collected at days 2, 3, 5.

**Follow-up and aflatoxin measurement**
- Follow-up sample at day 70 (5 weeks after discontinuation of the treatments)
- Fecal and urinary aflatoxin M$_1$ and Q$_1$ concentrations were measured by HPLC. AFB-N7-guanine was used as a validated biomarker for reduction in HCC.

**Study days**
- Baseline (days: 1, 2, 3, 5)
- Probiotic/placebo (days: 21, 35)
- Follow-up (day: 70)
Principle Metabolites of Aflatoxin B1 and Potential Biomarkers

Aflatoxin B1

1α2, 3α4 (3α5)

Aflatoxin-exo 8,9-Epoxide

1α2

Aflatoxin-end 8,9-Epoxide

GST

DNA

Aflatoxin-dialdehyde

Aflatoxin-dihydriodiol

Aflatoxin-alcohol

Aflatoxin-glucuronide

Aflatoxin albumin

Urine

Blood
Probiotic supplementation reduces the urinary excretion of AFB$_1$-N$^7$-guanine, a biomarker of biologically effective dose of exposure to AFB$_1$.

What our findings mean?