Clinical and translational studies of the anti-colorectal cancer activity of the ω-3 polyunsaturated fatty acid eicosapentaenoic acid

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The long natural history of colorectal carcinogenesis

adenoma (polyp)  adenocarcinoma (cancer)

benign  malignant
Colorectal Cancer (CRC) prevention strategies

• Screening
• Surveillance
• Chemoprevention
• Lifestyle/behaviour modification
  – Body weight
  – Diet
  – Alcohol
  – Smoking
The ideal CRC chemoprevention agent

The use of natural or synthetic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer – Sporn 1976

• Effective
  – Colorectal cancer
  – Other malignancies
  – Other diseases (improved life-expectancy)

• Safe and well tolerated

• Easy to use and acceptable

• Inexpensive
Evidence that $\omega$-3 PUFAs have CRC chemopreventative efficacy

- Epidemiological observations
- Rodent models of colorectal carcinogenesis
- Clinical trials of $\omega$-3 PUFAs
Evidence that dietary ω-3 PUFA intake reduces CRC risk is not convincing

- “Limited, but suggestive, evidence that dietary fish intake reduces CRC risk”
- 35 cohort studies
  - Approx. 50% have reported decreased risk
- >50 case-control studies
- Systematic Review of cohort studies
  - Only 1 of 9 studies demonstrated a significant reduction in CRC risk in the highest ω-3 PUFA intake category

2nd Expert report WCRF/AICR 2007 and WCRF/AICR CUP 2011
JAMA 2006;295:403-15
Pre-clinical models of early stages of colorectal carcinogenesis

- Chemical carcinogenesis
  - Azoxymethane (AOM)
  - Dimethylhydrazine (DMH)
  - End-points
    - Aberrant crypt focus (ACF)
    - Tumour (adenoma/adenocarcinoma)

- \(Apc^{\text{Min}/+}\) mouse model of familial adenomatous polyposis (FAP)
  - Multiple adenomas in SI and colon after loss of second \(Apc\) allele
Pre-clinical evidence that $\omega$-3 PUFAs have CRC chemopreventative efficacy

• Chemical carcinogenesis models (15 rat/2 mouse)
  – 4-20% (v/w) fish oil in chow
  – 20-50% reduction in tumour incidence
  – 30-70% reduction in ACF or tumour multiplicity

• $Apc^{Min/+}$ and $Apc^{\Delta 716}$ mouse models
  – 1-12% (v/w) fish oil in chow
  – 40-80% reduction in adenoma multiplicity

• Usually EPA/DHA mix

• EPA = DHA
  – 6 single $\omega$-3 PUFA studies
  – 1 direct comparison ($Apc^{Min/+}$)

*Gut* 2011 doi 10.1136/gut.2010.233718
EPA as the free fatty acid reduces intestinal adenoma multiplicity in \( Apc^{Min/+} \) mice

- 99% pure EPA as the free fatty acid (FFA)
- AIN-93G diet with soybean oil
- 12 weeks
- \( n=8 \) each group
Why a discrepancy between the human observational and pre-clinical data?

- Methodological weaknesses in epidemiological studies
  - Subjective dietary measurements
  - Variable definitions of fish intake
- ‘Pharmacological’ treatment dose versus dietary ω-3 PUFA
  - 100 g ‘oily’ fish (salmon or sardines) = 1-2 g ω-3 PUFA
  - 100 g ‘lean’ fish (cod or haddock) = 0.25 g ω-3 PUFA
  - 2 g ω-3 PUFA per day is equivalent to eating 7-10 ‘oily’ fish portions per week
- Rodent models do not reflect human colorectal carcinogenesis
  - The same models have predicted efficacy of other agents eg. coxibs
- Confounding effect of reduced (pro-tumorigenic) ω-6 PUFA intake in rodent models
  - Some reports have controlled for ω-6 PUFA (corn oil) intake and demonstrated ω-3 PUFA efficacy
Human mucosal biomarkers of CRC risk

- No prospectively validated biomarker!

- Epithelial cell proliferation index (PI)
  - Whole crypt microdissection
  - Ki-67 (MIB-1)/PCNA IHC

- Epithelial cell apoptosis index (AI)
  - H&E apoptotic body counting
  - Neo-cytokeratin 18 IHC

- Tissue ω-3 and ω-6 PUFA content by GC-MS
### Human biomarker studies of ω-3 PUFA therapy

<table>
<thead>
<tr>
<th>Study (author/year)</th>
<th>Design Patient group</th>
<th>N</th>
<th>ω-3 PUFA daily dose</th>
<th>Treatment Duration</th>
<th>Primary outcome</th>
<th>Mucosal PUFA content</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti 1992</td>
<td>R, DB, PC ‘sporadic’ adenoma</td>
<td>24</td>
<td>7.7 g FO1</td>
<td>12 wk</td>
<td>PI</td>
<td>↑EPA &amp; ↓AA</td>
<td>62% ↓PI</td>
</tr>
<tr>
<td>Bartoli 1993</td>
<td>R, DB, PC ‘sporadic’ adenoma</td>
<td>40</td>
<td>2.5-7.7 g FO1</td>
<td>30 days</td>
<td>PI</td>
<td>Dose dependent ↑EPA/DHA &amp; ↓AA</td>
<td>Dose dependent 40-70% ↓PI</td>
</tr>
<tr>
<td>Bartlam 1993</td>
<td>DB crossover trial Healthy volunteer</td>
<td>12</td>
<td>4.4 g FO2</td>
<td>4wk +4 wk</td>
<td>PI</td>
<td>ω-3 PUFA ↔ ω-6 PUFA ↓ (NS)</td>
<td>16% ↓ PI &amp; 35% ↓ mucosal PGE₂</td>
</tr>
<tr>
<td>Anti 1994</td>
<td>R, DB, PC ‘sporadic’ adenoma</td>
<td>60</td>
<td>2.5-7.7 g FO1</td>
<td>30 days</td>
<td>PI</td>
<td>Dose dependent ↑EPA/DHA &amp; ↓AA</td>
<td>Dose independent 50-70% ↓</td>
</tr>
<tr>
<td>Huang 1996</td>
<td>R, DB, PC Dukes A/B CRC or severely dysplastic polyp</td>
<td>27</td>
<td>7.2 g FO3</td>
<td>6 months</td>
<td>PI</td>
<td>↑EPA/DHA &amp; ↓AA</td>
<td>71% ↓ PI (only in patients with high baseline PI)</td>
</tr>
<tr>
<td>Gee 1999</td>
<td>R, PC, single blind Awaiting CRC surgery</td>
<td>51</td>
<td>2.4 g FO4</td>
<td>7-21 days pre- and 8-12 wk post- surgery</td>
<td>PI</td>
<td>↑EPA/DHA ↑ ω-3 : ω-6 ratio</td>
<td>No effect on PI at surgery or 12wk post-op</td>
</tr>
<tr>
<td>Cheng 2003</td>
<td>R, C, open label Previous CRC/adenoma</td>
<td>41</td>
<td>Dietary advice +/- 500 mg FO5</td>
<td>2 years</td>
<td>PI/AI</td>
<td>Not assessed</td>
<td>PI↔, 50% ↑AI, 50% ↑ Bax, COX2 ↔</td>
</tr>
<tr>
<td>Courtney 2007</td>
<td>R, single blind ‘sporadic’ adenoma</td>
<td>30</td>
<td>EPA 2 g as FFA</td>
<td>3 months</td>
<td>PI/AI</td>
<td>↑EPA/DHA &amp; ↓AA</td>
<td>20% ↓ PI 7x ↑ AI</td>
</tr>
<tr>
<td>West 2009</td>
<td>R, DB, PC ‘sporadic’ adenoma</td>
<td>152</td>
<td>EPA 1 g or2 g as FFA</td>
<td>6 months</td>
<td>PI/AI</td>
<td>↑EPA/DHA &amp; ↓AA</td>
<td>13% ↓ PI 57% ↑ AI (NS)</td>
</tr>
</tbody>
</table>

FO1 = 54% EPA/46% DHA as ethyl esters  
FO2 = 48% EPA/44% DHA, as triglycerides  
FO3 = 55% EPA/30% DHA/15% other ω-3 PUFAs  
FO4 = 58% EPA/42% DHA  
FO5 = 20% EPA/80% DHA
Polyp (adenoma) Prevention Trials in CRC chemoprevention research

- Colorectal adenoma number and characteristics (size, ‘advanced’ features) are established as biomarkers of future CRC risk
- Relatively short trials are feasible
  - Sigmoidoscopic/colonoscopic surveillance in FAP
  - Colonoscopic surveillance programmes for ‘sporadic’ neoplasia (3-5 years)
- Efficacy in Polyp Prevention Trials mirrored by long-term (10-15 years) effects on CRC incidence for aspirin
Familial adenomatous polyposis

- Autosomal dominant mutation of the \( APC \) gene
- Initiation of colorectal carcinogenesis after loss of the second \( APC \) allele
- Molecular pathology is identical to ‘sporadic’ colorectal adenomas
- Classical (100s) and attenuated (10-100) phenotypes
- 100% penetrance for CRC by 4-5\(^{th}\) decade
- Surgical options
  - colectomy and ileo-rectal anastomosis (IRA)
  - pan-proctocolectomy
- Endoscopic surveillance of rectal stump needed every 6-12 months after IRA

Endoscopic surveillance of the rectal stump
Trials of $\omega$-3 PUFAs in patients with FAP

- **Japanese case series**
  - n=5
  - Previous colectomy or >30 polyps
  - 2.2 g DHA + 0.6 g EPA for 1-2 years
  - No significant change in polyp number

- **Phase III DBRCT**
  - n=58
  - Previous colectomy and IRA undergoing surveillance
  - EPA-FFA 2 g daily or placebo for 6 months

_Gut_ 2010;59:918-25
Endoscopic Measurements

- Polyp number and diameter in tattooed area
  - comparable photos from DVDs
  - assessed by 2 blinded Endoscopists
  - biopsy forceps as magnification guide

- DVDs reviewed by 5 Endoscopists
  - Independent of photo review
  - Blinded to treatment & viewing order
  - Scored overall global polyp burden as:
    - Better (+1); Same (0); Worse (-1)
  - Mean score calculated
63 FAP patients assessed for eligibility

Excluded (n = 5) Not meeting inclusion criteria

Placebo (n = 29)
Analysed (n = 27)

Randomisation (n = 58)

EPA 2 g daily (n = 29)
Analysed (n = 28)
# Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=27)</th>
<th>EPA-FFA (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at study entry (y)</strong></td>
<td>42.5 (13.8)</td>
<td>39.5 (11.4)</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>15 (55.6)</td>
<td>13 (46.4)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>12 (44.4)</td>
<td>15 (53.6)</td>
</tr>
<tr>
<td><strong>Time since colectomy (y)</strong></td>
<td>15.4 (9.3)</td>
<td>15.9 (9.6)</td>
</tr>
<tr>
<td><strong>Length of rectal remnant (cm)</strong></td>
<td>20.6 (4.0)</td>
<td>20.4 (5.7)</td>
</tr>
</tbody>
</table>
### Number of Polyps in Focal Area

<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean (SD)</th>
<th>Change 0-6 months Mean [95% CI]</th>
<th>Difference between treatments Mean [95% CI]</th>
<th>$P$ value (ANCOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4.50 (2.63)</td>
<td>0.54 [-1.78; -0.35]</td>
<td>-1.06 [-1.78; -0.35]</td>
<td>0.0046</td>
</tr>
<tr>
<td>EPA-FFA 2g/day</td>
<td>4.13 (2.47)</td>
<td>-0.52 [-1.02; -0.02]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Percentage Change in Number of Polyps

Δ No of polyps (%)

Placebo: 9.7 [-2.6; 22.0]
EPA-FFA: -12.6 [-24.7; -0.6]
Net change: -22.4 [-39.6; -5.1]

P = 0.0122
Percentage Change in Size of Polyps

$\Delta$ Total polyp diameter (%)

- **Placebo**
  - 17.3 [-1.7; 36.2]

- **EPA-FFA**
  - -12.6 [-30.6; 5.46]

- **Net change**
  - -29.8 [-56.1; -3.58]

$P = 0.0270$
Change in Global Polyp Burden

Mean score

Better

Worse

Placebo

EPA-FFA

Net change

-0.34 [-0.56; -0.11]

0.09 [-0.14; 0.32]

0.42 [0.10; 0.75]

P = 0.011
Percentage Change in Number of Polyps

# Adverse Events

<table>
<thead>
<tr>
<th>Condition</th>
<th>Placebo (n = 29)</th>
<th>EPA-FFA (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Upper abdominal pain</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Epigastric discomfort</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>
# Mucosal PUFA content

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Placebo (n=26)</th>
<th>EPA-FFA (n=26)</th>
<th>Difference EPA-FFA - Pla (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (SD)</td>
<td>6 months (SD)</td>
<td>Change (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.73 (0.72)</td>
<td>1.30 (1.09)</td>
<td>0.54 (-0.06, 1.13)</td>
<td>0.97 (0.89) 2.50 (1.98)</td>
</tr>
<tr>
<td>DPA</td>
<td>0.75 (0.65)</td>
<td>0.76 (0.66)</td>
<td>0.04 (-0.20, 0.28)</td>
<td>0.64 (1.15) 1.17 (0.69)</td>
</tr>
<tr>
<td>DHA</td>
<td>1.39 (0.61)</td>
<td>1.42 (1.05)</td>
<td>-0.11 (-0.46, 0.24)</td>
<td>1.92 (1.30) 1.71 (0.94)</td>
</tr>
<tr>
<td>AA</td>
<td>9.63 (2.18)</td>
<td>9.88 (1.86)</td>
<td>0.61 (-0.10, 1.31)</td>
<td>8.47 (1.68) 8.82 (1.91)</td>
</tr>
</tbody>
</table>

Data are the mean % of the total mucosal fatty acid pool measured by GC-MS

- **Significant 2.6-fold increase in rectal mucosal EPA levels**
- Increase in DPA (but not DHA) levels
- No significant change in AA content
EPA-FFA has chemopreventative efficacy in FAP

• EPA-FFA 2 g daily for 6 months
  – Reduces rectal adenoma number and size
  – Similar magnitude effect as for the selective COX-2 inhibitor celecoxib
  – Is safe and well-tolerated
  – Leads to EPA incorporation into rectal mucosa
  – ? Efficacy against ‘sporadic’ colorectal neoplasia

*Gut* 2010;59:918-25
seAFOod Polyp Prevention Trial

• Double-blind randomised placebo-controlled trial in English Bowel Cancer Screening Programme

• 2 x 2 factorial design
  – EPA free fatty acid 2 g daily
  – aspirin 300 mg daily

• Intervention for 12 months

• ‘high risk’ patients due for surveillance colonoscopy at 1 year
  – ≥ 5 small polyps
  – ≥ 3 polyps with at least one ≥ 1 cm

• www.seafood-trial.co.uk
seAFOod Polyp Prevention Trial

• 1º endpoint – number of patients with a polyp(s) at one year
  – 768 evaluable patients to detect a minimum 18% decrease in adenoma ‘recurrence’
  – Assuming 15% drop-out this increases to 904 patients
  – Assuming 40% ineligibility (incl aspirin use, need for re-look), we need to identify 1507 ‘high risk’ patients

• 2º endpoints – polyp number, ‘advanced’ lesions, location, AEs

• Exploratory end-points - lipid biomarker and genomic studies
  – Erythrocyte and mucosal EPA, DPA and AA levels by GC-MS
  – Plasma and mucosal ‘lipidomic’ analysis by LC-MS/MS
    • PGE₂, PGE₃, 18R-HEPE, resolvin E1/2
  – Urinary PGE-M by LC-MS/MS
  – COX-2 immunohistochemistry on FFPE polyp tissue
  – Genomic DNA for genotype studies
Mechanisms of anti-cancer activity of EPA

- inhibition of cyclooxygenase (COX) activity
- production of novel anti-inflammatory lipid mediators
  - E-type resolvins (requirement for aspirin?)
- direct fatty acid signalling via GPCRs
  - Stromal macrophage GPR120
- alteration of membrane dynamics and receptor function
  - EGF receptor
- increased cellular oxidative stress leading to apoptosis
- Anti-angiogenesis
- Modulation of host anti-tumour immune surveillance
Cyclooxygenase (COX) isoforms

Arachidonic acid

NSAIDs

COX

PGH₂

PGI₂

TXA₂

stomach and endothelium

kidney and intestine

normal

COX-1

COX-2

pathological e.g. RA joint

growth factors cytokines tumour promoters

PGs

PGEs

PGE₂ is pro-tumorigenic in CRC
COX-dependent mechanisms of action

‘western’ diet/untreated

AA → COX-1 → PGE₂

AA → COX-2 → PGE₂

dietary/therapeutic EPA

EPA → COX-1 → ↓PGE₂

EPA → COX-2 → PGE₃

PGE₃ is a partial agonist at the EP4 receptor

LoVo human CRC cells

EPA-FFA as adjuvant therapy for prevention and/or treatment of CRC liver metastasis (CRCLM)

• An unmet clinical need
• Conflicting data from animal models of CRCLM
• Efficacy and mechanism of action of EPA-FFA in a BALB/c MC-26 mouse CRC cell model
• EPA for Metastasis Treatment (EMT) Study
  – Phase II DBRCT of EPA-FFA 2 g daily prior to liver surgery (NCT01070355)

Gut 2011 doi 10.1136/gut.2010.233718
EPA for Metastasis Treatment (EMT) Study

- Phase II DBRCT
- NCT01070355
- EPA-FFA 2 g daily or placebo prior to liver surgery for CRCLM (2-8 weeks)
- N=88, LPLV Oct 2011
- 1º endpoint – tumour MIB-1 proliferation index
- 2º endpoints
  - Safety & tolerability (AEs)
  - Platelet function studies
  - Apoptosis index, microvessel density
  - Tumour PUFA content
  - Tumour PGE$_2$/PGE$_3$ levels
  - Urinary PGE-M
  - COX-2 expression
  - PBMC activation (NFκB activation, PGE$_2$ production)
Summary

• The ω-3 PUFA EPA has chemopreventative efficacy in animal models and a RCT in FAP patients
• A RCT of EPA-FFA for prevention of ‘sporadic’ colorectal neoplasia in high risk patients is underway
• Mechanistic pre-clinical and human biomarker studies will provide insights into its mechanism(s) of action
• A Phase II RCT of EPA-FFA in CRCLM patients will report in 2012 – test use as adjuvant therapy in CRC patients?