Clinical trial: protective effect of a commercial fish protein hydrolysate against indomethacin (NSAID)-induced small intestinal injury
Marchbank, T; Limdi, JK; Mahmood, A; Elia, G; Playford, RJ

For additional information about this publication click this link.
https://qmro.qmul.ac.uk/jspui/handle/123456789/178

Information about this research object was correct at the time of download; we occasionally make corrections to records, please therefore check the published record when citing. For more information contact scholarlycommunications@qmul.ac.uk
Clinical trial: protective effect of a commercial fish protein hydrolysate against indomethacin (NSAID)-induced small intestinal injury.

T. MARCHBANK1*, J. K LIMDI1*, A. MAHMOOD2, G. ELIA3 & R. J PLAYFORD1.

1. Centre for Gastroenterology, Institute of Cell and Molecular Science, Barts & The London School of Medicine, Queen Mary University of London, London, UK, 2. Epsom & St Helier NHS Trust, Carshalton, Surrey UK, 3. Histopathology Unit, Cancer Research UK-London Research Institute, 44, Lincoln's Inn Fields, UK.

Correspondence Prof. RJ Playford,
Professor of Medicine,
Barts & The London,
Queen Mary's School of Medicine & Dentistry,
Turner Street,
London E1 2AD, UK

Telephone +44(0)20 7882 2260
Fax +44 (0)20 7377 7607
Email r.playford@qmul.ac.uk

**Running title**: Fish protein hydrolysate and intestinal repair
Abbreviations: HPLC; high pressure liquid chromatography, NSAID; non-steroidal anti-inflammatory drugs

Key words: Repair, gut growth, injury, nutriceutical, clinical trial

ABSTRACT

Background: A partially hydrolysed and dried product of pacific whiting fish is marketed as a health food supplement supporting ‘intestinal health’. We examined whether it influenced the small intestinal damaging side effects of the non-steroidal anti-inflammatory drug, indomethacin.

Methods: Eight human volunteers completed a double-blind, placebo-controlled, crossover protocol of clinically relevant dose of indomethacin (50 mg tds po for 5 days) with 7 days of fish hydrolysate or placebo starting 2 days prior to indomethacin. Changes in gut permeability were assessed using 5 h urinary lactulose: rhamnose (L/R) ratios.

Results: Fish hydrolysate given alone did not affect permeability. In the main study (N=8), baseline values were similar for both arms (0.28 +/- 0.05 and 0.35 +/- 0.07). Administration of indomethacin (+ placebo) caused a five-fold rise in L/R ratios (increasing to 1.54 +/- 0.35) whereas L/R ratios in the same subjects ingesting indomethacin + fish hydrolysate, was only 0.59 +/- 0.14, (p <0.01 vs. indomethacin alone). Dyspeptic symptoms occurred in 4/8 subjects taking indomethacin alone but 0/8 when hydrolysate was co-administered.

Conclusion: Natural bioactive products (nutriceuticals), such as this, may provide a novel approach to the prevention and treatment of NSAID-induced and other gastrointestinal injurious conditions.
INTRODUCTION

There is currently a resurgence of interest in the use of natural bioactive products (‘nutraceuticals’) by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders\(^1\). Unfortunately, current evidence of the scientific validity of many of these traditional and commercial compounds is severely limited.

One product of interest, that is already commercially available, is a fermented fish product derived from the controlled proteolytic yeast fermentation of pacific whiting (Merluccius productus). Fermentation is a commonly used process in the standard food industry as well as in the bioactive food (nutraceutical) field. Fermentation of foodstuffs has many effects, including partial degradation of protein constituents which, as well as potentially aiding absorption from the gut, may also influence its biological activity.

Fish hydrolysate is claimed to be beneficial for a variety of gut conditions and we have previously shown it to be capable of stimulating proliferation and migration (restitution) of HT29 cells \textit{in vitro}\(^2\). However, data examining its ability to influence gut integrity in human studies is severely limited. We chose to examine the potential activity of this fish hydrolysate against the damaging effects of the non-steroidal anti-inflammatory drug (NSAID) indomethacin as NSAIDs are some of the most widely prescribed group of drugs worldwide. Despite the undoubted efficacy of NSAIDs, side effects, including peptic ulceration and small intestinal injury are common and novel therapies are still required\(^3\).

We, therefore, performed a series of studies to analyse whether ingesting this fish hydrolysate product could influence the damaging effect of indomethacin on small intestinal integrity, as
assessed by following changes in permeability (leakiness) of the small intestine in a human clinical trial.

MATERIALS & METHODS

All chemicals were purchased from Sigma (Poole, Dorset) unless otherwise stated.

Ethics

Human studies were approved by appropriate regulatory authorities. Clinical trial REC reference number 05/Q0406/97.

Hydrolysed fish protein concentrate

The dried fish protein hydrolysate preparation studied, Seacure®, was donated by Proper Nutrition, Inc., Reading, PA, U.S.A (for details of preparation and constituents see ref 2). The fish protein hydrolysate contains 75-80% protein constituents (60% peptides and 40% amino acids and the major amino acid constituents are glutamine (approx. 14-16% of total), asparagine (10%) and lysine (10%) and 6-10% fish oils.

Placebo

A placebo was produced that was identical in colour and smell using rice flour with 1% sea cucumber.

Clinical Study
Background to method

Assessment of intestinal permeability by quantitating unmediated absorption of at least two sugars of different sizes provides a sensitive index of intestinal damage\(^4\). To maintain consistency with our previous studies (including osmolality), we administered a lactulose, rhamnose, mannitol mixture but again only present lactulose/rhamnose ratios for reasons previously described\(^5\). This is considered appropriate as this specific combination has been recommended for assessing enteropathy induced by NSAIDs\(^6\).

Protocol

Following an overnight fast, subjects emptied their bladders and then drank a standardised sugar solution containing lactulose 5g, mannitol 2g, and rhamnose 1g in a total of 450ml water (calculated osmolality 69 mOs). Subjects were allowed unlimited intake of fluid after the first hour of the test to ensure adequate urine output. The urine was collected and pooled over the next 5 hours and total volume recorded. Aliquots were centrifuged briefly to remove gross debris and the supernatant frozen at -20\(^0\)C until later analysis.

Analyses

Analyses of sugar content within the urine were based on the methods and equipment used by our group previously\(^5\). The various sugars were separated using high pressure liquid chromatography (HPLC) and quantitated using a pulsed amphometric detector. Using this technique, sugars are oxidised on the gold electrode at the working potential (P 0.05V), the current produced being a measure of the amount of sugar present in the sample\(^7\).

Study protocols

Preliminary studies:
A) To determine the reproducibility of results, a single individual performed permeability studies for 4 separate days (while not taking any test treatment or NSAIDs). These samples were assayed to determine intra-patient variation and gave a coefficient of variation of about 8%. In addition, a single sample was measured 6 times to determine intra-assay variation and gave a coefficient of variation of 5%. These coefficients of variation were similar to those reported by us previously5.

B) To examine whether fish hydrolysate influenced permeability under basal conditions, four subjects underwent an initial permeability assessment and then ingested fish hydrolysate for seven days with a further assessment on the final day.

Main study:
10 volunteers (25-40 yr., 5M, 5F) who were not taking NSAIDs or suffering from conditions likely to affect intestinal permeability e.g. coeliac disease or previous intestinal surgery, were entered into the study. Subjects abstained from alcohol consumption and ingestion of any NSAID, including aspirin, for one week prior to starting the study and throughout the remainder of the test period. Subjects were asked to record and report if they had any upper abdominal discomfort (‘dyspepsia’) prior to starting the protocol or during the two arms of the study.

Each subject undertook a total of 6 permeability assessments (Fig 1). Each arm of the study comprised 3 collections on day -2, 0, and 7. For both arms, samples collected on days -2 and 0 were ‘baseline’ analyses when the volunteer was not taking any test substance. After urine collection on day 0, volunteers took test substance comprising fish hydrolysate (1g) or placebo capsule three times daily for a week. Fish hydrolysate and placebo capsules were indistinguishable in terms of appearance and taste.
After the weeks course of test substance (day 7), the third urine collection for that arm of the study was completed. Following a two-week washout period, each participant repeated the protocol with the other test capsule. The active and placebo capsule arms of the study were administered in random order. In addition, in both arms of the study, for the last 5 days of the study (days 2-7) participants also received an NSAID (indomethacin 50mg tds, see Fig 1).

Statistics
All values are expressed as the mean +/- SEM. Two way ANOVA was used with presence of NSAID and presence of fish hydrolysate as factors. Where a significant effect was seen (p<0.05), individual comparisons were performed using t-tests based on the group means, residual and degrees of freedom obtained from the ANOVA, a method equivalent to repeated measures analyses.

RESULTS

Preliminary studies.
In the four subjects who took fish hydrolysate for 7 days without taking an NSAID, there was no change in intestinal permeability (lactulose:rhamnose ratio was 0.53 +/- 0.13 before treatment vs. 0.54 +/- 0.13 after treatment, Fig 2).

Main study.
Withdrawal: Two female volunteers (who started on the indomethacin plus placebo phase) were unable to tolerate the indomethacin due to onset of abdominal discomfort and withdrew from the study within 2 days of starting. They have, therefore, not been included in the analyses. The remaining 8 subjects completed the study.
Dyspepsia:
At the beginning of the study, 7/8 subjects who completed the study had no dyspeptic (abdominal discomfort) symptoms and one subject had longstanding mild dyspepsia. There was no worsening or onset of symptoms in any of the 8 subjects when they took indomethacin plus fish hydrolysate. In contrast, 4/8 complained of onset or worsening of their dyspeptic symptoms when taking the indomethacin with placebo.

Permeability
Baseline permeability values were similar at the beginning of each study arm and at the first and second (baseline) assessments within a study arm (Fig 3). Permeability increased about five-fold in response to indomethacin during the placebo arm (rising from 0.28 +/- 0.05, initial baseline value, to 1.54 +/- 0.35 at day 7, p < 0.01). In contrast, when the subjects were also taking the fish hydrolysate, the rise in permeability caused by indomethacin was virtually completely abrogated in 7/8 of the subjects and was markedly truncated in the 8th individual (rising from 0.35 +/- 0.07, initial baseline value, to 0.59 +/- 0.14 at day 7). Statistical analyses, using presence of fish hydrolysate and time as factors showed significant effects of time (i.e. indomethacin administration, F 2,42= 13.69, p < 0.0001), presence of fish hydrolysate (F1, 42= 4.34, p = 0.0433) and an interaction between the two. F2, 42= 6.27, p = 0.004). This showed that the rise in intestinal permeability caused by indomethacin was truncated by the presence of fish hydrolysate. Subsequent t-test comparisons based on mean square error of residual from ANOVA showed that permeability values at day 7 of both arms of study were significantly different (p < 0.01). The order in which placebo and fish hydrolysate was administered did not influence results (although numbers are too small to perform detailed statistical analysis).
DISCUSSION

We have shown, for the first time, a commercially available fish hydrolysate preparation reduces the degree of small intestinal damage caused by the NSAID indomethacin in a pilot human clinical trial.

Several methods are available to determine the degree of small intestinal injury induced by NSAIDs in humans, all of which have their drawbacks; Enteroscopy is an invasive procedure, $[^{111}\text{In}]$-labelled white cells require radioactive exposure and measurement of the neutrophil marker, calprotectin in the stool is still at a relatively early stage of development. Measurement of gut permeability is a safe, sensitive and simple investigation to perform but, as for all the other methods of assessment that do not involve direct visualisation, it is not readily equated to a score that uses visualisation, such as number of erosions. Measurement of intestinal permeability has been used previously to assess the degree of small intestinal damage in patients with coeliac disease and Crohn's disease and NSAID usage. The five-four rise in permeability found in the control arm of our main study is in keeping with our, and others, published works.

Non-steroidal anti-inflammatory drugs are used worldwide but side effects such as dyspepsia, peptic ulceration and enteropathy are common and only partially relieved by acid suppressants. There is, therefore, a need for novel therapies. Indomethacin causes damage to the gastrointestinal tract by several mechanisms including reduction of mucosal prostaglandin levels, reduction of mucosal blood flow, stimulating neutrophil activation, and stimulating apoptosis. It is likely that many of these mechanisms will be influenced by the numerous factors present in the fish hydrolysate. We have previously demonstrated pro-proliferative and pro-migratory (restitutive) activity of this fish hydrolysate product in vitro, and the current studies expanded these in vitro results to show this products efficacy in a clinically relevant model.
The molecules responsible for the protective effects of the hydrolysate against indomethacin-induced injury seen in the current study are incompletely defined. It is unlikely that this effect was due to the hydrolysate influencing indomethacin adsorption as we have shown previously that the hydrolysate can reduce (systemically administered) indomethacin-induced gastric injury in rats². Our previous studies suggested glutamine within the fish hydrolysate played an important part, accounting for about 34% of the pro-proliferative activity seen using an in vitro model and may also have contributed to antioxidant activity via stimulation of glutathione production. Many of the amino acid constituents of the fish protein hydrolysate remain in the intact protein or partially cleaved form and it is also possible that these intact proteins have direct bioactivity as shown in partially digested bovine milk.¹⁴ The fish hydrolysate contains about 6-10% fish oils². In addition to increasing migration¹⁵, and affecting prostaglandin production, these oils may contribute to anti-apoptotic effects¹⁶. Further work is required, however, to determine in detail how these effects are mediated.

Hundreds, if not thousands, of products are currently marketed as ‘health food supplements¹⁷. 72% of the American population use one or more health supplement products regularly and 57% considered that these therapies reduced their need for drugs and other medical therapies. This results in an annual turnover of £9 billion in the USA and £1.2 billion in the UK, with an 8% annual growth¹⁷. The sources of these ‘natural’ products are diverse and include bacteria, plant, animal, and marine origins¹,¹⁸. Unfortunately, current evidence of the scientific validity of most of these traditional and commercial compounds is severely limited and the level of evidence used in support of their claims often falls well below that acceptable in the medical and scientific community.
The fish hydrolysate studied in this article is already marketed in the USA as an ‘over the counter’ health food supplement and, as with many of these products, its major marketing strategy is via patient validatory statements. There have, however, been some limited scientific studies. For example, Englender et al. reported that use of the fish protein hydrolysate, at a dosage of 3g per day for 60 days, reduced the symptoms of occasional diarrhoea and constipation, and alleviated bloating.

The positive effect in the current study is of particular interest as the doses of NSAID and fish hydrolysate used are at standard clinical dose and manufacturers recommended dose respectively. These results emphasise that the division between “food products” and “drugs”, when considered in terms of biological activity (and concerns about potential safety aspects), is far from clear and products such as this should be considered as a “nutriceuticals” or functional foods. Although not directly relevant for this fish hydrolysate product, the distinction between ‘natural’ and ‘artificial’ is also blurred if a single compound is isolated and clinically used, even though it was from a ‘natural’ source initially.

Further study of products based on fish hydrolysate for the prevention and treatment of other injurious conditions of the bowel, such as inflammatory bowel disease, necrotising enterocolitis and chemotherapy induced mucositis etc., where therapy is suboptimal and novel approaches are required, appear justified.

**Declaration of funding interests:** This work was partially funded by Proper Nutrition, Inc., who are the manufactures of the Seacure product used. They were not involved with the data analyses. This work was partially funded by the Wexham Park Gastrointestinal trust grant number 2004/6772.
REFERENCES


15. Ruthig DJ, Meckling-Gill KA. Both (n-3) and (n-6) fatty acids stimulate wound healing in the rat intestinal epithelial cell line, IEC-6. *J Nutr* 1999;129:1791–8.


FIGURE LEGENDS

Fig 1. Protocol for small bowel permeability trial.
Healthy volunteers participated in a double-blinded randomised controlled cross-over protocol. Each arm comprised 3 urine collections (2 baseline). In each arm, volunteers took fish hydrolysate (1g) or placebo capsule three times daily for 7 days with indomethacin (50 mg three times daily) for the final 5 days.

Fig 2. Effect of fish hydrolysate on permeability under basal conditions.
Four subjects underwent an initial permeability assessment and then ingested fish hydrolysate for seven days, without taking an NSAID, with a further assessment on the final day. Each line represents one individual’s permeability result expressed as lactulose/rhamnose ratio. There was no change in intestinal permeability in any of the subjects as result of taking fish hydrolysate. (Note same scale as in Fig 3.)

Fig 3. Effect of fish hydrolysate on indomethacin-induced permeability in a pilot clinical trial.
8 individuals completed protocol shown in Fig 1. Each colour line represents one individual’s permeability result expressed as lactulose/rhamnose ratio. Black bars show mean values for each stage. Both arms were performed in random order. When taking placebo, volunteers had a five-fold increase in mean lactulose/rhamnose ratios in response to indomethacin administration (p < 0.01) whereas no significant increase was seen if fish hydrolysate was also being taken.
Day -2 0 2 7 Two week washout -2 0 2 7
Test substance (fish hydrolysate or placebo)
NSAID (Indomethacin)
Urine collection