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The potential of cod hydrolyzate to inhibit blood pressure in spontaneously hypertensive rats

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ABSTRACT

Hypertension is an independent yet controllable risk factor for cardiovascular diseases. Synthetic angiotensin-converting enzyme (ACE) inhibitors used to treat hypertension are often associated with adverse effects, and the interest in diet-related inhibitors is increasing. We hypothesized that North Atlantic fish hydrolysate might inhibit ACE, thus preventing hypertension. We assessed the ACE inhibitory potential of various North Atlantic fish species and evaluated the effect of dietary supplementation of fish hydrolysates on the blood pressure of spontaneously hypertensive rats. Fish samples were hydrolyzed using simulated gastrointestinal digestion, and ACE inhibitory activity was evaluated using an ACE inhibitory activity assay. In vivo anti-hypertensive effects were evaluated by administering hydrolysates of wild Atlantic cod (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.), or farmed Atlantic salmon (*Salmo salar* L.) to 10-week-old male, spontaneously hypertensive rats for 4 weeks. The dosing was 200 mg/kg body weight for 21 days, followed by 500 mg/kg body weight for 7 days. Water and Captopril (20 mg/kg body weight) were administered as the negative and positive controls, respectively. The analyzed fish hydrolysates exhibited a 50 % ACE inhibition coefficient (IC_{50}) of 1 to 2.7 μg/mU ACE. Fish hydrolysate supplements did not significantly inhibit the increase in blood pressure during the experimental period. The group receiving cod supplement had a lower (not significant) increase in blood pressure compared to the other groups. Although further studies are necessary to verify the antihypertensive effect of cod, the results obtained in this study indicate the potential that cod hydrolysate may have in inhibiting hypertension.

1. Introduction

Hypertension, or elevated blood pressure (BP), is a worldwide health issue [1], with an estimated 1.56 billion individuals being affected by 2025 [2]. It is a major independent risk factor for cardiovascular diseases [3] which are associated with myocardial infarction, stroke, and heart failure. Among other factors, angiotensin converting enzyme (ACE) plays an important role in increasing BP by converting the inactive angiotensin 1 into the potent vasoconstrictor angiotensin 2 [4], in addition to deactivating the potent vasodilator bradykinin [5]. Therefore, inhibition of ACE may prevent a BP increase. Synthetic ACE inhibitors are traditionally used to treat hypertension and heart failure [6]. These potent

Abbreviations: ACE, angiotensin converting enzyme; BP, blood pressure; HHL, hippuryl-histidyl-leucine; SHR, spontaneously hypertensive rats.

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therapeutics are often associated with adverse effects such as cough, taste alterations, skin rashes, and renal dysfunction [7], and so, novel therapeutic approaches are needed. Dietary interventions with a particular focus on bioactive components, for instance peptides which prevent mild hypertension through inhibition of ACE activity [8], are a promising antihypertensive treatment strategy. Several putative modes of action strategies, including blocking parts of the active site or altering enzyme conformation, therefore preventing substrates from binding to the active site, may explain the bioactivity of ACE inhibitory peptides [9]. Numerous in vitro studies have indicated the ACE inhibitory effect of fish hydrolysate [9], but only a few animal studies have been carried out and a very small number of human clinical trials have been published. Thus, solid evidence on the correlation between consumption and the prevention of mild hypertension is limited, so dietary recommendations of fish consumption are still mainly linked to the marine polyunsaturated fatty acids EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3) [10]. We hypothesized that commonly consumed Atlantic fish species contain peptides that may inhibit ACE, and thus may prevent hypertension. The spontaneously hypertensive rats (SHR) strain is the most commonly used model for hypertension studies [11]. In addition to spontaneous development of hypertension, the strain is also prone to other complications similar to those observed in humans, such as cardiac hypertrophy, cardiac failure, and renal dysfunction [11], making the model clinically relevant. The present study was designed to assess the in vitro ACE inhibitory potential of hydrolysates produced from North Atlantic fish species by a simulated gastrointestinal digestion in vitro and to evaluate the potential antihypertensive effects of the fish hydrolysates through dietary supplementation in an SHR model.

2. Methods and materials

Frozen fillets of haddock (*Melanogrammus aeglefinus* L.), saithe (*Pollachius virens* L.), Atlantic cod (*Gadus morhua* L.), whiting (*Merlangus merlangius* L.), plaice (*Pleuronectes platessa* L.), Atlantic halibut (*Hippoglossus hippoglossus* L.), and Greenland halibut (*Reinhardtius hippoglossoides [Walbaum]*) caught in the waters off the coast of the Faroe Islands as well as farmed Atlantic salmon (*Salmo salar* L.) were kindly provided by Norðfra, Faroe Islands and transported to the Norwegian College of Fishery Science, University of Tromsø, Norway. The samples were stored in plastic bags at –50 °C until analysis. Prior to analysis, visible fat was removed from the belly flaps and dorsal fin areas and the fillets were minced. Fish collagens were produced from saithe skins and kindly provided by Seanergy Inc, Eiði, Faroe Islands (batch numbers: 023, 024, 025, 027, 029, 030, 031, 032).

2.1. *In vitro gastrointestinal digestion*

The in vitro gastrointestinal digestion model described by Dragnes et al [12] was used to hydrolyze the fish samples. Approximately 12.5 g fish sample were mixed with 50 mL water, and the pH was adjusted to 2 with 3 mol/L HCl. After addition of 50 mg pepsin (Sigma Chemical Co, St. Louis, MO, USA, P6887) that was dissolved in 1 mL water, the mixture was incubated for 2 hours at 37°C at 200 rpm to simulate the stomach phase. The pH was then adjusted to 6.5 with 3 mol/L NaOH, and intestinal digestion was simulated by addition of 2 ml of a solution containing 50 mg of trypsin (Sigma T1426) and chymotrypsin (Sigma C4129), respectively. After 4.5 hours of digestion, the samples were frozen, freeze-dried, and finely ground. The samples for in vitro analysis of ACE inhibitory effect were kept frozen at –55°C until analysis, while the samples for in vivo antihypertensive activity determination in SHR were kept refrigerated (4°C) until administration.

2.2. *Angiotensin-converting enzyme inhibition assay*

Angiotensin-converting enzyme inhibition assay was performed by measuring the end product, hippuric acid, after an enzymatic reaction between ACE (Sigma A6778) and the substrate hippuryl-histidyl-leucine (HHL) (Sigma H1635) [4]. Freeze-dried samples were dissolved in distilled water to a concentration of 20 mg/mL and diluted to a suitable concentration. A sample of 25 μL was pre-incubated with 100 μL substrate (2 mmol/L HHL in 100 mmol/L sodium borate buffer, pH 8.3) at 37 °C for 10 min. Addition of 50 μL (5 mU) ACE (Sigma) started the enzymatic reaction that was carried out on a shaker at 37 °C for 30 min. The reaction was stopped by adding 215 μL 1 mol/L HCl. The end product, hippuric acid, was then measured by quantitative HPLC analyses, as previously described [12], and the amount of sample needed to inhibit 50% ACE activity was defined as the IC_{50} value and presented as μg/mU ACE.

2.3. *In vivo experiments with spontaneously hypertensive rats*

Fifty ten-week-old, male SHR rats (NCrl, specific pathogen free, 283 ± 10 g) with tail systolic BP over 150 mm Hg, were obtained from Charles River Laboratory (Germany). After an acclimation period of 20 days, the rats were individually identified by tail numbering and allotted to 5 different experimental groups (n = 10 per group). The groups received oral treatment with hydrolysates from cod, haddock, and salmon, along with water as negative control and Captopril (Sigma) as positive control. The daily dosages of fish hydrolysates were 200 mg/kg body weight from day 0 to 21 and 500 mg/kg body weight from day 22-28. Captopril was administered at a dosage of 20 mg/kg body weight. The test items were dissolved in sterile water and diluted to concentrations yielding correct daily doses of 10 ml/kg body weight from day 0-21 and 20 ml/kg body weight from day 22-28, based on the most recent body weight data. The dosing solutions were prepared daily and used within 4 hours. The fish hydrolysates or control agents were administered by gavage using a 5 and 10 ml syringe (for doses of 10 mL/kg and 20 ml/kg, respectively) and rat feeding tubes. Using a rat non-invasive tail measurement method (MRBP tail cuff equipment, IITC Life Science with IITC BP, Software v. 1.59), heart rate, systolic, diastolic, and mean BP were measured on day 0 (one day prior to first dosing day) and 6 hours after administration