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[^{123}I]Iodooctyl fenbufen amide as a SPECT tracer for imaging tumors that over-express COX enzymes

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ABSTRACT

This study is concerned with the development of an agent for single photon emission computer tomography (SPECT) for imaging inflammation and tumor progression. [^{123}I]Iodooctyl fenbufen amide ([^{123}I]IOFA) was prepared from the precursor *N*-octyl-4-oxo-4-(4′-(trimethylstannyl)biphenyl-4-yl) butanamide with a radiochemical yield of 15%, specific activity of 37 GBq/μmol, and radiochemical purity of 95%. Analysis of the binding of [^{123}I]IOFA to COX-1 and COX-2 enzymes by using HPLC and a gel filtration column showed a selectivity ratio of 1:1.3. An assay for the competitive inhibition of substrate transfer showed that IOFA exhibited a comparable IC_{50} value compared to fenbufen. In the normal rat liver, a lower level and homogeneous pattern of [^{123}I]IOFA radioactivity was observed by SPECT. In contrast, in the rat liver with thioacetamide-induced cholangiocarcinoma, a higher uptake and heterogeneous pattern of [^{123}I]IOFA radioactivity was seen as hot spots in tumor lesions by SPECT imaging. Importantly, elevated COX-1 and COX-2 expressions from immunostaining were found in the bile ducts of tumor rats but not of normal rats. Therefore, [^{123}I]IOFA was found to exhibit the potential for imaging tumors that over-express COX.

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) have been well recognized for their anti-inflammatory efficacy and cancer-preventing effects. Chronic inflammation is associated with an increased risk of cancer for individuals with inflammatory bowel diseases [1]. Hence, cancer prevention by using NSAIDs is a relevant concept, and reports on their use for this means can be found in literature [2,3]. Their inflammatory mechanism is mainly associated with inflammatory lipids that are catalyzed by the key enzyme, cyclooxygenase (COX) [4]. Three types of COX enzymes have been reported: (1) COX-1, which functions as a house-keeping enzyme and is constitutively expressed in most tissue types; (2) COX-2, which is a highly inducible enzyme under physiological conditions; and (3) a COX-1 splice variant that is termed COX-3 [5–7]. COX mediates the conversion of arachidonic acid (AA) into prostaglandins (PG) via two sequential steps: initial oxidation to PGG2 by using a cyclooxygenase enzyme and subsequent reduction to an unstable endoperoxide intermediate PGH2 by peroxidase (POX) [8]. The two reactions occur in spatially distinct but mechanistically coupled active sites. The cyclooxygenase active site is located at the end of a long hydrophobic channel that is broad near the membrane-binding domain (the lobby) and narrows as it extends toward the interior of the protein [9,10]. The POX active site is located on the surface of the protein near the heme cofactor. Prostaglandin endoperoxide synthase-1 and -2 (PGHS-1 and -2) are also known as COX-1 and COX-2, respectively [11]. PGHSs are composed of two monomers. Each monomer has a physically distinct COX and POX active site. The two monomers have different conformations, and they function cooperatively during catalysis in solution. It is clear

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that the COX reaction is activated through a radical cation intermediate of a heme–monomer complex that is derived from the oxidation of the heme–monomer complex by POX. The key rests in the fact that the Tyr^{385} radical should be formed via POX before the oxidation of AA by COX. COX and POX could maintain their reaction pathways independently. However, the reaction cycle of COX is sensitive and could be terminated by various factors, whereas POX activity is relatively unaffected and can last longer.

The mechanism of inhibition of COXs by traditional NSAIDs is complicated because AA and the inhibitor interact with COX reciprocally. In PGHS-1, the conformations of the two AAs in the AA-COX complexes of the dimer are symmetrical. In contrast, the two conformations of AAs in PGHS-2 are antisymmetrical [12]. PGHS-2 functions as a conformational heterodimer with an allosteric monomer (E_{allo}) and a catalytic (E_{cat}) monomer. For example, some non-substrate fatty acids could bind E_{allo} to stimulate the rate of AA oxygenation. Some COX inhibitory NSAIDs and some COX-2 specific inhibitors function through E_{allo}, E_{cat}, or both E_{allo} and E_{cat} [13,14]. In many tumors, high prostaglandin levels are up-regulated by COX, whereas the degraded enzyme e.g., 15-prostagladin dehydrogenase (15-PGDH) plays a role in the negative regulation of prostaglandin levels.

The long-term uptake of NSAIDs and COX-1inhibitors is always accompanied by adverse side effects such as gastrointestinal toxicity [15,16]. Specific COX-2 inhibitors were developed to overcome this side effect. However, prolonged use of COX-2 inhibitors results in other side effects such as cardiovascular events. Furthermore, very high dosages of COX inhibitors or NSAIDs are frequently required to exhibit tumor inhibition effects, but only low dosages are required for inhibiting prostaglandin formation [1,17]. The antitumoral effects are thus probably not only mediated through the COX pathway but also through an NSAID-activated gene or growth differentiation factor 15 [1].

Because of the complexity of COX mechanisms such as dual functionality of the enzyme and close coupling of the two active sites, agents with unique inhibitory properties are still under development. Apart from the fact that aspirin irreversibly inactivates COX-2 by covalently modifying E_{cat}, other COX inhibitors have been developed such as (a) time-independent E_{allo} inhibitors (ibuprofen with 2-arachidonylglycerol and PGHS-2) or time-independent E_{allo} and E_{cat} inhibitors (ibuprofen with AA and PGHS-2), and (b) time-dependent E_{allo} inhibitors (naproxen with AA and PGHS-2) [11,13,14]. In addition, a number of studies have focused on developing COX-1 selective inhibitors with very low gastric ulcerogenic activities [18,19], because inhibiting COX-1 alone is not sufficient to cause gastric damage [20].

Imaging inflammation as well as tumor progression has attracted a great deal of attention recently [21–36]. As a diagnostic imaging probe for positron emission tomography (PET) or single photon emission computer tomography (SPECT), cytotoxicity is not a serious concern because only very low doses (lower than 1/100 the therapeutic dose) are administered to an individual within a short period [37]. Various PET and SPECT probes based on the structural characteristics of COX-2 [21–35] and COX-1 [36] specific inhibitors have been developed in the past few decades. However, only rare radiopharmaceuticals have been successfully applied to imaging inflammatory events [36]. The reason for this has been attributed to the instability of COX-2 enzymes or due to the very low absolute amount of COX-2 over-expression that is available to be detected [1].

Because the designs of these radiolabeled COX-2 inhibitors are mainly based on a triphenyl ring scaffold or biaryl scaffold that specifically target the COX-2 enzyme, other types of radiopharmaceuticals that target the COX-1 enzyme have been re-investigated [38]. Promising imaging results [39] encouraged us to prepare octyl fenbufen amide (OFA) [40–42], which are members of the NSAID family that do not exhibit COX selectivity (Fig. 1). Specifically, OFA was discovered through parallel solution phase synthesis. Moreover, the octyl group can be used to modify its antitumoral cytotoxicity ($EC_{50} = 20$ μM) in comparison with its parent fenbufen [42]. Arachidonyl ethanolamide (AEA) was reported to be poly-unsaturated fatty acyl amide that exerts immunomodulatory activity through unspecified anchoring to the active site of COX-2 (as well as COX-1) [43,44]. Because both AEA and OFA share a similar structural feature in terms of their eight-carbon chain length, the inhibition of cellular growth by OFA was postulated to be mediated through such an interaction. Hence, OFA might be a tracer suitable for imaging COX function. Taken together, OFA was chosen for radiolabeling and for imaging of COXs in vivo. Specifically, OFA was labeled with radioiodine ^{123}I for non-invasive in vivo imaging using SPECT.

2. Materials and methods

2.1. General

All of the reagents and solvents that we used were purchased from Sigma–Aldrich, Malingkrodt, Acros, Alfa, Tedia, or Fluka. CH_2Cl_2 and toluene were dried over CaH_2, and MeOH was dried over Mg and distilled prior to use. DMF and 1,4-dioxane were distilled under reduced pressure. The reagents and solvents were reagent grade. Preparation of organostannyl compound **3** was carried out in dried glassware under nitrogen at positive pressure. The eluents that were used for flash chromatography such as EtOAc, acetone, and *n*-hexane were of industrial grade and distilled prior to use; MeOH and $CHCl_3$ were reagent grade and used without further purification. Thin layer chromatography (TLC) was performed with MERCK TLC silica gel 60 F_{254} precoated plates. The starting materials and products were visualized with UV light (254 nm). Further confirmation was carried out by staining with 5% *p*-anisaldehyde, ninhydrin, or ceric ammonium molybdate under heating. Flash chromatography was performed using Geduran Si 60 silica gel (230–400 mesh). Melting points were measured with MEL-TEMP and were uncorrected. NMR spectroscopy, including ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz, DEPT-135), was performed on a Varian Unity Inova 500 MHz. D-solvents that were employed for NMR, including CD_3OD, $CDCl_3$, and C_6D_6, were purchased from Cambridge Isotope Laboratories, Inc. Low-Resolution Mass Spectrometry (LRMS) was performed on an ESI-MS spectrometer by employing a VARIAN 901-MS Liquid Chromatography Tandem Mass Q-Tof Spectrometer at the Department of Chemistry of National Tsing-Hua University (NTHU). High Resolution Mass Spectrometry (HRMS) was performed using a varian HPLC (prostar series ESI/APCI) coupled mass detector from a Varian 901-MS (FT-ICR Mass) and a triple quadrapole.

A commercial colorimetric COX (ovine) inhibitor screening assay kit was purchased from Cayman Chemical Ltd (760111). The assay kit contained both COX-1 and

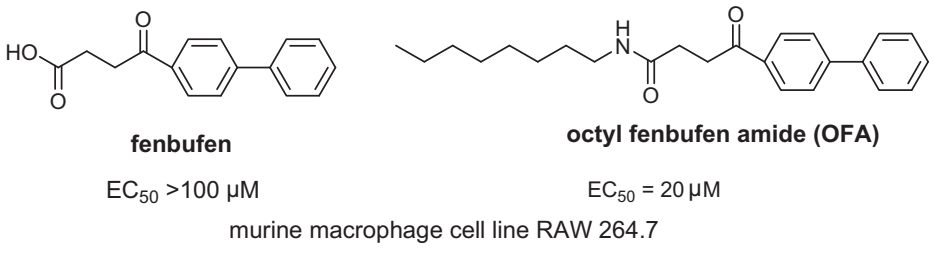

Fig. 1. Enhanced inhibition of cellular growth by octyl fenbufen amide.