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Research paper

Development of an assay for the quantification of type I collagen synthesis in the guinea pig

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Abstract

There is a need for a reliable assay for the quantification of collagen type I synthesis in the guinea pig, an important model for many connective tissue diseases. Procollagen type I C-terminal propeptide (PICP) is the established marker of type I collagen synthesis but, to date, no assay has been developed to measure PICP in guinea pig tissue extracts. A monoclonal antibody, known to cross-react with intact guinea pig procollagen type I (anti-PICP), was tested for its ability to bind soluble guinea pig PICP in crude skin extracts using a biosensor. Anti-PICP was immobilised to the surface of a sensor chip and antibody-antigen binding was detected using the phenomenon of surface plasmon resonance (SPR). The binding component in the SPR-immunoassay was identified as PICP by purification and N-terminal sequencing. Guinea pig PICP was purified from skin by gel filtration, ion exchange chromatography and lectin affinity chromatography. Purified PICP was then biotinylated and used with anti-PICP to develop a competition ELISA that was able to selectively and sensitively measure PICP in extracts of guinea pig connective tissue.

Keywords: Collagen type I synthesis; Guinea pig; PICP; Competition ELISA; SPR

Abbreviations: PICP, procollagen type I C-terminal propeptide; SPR, Surface plasmon resonance; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; GPSX, guinea pig skin extract; RU, response units; SDS, sodium dodecyl sulphate; CV, column volumes; mIgG, non-specific mouse monoclonal antibody BSA, bovine serum albumin; RAM, rabbit anti-mouse polyclonal antibody; anti-PICP, anti-human procollagen type I monoclonal antibody; TMB, 5,5′-tetramethylbenzidine hydrochloride; GPP, guinea pig protein; GPLX, guinea pig ligament extract; GPBX, guinea pig bone extract; MSX, mouse skin extract.

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1. Introduction

The guinea pig is used as a model for many connective tissue diseases such as scurvy, osteoarthritis, atherosclerosis and asthma. In each case, determining the rate of collagen type I synthesis is paramount to an understanding of the pathogenesis (Oyamada et al., 1990; Ivanov et al., 1997; Morishima et al., 2001; Anderson-MacKenzie et al., 2002). In all published work in which collagen type I synthesis has been estimated in the guinea pig, methods such as quantification of collagen type I mRNAs, immunochemical staining of procollagen type I or determination of incorporated tritiated proline have been used (Form et al., 1984; Bylander et al., 1987; Oyamada et al., 1990; Bergsteinsdottir et al., 1993; Kim et al., 1994; Ivanov et al., 1997; Morishima et al., 2001; Lavoie et al., 2002). There has been no reported use of an assay for the quantification of the established marker of collagen type I synthesis, procollagen type I C-terminal propeptide (PICP), in guinea pig tissue or serum.

During the biosynthesis of fibrillar collagen, the amino and carboxyl terminal propeptide domains are cleaved, by specific extracellular tissue endopeptidases, after secretion but prior to fibril aggregation. These highly stable and soluble propeptides are released into the interstitial fluid, and later circulate in the blood supply. As PICP is present at a stoichiometric ratio of 1:1 to each collagen molecule synthesised and is not further metabolised until breakdown in the liver, it is an excellent marker for collagen synthesis. PICP has now been established as the gold standard for determining collagen type I synthesis levels in many fields of interest, particularly bone research (Delmas, 1995; Risteli et al., 1995; Bikle, 1997; Mansell and Bailey, 1998). Determination of PICP content is a more reliable and convenient way of assessing collagen type I synthesis than the methods mentioned previously (Nacher et al., 1999), especially if sample size is limited.

McDonald et al. (1986) raised a monoclonal antibody (mAb) against intact human procollagen type I (anti-PICP) and showed that the epitope resided in the carboxy-terminal propeptide domain, and that the antibody was capable of recognising the cleaved human PICP fragment. The McDonald anti-PICP is used in both the enzyme-linked immunosorbent assay (ELISA, Quidell, Oxford, UK) and the radioimmunoassay (RIA, Orion Diagnostica, Oulu, Finland) for PICP quantification. Neither of these commercially available assays have been successfully used to quantitate guinea pig PICP, but the McDonald anti-PICP has been used for immunochemical staining of intact insoluble procollagen type I in the guinea pig (Bergsteinsdottir et al., 1993). Our study was therefore designed to determine whether this anti-PICP cross-reacts with soluble guinea pig PICP, a prerequisite for future assay development.

Antibody-antigen binding can be detected using a biosensor (Karlsson and Stahlberg, 1995; Ravanat et al., 1998; Hseih et al., 1998). Biosensors do not require a secondary recognition step, but use a phenomenon known as surface plasmon resonance (SPR) to detect a change in mass on the surface of an antibody coated sensor chip, which occurs in the event of antigen binding (Quinn et al., 1997; Nice and Catimel, 1999; Mullett et al., 2000). Anti-PICP was therefore tested using an SPR immunoassay to determine its cross-reactivity with soluble PICP in guinea pig tissue extract. The binding component of the crude tissue extract was identified as PICP by purification and N-terminal sequencing. Purified guinea pig PICP was then biotinylated and used to develop a competition ELISA.

2. Materials and methods

2.1. Reagents

Acrylamide and electrophoresis equipment was purchased from Biorad (Herts, UK). All chromatography columns were supplied by Amersham Biosciences (Bucks, UK). Unless otherwise stated all other chemicals were purchased from Sigma (Dorset, UK).

2.2. Tissue extraction

Three-week-old guinea pigs (Dunkin-Hartley Harlan Olac/Interfauna) were culled, shaved and the belly skin removed. The skin was diced and then reduced to a fine powder in liquid nitrogen using a custom-made freezer mill. The powdered skin was freeze dried overnight and extracted in 20 mM triethanolamine 0.1% Brij 35 and mammalian tissue protease inhibitor