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Determination of Chondroitin-6-Sulphate by a Competitive Enzyme Immunoassay Using a Biotinylated Antigen

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Dedicated to Prof. Dr. Dr. Helmut Greiling on the occasion of his 65th birthday

Summary: A competitive enzyme immunoassay was developed to determine chondroitin-6-sulphate in body fluids and cell cultures. The assay uses a monoclonal anti-chondroitin-6-sulphate antibody, immobilised to microtitre plates, and it involves a competitive binding reaction between chondroitin-6-sulphate in the samples and the biotinylated antigen.

This assay enables the quantification of chondroitin-6-sulphate in the low concentration range of 16–120 μg/l. The intra-assay and inter-assay coefficients of variation are below 6.5% and 9.0%, respectively. More than 90% of chondroitin-6-sulphate was recovered when added to 0.1 mol/l phosphate-buffered saline, an albumin solution (40 g/l in phosphate-buffered saline) and cell culture medium (containing 100 ml/l foetal calf serum).

Chondroitin-6-sulphate was also determined in sera of healthy male (n = 90) and female (n = 90) blood donors. The normal range was 55–169 μg/l. In men the mean value was estimated at 102.2 ±37.1 μg/l and in women at 98.7 ± 26.4 μg/l. No age or sex dependence was observed.

The urine excretion of chondroitin-6-sulphate in men (n = 16) was 44.5 ± 21.1 mg/kg creatinine (mean ± standard deviation) and in females (n = 10) 53.5 ± 21.3 mg/kg creatinine. The clearance rate in men was 0.41 ± 0.22 ml × min^{-1} and in women 0.38 ± 0.15 ml × min^{-1}. No sex dependence was found.

Furthermore, the enzyme immunoassay was modified to measure the specific incorporation of a radioactively labelled precursor ([^{14}C]galactosamine) into chondroitin-6-sulphate. This modification rapidly gives information on the cellular glycosaminoglycan synthesis in cell culture. Using this method our experiments with cultivated human chondrocytes showed that the synthesis of chondroitin-6-sulphate decreased in the presence of interleukin-1α (60.0% less), tumour necrosis factor α (64.4%), γ-interferon (21.6%) and lipopolysaccharide (53.4%).

Introduction

Chondroitin sulphate consists of repeated disaccharide units containing N-acetyl-*D*-galactosamine and *D*-glucuronic acid. This glycosaminoglycan is sulphated either on the C4 or C6 position of the amino sugar (1) and attached to a core protein to form proteoglycans. The macromolecule is synthesised in the *Golgi* apparatus, transported to the cell surface and secreted into the extracellular space (2). Chondroitin sulphate is a major and ubiquitous component of the extracellular matrix of connective tissues (1). Several methods for the determi-