Inhibition of the metabolism of t-fucose in aortic tissue and cultured arterial wall cells

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Much interest has been focused on endothelial and smooth muscle cells of the blood vessel wall, because the development of several pathogenic vascular diseases like thrombosis and atherosclerosis is closely connected with metabolic changes in these cells [1]. The glycoconjugate metabolism of these cells is only poorly understood. In an earlier investigation, we have reported on the biosynthesis of N-acetyll


termed by the addition of 70% (v/v) ethanol and the liberated from precipitated cell protein by hydrolysis and a spectrophotometric method used to determine the amount of GDP-fucose. The pathway for t-fucose synthesis de novo uses GDP-mannose as a precursor to form GDP-fucose, whereas in the second pathway, a specific kinase acts on free fucose to form fucose 1-phosphate, which is further converted to GDP-fucose. Both pathways have been investigated and the enzymes involved characterized in cultured endothelial and smooth muscle cells. Aortic tissue cells were isolated from porcine aortas and cultured as described previously [3]. Fucokinase (EC 2.7.1.52) activity was estimated by measuring the rate of formation of t-[14C]fucose-1-phosphate from t-[14C]fucose. For the estimation of GTP: fucose-1-phosphate guanylyltransferase (EC 2.7.7.30) 14C-labelled fucose-1-phosphate was prepared from GDP-fucose by enzymic conversion with phosphodiesterase of Cratulus auricularius. Aortic tissue or arterial wall cells were homogenized in 0.2 M-Tris buffer containing 75 mM-nicotinamide and 10 mM-MgCl2, and the 10000 g supernatant used immediately in the assay. The incubation of the assay mixture was terminated by the addition of 70% (v/v) ethanol and the reaction products separated by paper chromatography using ethanol/ammonium acetate, pH 3.8 (5:2, by vol.). The assay for conversion of GDP-mannose to GDP-fucose was performed as described by Ripka et al. [4]. For the measurement of protein-bound t-fucose, the deoxyguar was liberated from precipitated cell protein by hydrolysis and a spectrophotometric method used to determine the amount of t-fucose liberated [5].

The different enzymes necessary for the synthesis de novo of GDP-fucose, but also for the utilization of free fucose, were present in aortic tissue and cultured arterial wall cells. Under standard assay conditions of fucokinase, maximal enzyme activity was obtained at a concentration of about 6 x 10^{-5} M-t-fucose. By Lineweaver-Burk analysis a K_m of 1 x 10^{-3} M was determined. The pH optimum is at 8 with sharp declines in the enzyme activity above and below this value. Addition of Ca^{2+} to the standard reaction mixture led to a dose-dependent decrease in the enzyme activity. A cell-proliferation-dependent influence on enzyme activity was observed, with highest activity found at the confluent stage. Cultured smooth muscle cells expressed lower fucokinase activity than endothelial cells (3.2 ± 0.8 nmol/h per mg of protein versus 8.7 ± 1.8 nmol/h per mg of protein).

GTP: fucose-1-phosphate guanylyltransferase exhibited a significantly higher activity compared with fucokinase. Maximal enzyme activity was obtained at a concentration of 5 mM-fucose-1-phosphate. From these data a K_m of 1 x 10^{-3} M was calculated. The estimated specific activities of 121 ± 19 nmol/h per mg of protein for endothelial cells and an even higher enzyme level in smooth muscle cells (205 ± 64 nmol/h per mg of protein) indicates that this enzyme is considerably more active in arterial wall cells than in aortic cells.

For the synthesis de novo of GDP-fucose from GDP-mannose by arterial wall cells were found to be at pH 8 in the presence of 5 μM-GDP-mannose and 15 mM-Mg^{2+}. Under these conditions, a specific activity of 2.6 ± 0.3 nmol/h per mg of protein was estimated in confluent endothelial cells. Smooth muscle cells produced 1.1 ± 0.1 nmol of GDP-fucose/h per mg of cell protein. Under conditions in vitro it is possible to follow the conversion of free t-fucose to fucose-1-phosphate and GDP-fucose. The conversion of t-fucose into soluble intermediates amounted to about 50% during an incubation time of 5 h. Pretreatment of cultured arterial wall cells with increasing doses of nicotine, a component of tobacco smoke and possible risk factor in the development of cardiovascular disease, largely influenced the conversion of t-fucose in the cell-free system. Treatment of endothelial cells with 1 mM-nicotine for 18 h led to a 20% reduction of fucose-1-phosphate and a 58% reduction in GDP-fucose formation compared with control. A further reduced level of t-fucose conversion was observed after pretreatment with 5 mM-nicotine, 68% and 94% inhibition of fucose-1-phosphate and GDP-fucose formation, respectively. The influence of
nicotine is not a direct effect on enzyme activity, but is only observed after pretreatment of cells for at least 6 h.

The measurement of protein-bound L-fucose revealed that aortic tissue and cultured arterial wall cells contain a high level of this deoxysugar. There was no significant difference in the sugar content between endothelial cells (23.9 ± 8.9 nmol/mg of protein) and smooth muscle cells (23.9 ± 3.2 nmol/mg of protein). Total aortic tissue exhibits a level of 12.1 ± 2.1 nmol/mg of protein-bound L-fucose.

The results obtained indicate that the arterial wall is a highly active tissue of the body in respect to L-fucose metabolism.

Serum keratan sulphate levels rise in rheumatoid arthritis patients, but fall in ankylosing spondylitis patients compared with normal controls

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Summary

Serum levels of keratan sulphate (KS) were found to be significantly elevated in patients with destructive and predominantly seronegative rheumatoid arthritis (RA) compared with a control population. Levels in RA did not correlate with clinical or laboratory indices of joint activity or damage. Conversely levels were depressed in ankylosing spondylitis (AS) compared with controls.

Introduction

KS is a glycosaminoglycan which quantitatively is almost entirely confined to hyaline cartilage. Serum levels rise in cartilage breakdown and may be a useful marker of cartilage turnover in rheumatic conditions [1].

Methods

1. Patient selection. Out-patients with RA and AS who were being assessed in clinical and radiological studies had serum stored at −70°C for measurement of the KS level. Serum was also collected from adult controls who denied a history of joint disease.

2. Clinical assessment. In the RA study, data were collected on the level of joint pain and stiffness, the number of swollen and tender joints (Ritchie index), the hand functional index (HFI), a component of the Keitel function test (KFT) [2], the range of joint movement and the Stanford health assessment questionnaire (HAQ). A score for the degree of joint damage (DS) was derived depending upon the loss of joint movement (score 0–20). In the AS group, data were obtained for the degree of spinal pain, stiffness and loss of movement and the level of peripheral joint inflammation (swelling and increased warmth) and damage (loss of joint movement).

3. Laboratory assessment. The C-reactive protein (CRP) level was measured in all patients and the rheumatoid factor titre in PA patients.

4. Serum KS level. This was measured using an inhibition enzyme-linked immunosorbent assay (E.L.I.S.A.) technique [3]. Proteoglycan was prepared from the hyaline cartilage of the knee joint of a 73-year-old male cadaver and the SD4 monoclonal antibody was obtained from ICN Biomedicals Ltd.

Results

1. Rheumatoid arthritis. The patients are predominantly seronegative (35/42); 34 female, 8 male; mean age 59.7 ± 11.9 years, mean disease duration 15.3 ± 7 years. Serial determinations of KS gave reproducible results and freezing/thawing of the sample four to five times did not influence the KS level. The mean KS level in the RA patients was significantly increased compared with the controls (P < 0.001) (Fig. 1). The KS level in the RA patients did not correlate with the Ritchie index, Keitel, HAQ, peripheral joint damage or CRP.

2. Ankylosing spondylitis. The AS patients (24 male, 6 female) had a mean age of 43.8 ± 10.3 years and a mean disease duration of 23.8 ± 9.8 years. The serum KS level was significantly depressed in the AS patients compared with the controls (P < 0.001) (Fig. 1).

3. Correlation of clinical parameters with serum KS levels. The KS level in RA patients did not correlate with the Ritchie index, Keitel, HAQ, peripheral joint damage or CRP. There was a trend for RA patients with more severe joint damage to have higher KS levels. Similarly in AS, clinical

Fig. 1. Serum KS levels (ng/ml) in RA, AS and controls

RA (n = 42) versus controls (n = 19): 361 ± 177 versus 227 ± 72 (P < 0.001*). AS (n = 30) versus controls: 154 ± 51 versus 227 ± 72 (P < 0.01). *Statistics by an unpaired t-test.

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