α- and β-Mannosidoses

A. COOPER, C. E. HATTON, M. THORNLEY and I. B. SARDHARWALLA
Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital,
Pendlebury, Manchester M27 1HA, UK

Summary: Clinical, pathological and biochemical findings in the mannosidoses are described. Family studies showed granulocyte-rich white cell fractions to be the tissue of choice for carrier detection in β-mannosidosis. Metabolic labelling studies using [3H] mannose demonstrated accumulation of Manβ1-4GlcNAc in cultured skin fibroblasts from a patient with this condition. Alternative methods of egress from lysosomes were suggested for this compound by its secretion into culture medium and apparent reduction of storage with time in cultures. β-mannosidase deficient goats are not thought to be a true animal model of the human condition, as although they showed a similar enzyme deficiency, the clinical presentation is much more severe and the major storage material (Manβ1-4GlcNAcβ1-4GlcNAc) is different.

Defective lysosomal catabolism of glycopeptides has been widely described (Beaudet and Thomas, 1989). Deficiencies of lysosomal enzymes lead to accumulation of undegraded storage products. The mannosidoses are two such diseases due to deficiencies of α-mannosidase (EC 3.2.1.24) and β-mannosidase (EC 3.2.1.25). In α-mannosidosis, oligosaccharides containing α1-3 and α1-6 linkages accumulate (Strecker et al., 1976; Yamashita et al., 1979), whereas in human β-mannosidosis the major storage material is a disaccharide, Manβ1-4GlcNAc (Cooper et al., 1988; Dorland et al., 1988b). α-Mannosidosis is the more severe disorder (Beaudet and Thomas, 1989), with symptoms resembling the mucopolysaccharidoses, whilst in β-mannosidosis the major findings are mental retardation, deafness, and in older patients, angiokeratoma (Cooper et al., 1986; Dorland et al., 1988a). Pathological findings in both conditions include cytoplasmic vacuolation due to lysosomal storage (Kjellman et al., 1969; Sung et al., 1977; Cooper, unpublished observation). Animal models are known for both conditions. Bovine α-mannosidosis resembles the human disorder (Whitem and Walker, 1957; Jolly and Thompson, 1978) but caprine β-mannosidosis (Jones et al., 1983) is more severe. Results of family studies and metabolic labelling experiments will be described for β-mannosidosis and the differences between the human condition and the animal analogue will be discussed.

Materials and methods

White cells and plasma were prepared and skin fibroblasts cultured as previously described (Cooper et al., 1987; Cooper et al., 1988). Bio-Gel P-2 chromatography,
oligosaccharide TLC and mannosidase assays were accomplished as reported (Cooper et al., 1988). Lymphocyte-rich and granulocyte-rich fractions were isolated from heparanized blood by centrifugation on Histopaque (Sigma). Metabolic labelling studies were performed in 25 cm$^2$ tissue culture flasks. Cultures of controls and mutant cells were subcultured into 10 flasks and just prior to confluence culture medium was replaced with medium supplemented with 10 μCi/ml [D-2-3H]$\text{mannose}$ (Amersham International). After a four day ‘pulse’, labelled medium was removed and cells were washed three times with phosphate buffered saline (PBS). Cultures were maintained in unlabelled medium and flasks harvested in duplicate, immediately (day 0), and at days 1,3,5 and 11 (chase). Cells were harvested by scraping, washed three times with PBS, and disrupted by sonication as previously described (Cooper et al., 1988). After centrifugation (4000 g, 10 min), supernatants were applied to Bio­Gel P-2 columns (1.5 × 100 cm) and eluted with water. 1 ml fractions were collected and the radioactivity in 25 μl aliquots in 4 ml Unisolve (Koch-Light) was determined in a Rack-Beta 1216 scintillation counter (Pharmacia-LKB). Culture medium from the chase period was deproteinized by addition of an equal volume of 5% (w/v) trichloroacetic acid. The supernatant obtained by centrifugation was chromatographed on Bio-Gel P-2 as described. Mutant cells were also labelled with medium containing 1 μCi/ml D–[U-14C]$\text{glucosamine}$ (Amersham International) and 10 μCi/ml tritiated mannose for a seven day pulse and harvested after a one day chase in unlabelled medium. Dual-labelled trisaccharide was digested for 24 h with endoglycosidase H (Sigma) in 0.1 mmol/L citrate/0.2 mmol/L phosphate buffer, pH 4.0. Reaction products were isolated by Bio-Gel P-2 chromatography. Undigested trisaccharide was further incubated with α-mannosidase (Sigma) under identical conditions.

ANIMAL MODELS OF THE MANNOSIDOSES

Animal models of α-mannosidosis include Aberdeen Angus cattle (Whittem and Walker, 1957; Jolly et al., 1980) and cats (Burditt et al., 1980b). The former is of considerable economic importance. α-Mannosidosis may also be induced by ingestion of Swainsonine, a potent α-mannosidase inhibitor (Daniel et al., 1984).

A deficiency of β-mannosidase in anglo-nubian goats was first described in 1981 (Jones et al., 1981; Healy et al., 1981), five years prior to the disorder being detected in man (Cooper et al., 1986; Wenger et al., 1986). In the caprine species the presentation is much more severe. Findings include facial dysmorphism involving doming of the skull, ocular abnormalities, folding of the ears, and extensive demyelination of the central nervous system; severe neurological symptoms, including intention tremor and inability to stand; joint hyperextension, muscle atrophy, mental retardation, nerve deafness and death in the neonatal period (Jones et al., 1983). There is marked cytoplasmic vacuolation of all tissues, including the brain (Lovell and Jones, 1983; Malachowski and Jones, 1983; Render et al., 1988).

J. Inher. Metab. Dis. 13 (1990)