Lethal osteogenesis imperfecta: abnormal collagen metabolism and biochemical characteristics of hypophosphatasia

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Abstract. We have examined collagen from a patient with the rare type IIC form of perinatally lethal osteogenesis imperfecta, in whom biochemical characteristics of hypophosphatasia were also apparent. In addition to normal α1(I) and α2(I) chains, there were chains overmodified along their lengths. Unexpectedly, the thermal stability of molecules containing these chains was normal. This suggests the existence of a structural mutation causing delayed triple helix formation, situated in either the α1(I) or α2(I) C-terminal propeptide. Since collagen synthesised by fibroblasts from each of the patient’s parents was normal, the mutation was probably newly arising and dominant. In contrast to other reported cases of lethal osteogenesis imperfecta, not only was the secretion of collagen by cultured fibroblasts considerably retarded, but that of non-collagenous proteins was also severely impaired.

Key words: Osteogenesis imperfecta type II – Collagen type I – Genetic defect – Alkaline phosphatase

Introduction

Osteogenesis imperfecta is a group of heritable disorders characterised by generalised connective tissue defects, although the principal clinical manifestation is an abnormal fragility of bone [13, 26]. A working classification on clinical and genetic criteria [23, 24] is generally accepted. Infants with the most severe form, the type II, or perinatally lethal variant, itself a heterogeneous entity [25], usually die at birth, or shortly after. The molecular basis of type II OI appears to reside in abnormalities of type I collagen [19]. Here are described abnormalities of structure and metabolism of type I collagen in an infant with the rare variant, type IIC. In addition to normal α1(I) and α2(I) chains, chains which had been overmodified along their lengths were present. In contrast to previously reported cases of type II OI (see Discussion) the thermal stability of molecules containing these latter chains was normal. The findings suggest the existence of a structural mutation delaying triple helix formation, which may be situated within the C-terminal propeptide region of a pro-α1(I) or pro-α2(I) chain, rather than in the helical region of the molecule. Surprisingly, the patient also displayed biochemical characteristics of hypophosphatasia [20]. A brief account of some of this work has already been given [22].

Materials and methods

Details of most of the methodology are provided in [27]. Modifications and additional methods are described below.

Electron microscopy. Skin obtained at autopsy from the patient and controls was fixed at 4°C with 4% (v/v) glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4. Thoracic vertebral bone was fixed in 4% (w/v) neutral buffered formaldehyde, post-fixed in 2% (w/v) osmium tetroxide in 0.2 M collidine, then dehydrated, first in ethanol and then in propylene oxide. It was embedded in Spurr’s low-viscosity medium and ultra-thin sections were stained with uranyl acetate and lead citrate.

Extraction of collagen from skin. Skin was finely chopped and subjected to digestion at 4°C in 0.5 M acetic acid containing peptic. The extract was dialysed against 0.5 M acetic acid, clarified by centrifugation and lyophilised.

Dermal fibroblast cultures. Fibroblast cultures were established from skin from the patient and each of her parents and from a 3-year-old control, and grown in EMEM with Earle’s salts, containing 10% (v/v) fotal calf serum.

Preparation of labelled collagens. Cells at early confluency were pre-incubated in growth medium supplemented with ascorbic acid and then labelled in serum-free medium containing [3H]proline (10 μCi/ml) and [3H]glycine (10 μCi/ml). The cell layer was scraped into the medium and the pH was adjusted to 2 with 6 M HCl. Pepsin was added to 100 μg/ml and digestion was allowed to proceed for 16 h at 4°C, being terminated by adjusting the pH to 8. Collagens were precipitated by the addition of NaCl to 20% (w/v), collected by centrifugation, resuspended in, and dialysed against, 0.5 M acetic acid, and lyophilised.

Preparation of labelled unhydroxylated procollagen (procollagen). Cells at early confluency, which had been pre-incubated in medium containing ascorbic acid, were labelled with
Determination of alkaline phosphatase. Alkaline phosphatase activity in plasma from the patient and her parents was measured as described [29]. An indication of the proportions of the bone and liver isoenzyme forms in the patient's plasma was obtained by measuring alkaline phosphatase activity following heating of the sample at 56°C for 10 min [17]. The total activity and the isoenzyme forms present in plasma from the patient's parents were quantified using specified conditions [14].