EXPRESSION OF HUMAN PROCOLLAGEN (I) IN MILK OF TRANSGENIC MICE

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Introduction

Collagens used for medical devices and theraeutic applications are isolated from tissues of farm animals. Although it would be advantageous to supply human rather than non-human collagen for therapeutic purposes, the sources for purified human collagen are limited and the only reliable source is human placenta or cell culture. The production of recombinant collagen is made troublesome by the neccessity for a multiplicity of posttranslational modifications, carried out by at least eight different enzymes which are present only in cells which natively produce collagen.

This has limited attempts at recombinant production to cells which natively produce collagen resulting in chimeric forms of the protein consisting of both human and host cell collagen. This complication can be avoided by expressing the protein in cells that normally do not produce fibrillar collagens. Type I collagen has a three-stranded helical structure and contains two α1(I) chains and one α2(I) chain. Transgenic mice were generated containing the human type I procollagen genes (α1 and α2) under the transcriptional control of a mammary gland specific gene promoter. These transgenic mice express human type I procollagen trimers at high levels in their milk.

Expression of human procollagen in milk

Transgenic mice were generated containing only the α1 transgene, and double transgenic mice were generated containing both α1 and α2 transgenes. To determine expression levels of recombinant procollagen, milk samples were analyzed by SDS-PAGE and immunoassay (ELISA). In milk samples from mice containing only the α1 transgene, up to 10 mg/ml of homotrimeric α1(I) procollagen was detected. Expression was detectable at variable levels in all mouse lines containing one or more intact copies of the transgene. In milk samples from mice containing both α1 and α2 transgenes, up to 3 mg/ml of heterotrimeric procollagen (I) was detected.

Specificity of transgene expression

Northern blot analysis of RNA isolated from various tissues of transgenic mice was performed to investigate the transgene expression pattern. Transgene derived transcripts (4,8 and 5,8 kb) were of the expected length, no aberrantly sized transcripts were observed. Furthermore, transgene expression was limited to the lactating mammary gland. The amount of transgene derived RNA and the amount of protein secreted into the milk appeared to correlate.

Characterization of human procollagen (I) expressed in milk of transgenic mice

Mouse milk samples from mice containing the α1 transgene were analyzed by SDS-PAGE under reducing conditions. High levels of transgene derived human α1(I) procollagen were detected. On a reducing gel a band was observed in transgenic mouse milk with a molecular weigth of approximately 160 kD. This is the expected size for α1(I) procollagen. Milk samples from mice containing both α1 and α2 transgenes contained, in addition to the α1(I) procollagen band, an α2(I) procollagen band running at 140 kD.

The identity of the 160 kD and 140 kD bands was confirmed by Western blot analysis. Two independent polyclonal antibodies raised against the C-propeptide and N-propetide of human α1(I) procollagen cross-reacted with the 160 kD band, while antibodies recognizing the N-terminus of human α2(I) procollagen cross-reacted with the 140 kD band. Milk samples containing high levels of procollagen protein were incubated with bacterial collagenase. The recombinant human procollagen was completely digested, further substantiating that this protein was human procollagen.

To examine whether recombinant human procollagen ocurred in the triple helical form, its sensitivity to trypsin, chymotrypsin and pepsin was determined. The protein appeared to be resistant to the action of these enzymes, strongly suggesting that the procollagen propeptides were in a triple helical conformation.