Overexpression of insulin-like growth factor binding protein-1 in transgenic mice

Abstract Overexpression of insulin-like growth factor-1 binding protein (IGFBP-1) in transgenic mice has provided insight into the physiological role of this binding protein in modulating the metabolic and growth-promoting effects of the IGFs. IGFBP-1 transgenic mice demonstrate both intrauterine and postnatal growth retardation. Organ weight was proportionately reduced relative to body weight in most organs, with the exception of the brain, which was disproportionately small in transgenic mice. There were no gross neurological manifestations of the reduction in brain size. Transgenic mice also demonstrated fasting hyperglycemia, impaired glucose tolerance, and modest insulin resistance in skeletal muscle and hepatic tissue. From these data, we can conclude that overexpression of IGFBP-1 results in inhibition of IGF action and in profound impairment of brain development, modest inhibition of fetal and postnatal growth, and inhibition of the metabolic effects of the IGFs. Increased expression of IGFBP-1 has been documented in a variety of situations, such as fetal nutritional deprivation and hypoxia, and has been considered to be a marker of metabolic disturbances that cause fetal growth retardation. The observations in IGFBP-1 transgenic mice suggest expression of IGFBP-1 may itself contribute to the growth retardation and impaired fetal brain development.

Key words Hyperglycemia · Glucose intolerance · Diabetes · Metabolic effects · Insulin-like growth factors · Growth

Introduction

The insulin-like growth factor binding proteins (IGFBPs) are a family of structurally similar proteins that are widely expressed and present in the serum, other biological fluids, and tissue extracts. They bind the IGFs with affinities comparable to the IGF-I receptor, and thus modulate the bioavailability of the IGFs. Six members of the IGFBP gene family have been identified [1]. Although they share approximately 50% homology, predominantly at the N- and C-terminal ends, they are derived from separate genes and show different hormonal, developmental, and tissue-specific regulation of expression [2]. Of the IGFBPs that have been shown to be present in the circulation, IGFBP-3 is the most abundant and appears to be responsible for the majority of the IGF binding capacity present in plasma [3].

While both enhancement and inhibition of IGF action by the IGFBPs can be demonstrated in various in vitro assays, the emerging consensus is that they are mainly inhibitory. In addition, other non-IGF-dependent functions have been proposed for the IGFBPs. These include growth inhibition in the case of IGFBP-3 [4] and cell migration in the case of IGFBP-1 and -5 [5, 6].

An important function of the IGFBPs is to limit the hypoglycemic effects of circulating IGF-I and IGF-II [7, 8]. These growth factors are present in concentrations more than 100-fold higher than insulin and would, at these high levels, exert an effect at the insulin receptor if not for the presence of the IGFBPs in serum and tissue fluid. In this regard IGFBP-3 is the major serum IGFBP, and virtually all the IGFBP-3 present in the circulation is saturated with IGF-1 or IGF-II [3]. The IGF bound to IGFBP-3 in the ternary complex has a long half-life compared with free IGF or IGFBP present in lower molecular weight complexes [9]. IGFBP-1 has a lower affinity for IGFs and is present in much lower concentrations than IGFBP-3 and most of the other binding proteins [7]. However, IGFBP-1 is largely unsaturated and is acutely regulated by nutritional intake and a variety of hormones, most notably insulin [10].
The physiological role of the IGFBPs remains unclear. There appears to be considerable overlap and possibly some redundancy of function of the IGFBPs. This latter notion is supported by the limited phenotype in IGFBP-2 knock-out mice [11]. IGFBP-4 and IGFBP-3 null mutant mice have also been generated, and preliminary reports indicate that their phenotype is not particularly dramatic [12], possibly indicating some compensation for loss of function by the other binding proteins.

Transgenic mouse technology, with overexpression of particular binding proteins in a generalized or tissue-specific fashion, offers another opportunity to study the functions of the various binding proteins. In an attempt to determine the physiological roles of IGFBP-1 we have generated transgenic mice that overexpress IGFBP-1 in most, if not all, tissues [13].

**Generation of transgenic mice**

The methods used for the generation and characterization of the IGFBP-1 transgenic mice have been described in detail elsewhere and will not be reviewed here [13]. The transgene was constructed using approximately 5 kilobases of rat genomic DNA containing the entire coding region of the IGFBP-1 gene, including the 3' untranslated sequence, the 5' untranslated region, and 78 base pairs of 5' flanking DNA subcloned downstream of the mouse phosphoglycerate kinase promoter (PGK). This promoter has the advantage of generating ubiquitous transgene expression from very early in embryogenesis. Transgenic mice were generated by pronuclear injection of the PGK-IGFBP-1 fragment devoid of plasmid sequences into fertilized BL57J/CDA F1 zygotes. Founders were bred to homozygosity with wild-type CD-1 mice. Non-transgenic mice derived from the F1 cross of the founders with CD-1 mice were bred in a similar fashion to the transgenic mice to provide wild-type, non-transgenic mice of the same genetic background as the transgenic animals.

Four different IGFBP-1 transgenic mouse strains were developed. These strains had different transgene copy number, integration sites, and also had variable serum levels of rat IGFBP-1, the product of the transgene [13]. Serum levels of IGFBP-1 varied from below the limit of sensitivity of the assay in one “low-expresser” transgenic mouse strain, to as high as 80 ng/ml in the strain that showed the highest level of expression. In general, the phenotypic manifestation of IGFBP-1 overexpression correlated with the mean serum levels of IGFBP-1 in the different mouse strains.

**Growth and allometry in IGFBP-1 transgenic mice**

Transgenic mice of all three high-expressing strains were significantly smaller at birth (Fig. 1). The birth weight of transgenic mice of the founder that had undetectable serum IGFBP-1 concentrations was not significantly different from non-transgenic litter-mates [13]. This strain of transgenic mice was considered to be a low-expressing strain since in all tissues examined, transgene-derived IGFBP-1 mRNA, although detectable, was considerably less abundant than in the other transgenic strains. At 1 month of age transgenic mice were 6.3–7.9 g lighter than the non-transgenic mice, depending upon the level of expression in the individual transgenic strains.

Organ weights relative to total body weight for the IGFBP-1 transgenic mice have been discussed in detail elsewhere [13]. In most cases there was a proportionate reduction in organ size. The most-obvious exception was the marked reduction in brain size. There were no gross neurological manifestations of this apparent reduction in brain size, although detailed testing has not been performed. This reduction in brain weight was observed in all four transgenic strains, including the low-expressing transgenic strain. In the transgenic strain with the highest level of expression, the absolute brain weight was reduced to 60% of the wild type mice (299±17 mg vs. 499±11 mg). Brain DNA content was less markedly reduced, with transgenic mice having approximately 85% that of wild-type mice [14]. Myelin staining was generally reduced and there was a diminished thickness of the corpus callosum of transgenic mice compared with wild-type mice. The sizes of the hippocampus and dentate gyrus were significantly reduced [14]. The deficit in DNA...