Animal insulin-like growth factor binding proteins and their biological functions

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ABSTRACT: Insulin-like growth factor (IGF-I, IGF-II) action is influenced by until today known eight forms of insulin-like growth factor binding proteins (IGFBPs). They have been obtained not only from some human and animal tissues and body fluids but also from conditioned medium of cell cultures. An important biological property of the IGFBPs is their ability to increase the circulating half-life of the IGFs. They are able to act as potentiators of cell proliferation. As IGFBPs bind to cell surfaces, they may act either to deliver the IGFs to those surfaces for activation of specific receptors or to activate cell responses independently of receptor activation. Phosphorylation, glycosylation and proteolysis of IGFBPs influence their affinity to IGFs. The IGFBPs in the role of inhibitors may block the activity of the IGFs and be used for antimitogenic therapy. In the last time measuring of IGFBPs levels can be used for diagnosis determination of some endocrine diseases or in differential diagnostics.

Keywords: insulin-like growth factor; insulin-like growth factor binding protein

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1. INTRODUCTION

High-affinity, soluble IGFBPs are specific proteins able to form complexes with IGFs in extracellular and interstitial fluids of live organisms. They influence IGFs transport to receptors on cell surfaces and also IGFs effects on cell proliferation. IGFBPs are proteins with different size, they are produced by many different tissues, especially by the liver and they bind to IGF-I, IGF-II, but not to insulin (with the exception of IGFBP-7). IGFBPs modulate IGFs effects by endocrine, paracrine and autocrine mechanisms of regulation. The origin of this regulation may depend on IGFBPs recognition such as glycosylated binding protein or posttranslation modification such as phosphorylation. IGFBPs modify the IGFs effects by regulation of IGFs transport, IGFs concentration in specific tissues, IGFs interaction with cell surface receptors, potentiation or inhibition of IGFs function.

The aim of this review is to inform the veterinary community about new knowledge in IGFBPs function in live organisms and its application in *in vitro* techniques, in biomolecular and genetical methods not only in growth factor studies but also in laboratory diagnostics of some diseases. A similar topic was reviewed for domestic animals by Hossner *et al.* (1997) and partially was published by us elsewhere (Kostecká and Blahovec, 1999).

2. CLASSIFICATION OF IGFBPs

Eight well characterized forms of IGFBPs with different molecular weight, amino acid composition, binding properties and distribution in biological fluids have been identified. Expression of these multiple IGFBP species is under developmental, hormonal and nutritional control.

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IGFBP-1 (25 to 34 kDa) is growth hormone-independent, but its production is increased by somatostatin and its analogues, such as octreotide and lanreotide (Lewitt *et al.*, 1998). IGFBP-1 is the most important binding protein in amniotic fluid. It was originally isolated from human placenta as placental protein 12 (Koistinen *et al.*, 1986). Today we know that it is produced by stromal cells of the endometrium in the postovulatory cycle phase (when progesterone is increased) of non-pregnant women and decidual cells of the endometrial stroma of pregnant women (progesterone influence). IGFBP-1 is a major secretory product not only of the decidualized human but also baboon endometrium (Seppala *et al.*, 1994). The affinity of IGFBP-1 is from 5 to10 times higher to human IGF-I than to human or rat IGF-II (Frauman *et al.*, 1989). IGFBP-1 was also found in the serum at 100 times lower concentration than IGFBP-3. It seems that the complex IGF-IGFBP-1 (30 to 40 kDa) in amniotic fluid is identical with the complex in the serum. IGFBP-1 in amniotic fluid has higher affinity to IGF-I than to IGF-II (D'Ercole *et al.*, 1985). On the other hand, Wang *et al.* (1990) found that ovine amniotic fluid contains 38-kDa IGFBP with 30 times higher affinity to IGF-II than to IGF-I. This can be connected with species differences.

Human maternal serum IGFBP-1 levels increase and reach the top in the 18 to 21 week of pregnancy. There is a positive correlation between serum progesterone and IGFBP-1 of endometrium, but not of serum. It means that progesterone plays an important role in the regulation of tissue changes by paracrine or autocrine mechanisms and the changes in IGFBP-1 secretion may be correlated to differentiation of stromal cells (McCusker and Clemmons, 1992).

Hypoxia of sheep fetuses caused by a decrease in the blood flow in uterus is followed by an increase in fetal plasma IGFBP-1 concentration and decrease in IGFBP-2 concentration. These changes can influence the biological function of IGFs and tissue specificity in DNA synthesis rate. The same changes were described in rat fetuses on low protein diet and human fetuses in intrauterine growth retardation (McLellan *et al.*, 1992).

IGFBP-1 with protease cathepsin-L may play a role also in trophoblast invasion. Boomsma *et al.* (1994) found that in the case of cat implantation the IGFBP-1 increases while cathepsin-L decreases. Therefore IGFBP-1 may regulate the trophoblast growth while cathepsin-L may affect trophoblast invasion by digesting the extracellular matrix.

In animals (sheep and goats) besides IGFs there are also proteins with higher molecular weight that are mitogenically active in BP-A31 cell culture (Blahovec *et al.*, 1997). Their properties are the same or very similar to those of human IGFBP-1 and IGFBP-3 (Bla-hovec *et al.*, 1999, 2001a). The recent studies have however shown that these mitogenically active proteins are probably high molecular precursor forms of IGF-II (Blahovec *et al.*, 2001b).

IGFBP-2 (32 to 34 kDa) is present in human cerebrospinal fluid, seminal plasma and lymph, in rat amniotic and cerebrospinal fluid, in chicken vitreal fluid. Vitreal IGFBP-2 is synthesized locally and it is not derived of serum IGFBP because it differs by glycosylation and molecular weight (Schoen *et al.*, 1992). It was also obtained from a conditioned medium of *membrana granulosa* cells of porcine ovary follicle. These cells are probable sources of IGFBPs in the porcine follicular fluid (Mondschein *et al.*, 1990).

High concentrations of IGFBP-2 were observed in rat fetal tissues and in porcine fetal serum that decrease in postnatal life (Russell and Van Wyk, 1995). Lee *et al.* (1993) described an increase in porcine fetal serum IGFBP-2 in the second half of pregnancy and its decrease *post partum*. The changes in IGFBP-3 concentration were opposite. The same changes were found in human and sheep fetuses. In preference IGFBP-2 binds to IGF-II, which is an important regulator in fetal growth (Hossner *et al.*, 1997).

IGFBP-1 and -2 concentrations are twice higher in serum than in lymph and it seems that they are the main IGF transporters (McCusker and Clemmons, 1992).

A potential role of IGFBPs in transporting and targeting IGFs to their receptors is supported by the finding that abundant IGFBP-2 is associated with cell surface proteoglycans in the human developing brain (Werther *et al.*, 1998).

IGFBP-3 (53 kDa) is growth hormone-dependent, acid-stable subunit of 150-kDa complex, binds to IGF-I or -II with high affinity, can function as an inhibitor or activator of IGF-I stimulated DNA synthesis. Molecular weight of IGFBP-3 depends on glycosylation degree. Human and porcine IGFBP-3 has three and rat binding protein four N-glycosidic bonds. Glycosylated IGFBP-3 is able to bind to cell surfaces by a weak non-covalent sugar-sugar interaction. Free IGFBP-3 has from 3 to 10 times higher affinity to ligand than cell surface-associated IGFBP-3 (McCusker and Clemmons, 1992).

IGFBP-3 is a dominant binding protein in the blood in 40 times higher concentration than IGFBP-1 and with higher affinity to IGF-I. The majority of circulatory IGF-I is bound to IGFBP-3. IGFBP-3 serum levels are 10 times higher than in lymph (McCusker

and Clemmons, 1992). IGFBP-3 is present also in cerebrospinal fluid, in human and rat lymph, in porcine and rat colostrum and milk, in human and porcine follicular fluid, in human seminal plasma and last trimester amniotic fluid.

IGF-I and IGFBP-3 concentrations are low in porcine serum during fetal life, but they increase in postnatal life. IGF-II levels are higher than IGF-I levels during the whole fetal development (Lee *et al.*, 1993).

Membrana granulosa cells of porcine follicle produce not only IGFBP-2 and -3, but also lower molecular weight IGFBPs. IGFBPs amounts and forms differ in the differentiation degree of *membrana granulosa* cells (Mondschein *et al.*, 1990).

IGFBP-3 is the predominant mammary IGFBP and its concentration also declines in blood and milk during lactation. On the other hand, during the involution period lactoferrin is critically involved in the regulation of the IGF system because lactoferrin has the capacity to compete with IGF binding to IGFBP-3. In this process all transretinoic acid is necessary for the entry of exogenously added lactoferrin into the mammary cell nucleus, while IGFBP-3 entry into the nuclei of all transretinoic acid treated cells also required the presence of lactoferrin (Baumrucker and Erondu, 2000).

More recently IGFBP-3 has been assigned a role as a putative death-promoting factor (Butt and Williams, 2001).

IGFBP-4 was isolated in two forms with different molecular weight (29 kDa and 24 kDa) from the ovine blood plasma. IGFBP-4 with higher molecular weight is N-glycosylated like ovine IGFBP-3 (Hossner *et al.*, 1997). A high degree of identity of ovine IGFBP-4 to rat (90%), human (96%) and bovine (98%) IGFBP-4 was described. In adult sheep it is present especially in liver, kidney, lungs, heart and some fetus tissues (Carr *et al.*, 1994). IGFBP-4 (24 kDa) was also found in human, rat and porcine serum (McCusker and Clemmons, 1992). Liver is the major source of this serum BP (Russell and Van Wyk, 1995).

IGFBP-4 is produced by BPE-1 cell line based on bovine parathyroid microcapillary endothelial cells. Two different BP in a conditioned medium of this cell line were found: N-glycosylated IGFBP-4 with molecular weight of 28 kDa and N-nonglycosylated BP with molecular weight of 24 kDa (Yang *et al.*, 1993). Smooth muscle cells of rat vessels produce IGFBP-4 (24 kDa) and its from 5 to 6 times increase is caused by platelet-derived growth factor (PDGF) isoforms (Gianella-Neto *et al.*, 1992). IGFBP-4 blocks the effects of exogenously added IGF-I to cells (Russell and Van Wyk, 1995).

IGFBP-4 with anti-IGF-I activity is synthesized by rat granulosa cells during physiological atresia. The expression of IGFBP-4 may be physiologically relevant to granulosa cells apoptosis and follicle death (Erickson *et al.*, 1996).

IGFBP-5 (23 kDa) was purified by Andress and Birnbaum (1992) from human osteoblast-derived culture that increases mitogenesis by coincubation with IGF-I or IGF-II. IGFBP-5 stimulates osteoblast mitogenesis not only by association to IGF but also to osteoblast surfaces (extracellular matrix) without IGF presence or interaction to IGF receptor.

IGFBP-2 to -5 are paracrine factors in the regulation of IGFs effects in developing lungs (Wallen *et al.*, 1997). IGFBP-2 is dominant in the epithelium of newborns, IGFBP-3 in saccular stadium (until 3 days *post partum*) and IGFBP-5 in alveolar stadium (from 5 to 21 days *post partum*).

IGFBP-5 is present in rat blood serum together with IGFBP-6, in kidney, bone and endocrine tissues. It is produced by rat thyroid cell line FRTL-5 (Russell and Van Wyk, 1995).

In the rat ovary, Shimasaki *et al.* (1996) found that granulosa cells of atretic follicles express two IGFBPs, IGFBP-4 and -5, whereas no IGFBP transcripts were detected in the granulosa cells of healthy follicles. IGFBP-4 and -5 expressions in cultured rat granulosa cells are regulated by the follicle-stimulating hormone (FSH) and gonadotropin releasing hormone (GnRH) signal transduction pathways. Low doses of FSH stimulate IGFBP-4 and -5 production whereas high doses inhibit their production by inducing protease activity that hydrolyses BPs into smaller fragments that bind IGFs with low affinity. Erickson *et al.* (1998) suggest that the endogenous IGF-I/IGFBP system in granulosa cells may be at or near the apex in the regulatory mechanisms that determine whether a follicle develops or dies by atresia in the murine ovary.

IGFBP-6 (30 to 32 kDa) was isolated from the human cerebrospinal fluid and its affinity is 10 times higher to IGF-II than to IGF-I. It is present in human and rat tissues (Russell and Van Wyk, 1995). It is O-glycos-ylated and O-glycosylation inhibits the proteolysis of IGFBP-6 by chymotrypsin and trypsin. Enzymatic deglycosylation does not change the affinity properties of IGFBP-6 to IGFs. Glycosylation is probably important in secretion, stability and localization of IGFBP-6 (Bach *et al.*, 1992). IGFBP-6 is a relatively specific inhibitor of IGF-II action. It has not been shown to potentiate IGF actions (Bach, 1999).

IGFBP-7 together with IGFBP-8 have low affinity to IGFs. IGFBP-8 is probably a connective tissue

growth factor (Kim *et al*., 1997). IGFBP-7 is a high-affinity insulin binding protein. IGFBP-7 is a functional insulin-binding protein, it blocks insulin binding to the insulin receptor and insulin action in this way (Yamanaka *et al*., 1997).

Some cells produce different IGFBPs forms, for example human fibroblasts produce IGFBP-3, -4 and -5 (Camacho-Hubner *et al*., 1992). Bovine endothelial cells are a source of IGFBP-2, -3 and -4 (Moser *et al*., 1992).

Yallampalli *et al.* (1993) isolated 4 IGFBPs forms (24, 28, 30 to 32, 38 to 42 kDa) from rat uterus extracts. They observed that these BPs are regulated not only by growth hormone but also by estradiol.

Falconer *et al.* (1991) observed the IGFBPs release from two different regions of sheep placenta: basal region and chorion tissue. The tissues of basal region produce IGF-I and also two forms of IGFBPs that are probably IGFBP-1 and -3 because of their similar molecular weight. The chorion tissue releases only IGFBP-3. It is very interesting and it leads to speculation if this tissue does not produce any other somatomedins (e.g. IGF-II) or if these tissues are not a target for IGF-I from the basal region.

Sheep plasma contains two 40 to 50 kDa binding proteins at least. The competitive tracer binding studies indicated that one protein demonstrates mixed specificity for IGF-I and -II while the other strongly favours IGF-II. 150 kDa binding protein of mixed specificity for IGF-I and -II was present not only in fetal and mature plasma but also in mammary lymph, follicular fluid and vitreous humor. The greater than 200 kDa binding protein, which is IGF-II specific, is present in plasma from mature sheep, colostrum and follicular fluid as well as fetal sheep plasma. This may be the ovine equivalent of the soluble type-2 IGF receptor identified in rat serum (Hodgkinson *et al*., 1989).

3. FUNCTIONS OF IGFBPs

There are a lot of common features in IGFBPs effects, but there are more differences that prevent to define a general mechanism of IGFBPs function (Jones and Clemmons, 1995).

IGFBPs carry IGFs in the circulatory system, they can cause inhibition of IGFs effects or total inactivation of growth factor. In certain situations they are able to potentiate the IGFs effects, too. They not only transport IGFs molecules to their receptors but also adhere to the cell surface.

3.1. Carrier function

IGFs circulate in the blood serum as macromolecules. From 75 to 90% of total human serum IGF-I are bound to ternary protein complex with molecular weight about 150 kDa and with long half-life (from 12 to16 hours). From 10 to 25% of total IGFs form a binary complex with molecular weight 28 to 35 kDa and with short half-life (about 30 minutes) (Martin and Baxter, 1992; Gourmelen *et al*., 1994; Zapf, 1997). Less than 1% of total IGF-I circulates in free form (Blum *et al*., 1989) and its half-life is about 10 minutes (McCusker and Clemmons, 1992).

Ternary complex is composed of acid-labile subunit (ALS, alpha-subunit), IGFBP-3 (beta-subunit) and IGF-I or IGF-II (gamma-subunit). ALS is N-glycosylated protein with molecular weight 84 to 86 kDa. It is able to form complexes not only with IGFBP-3 (50 kDa) but also with smaller forms of IGFBP-3, which explains the existence of 120-kDa ternary complex. The reaction between IGFBP-3 and ALS needs the binding of IGF-I or -II to IGFBP-3, so the binary complex composed of ALS and IGFBP-3 cannot be formed. Serum ALS levels in rat newborns are 15 times lower than in adult rats and in human newborns 7 times lower than in adults. Rat serum contains 42 mg/l ALS and human blood serum ALS concentration is 25 mg l. ALS levels are dependent like the IGFs and IGFBP-3 concentrations on growth hormone and the peak concentrations are reached in puberty (Zapf, 1995).

Binary complex (40 kDa) composed of one form of the first four IGFBPs and IGF-I or -II, is able to cross capillary barrier and transport IGFs to target cells where the IGFs effect is modulated by IGFBPs (stimulation or inhibition), either directly by interaction with IGF-receptor or by change in phosphorylation (Zapf, 1995). IGF availability from binary complex does not depend only on IGF/IGFBP ratio in the blood circulation but also on IGFBP proteolysis in the interstitial space. It is true because the lymph contains 8 times higher proteolytic activity than blood serum for IGFBP-3 cleavage. So IGF can be loosed from the ternary complex in blood serum only by some proteolytic activity because this complex is not able to cross the capillary barrier and plays the role of IGF reserve (Zapf, 1995).

Ternary complex is the main IGF transporter in blood serum *post partum* and it is nearly fully saturated in comparison with the binary complex that is not saturated. Human and rat fetal serum contains the small complex in big amounts in comparison with the big one (McCusker and Clemmons, 1992).

Butler and Gluckman (1986) observed the circulating IGFBPs forms in the blood of sheep fetuses, newborns and adults. By using gel chromatography they observed that BP of adult sheep are eluted in 100 to 200 kDa molecular weight region and the fetal BP in 20 to 50 kDa region. Distribution of newborn BP was equable in all regions, the next two days 20 to 50 kDa BP and on day 3 to 7 from 100 to 200 kDa molecules prevailed in comparison with adult sheep. The considerable developmental changes of IGFBPs in sheep perinatal time are influenced by IGF-I availability for its biological function.

Bar *et al.* (1990) found that bovine endothelial cell binding proteins (ECBP) and amniotic fluid IGFBP-1 are able to cross rat heart capillary vessels and ECBP are localized preferentially in myocardial connective tissues in comparison with IGFBP-1 which has higher affinity to the myocardium. IGF-I like IGFBP-1 is also leaving the vascular spaces and is distributed into heart muscle during the heart perfusion by this IGF. They determined that ECBP but not IGFBP-1 has affinity to heparin. Now we can speculate if heart connective tissue proteoglycans contained heparin can cause the different tissue distribution of ECBP and IGFBP-1. Endothelial transport of BP and selective association to specific subendothelial tissues can play a role of the check point in the regulation of IGFs effects.

High levels of IGFBP-3 and IGFBP-1 in chorion fluid and relatively low levels in amniotic fluid mean that amnion is an effective barrier for the transport of binding proteins from chorion to amniotic fluid (Nonoshita *et al.*, 1994).

IGFBP-2 is able to cross endothelial barriers and it works as IGFs transporter (McCusker and Clemmons, 1992).

3.2. Potentiation of IGFs effects

Potentiation phenomenon of IGFs effects is characterized by these mechanisms (Zapf, 1995):

– phosphorylation (IGFBP-1)
– association to cell surface (IGFBP-3)
– association to extracellular matrix (IGFBP-5)
– proteolysis (IGFBP-3 and -5)

Posttranslation modifications of IGFBPs can influence their biological activity. These modifications of IGFBPs such as phosphorylation lead to a change of their affinity to IGFs. More phosphorylated form of IGFBP-1 of human amniotic fluid and decidua has higher binding affinity to IGFs than less phosphorylated form (Koistinen *et al.*, 1993). Westwood *et al.* (1994) identified the high phosphorylated form of IGFBP-1 also in the blood circulatory system of human adults and its concentration increase during pregnancy.

On the other hand, human hepatoma cells (HepG2) and human decidual cells produce phosphorylated isoforms of IGFBP-1, it means that IGFBP-1 is secreted as phosphoprotein and then it is dephosphorylated. Phosphorylated form of IGFBP-1 produced by HepG2 cells has 6 times higher affinity to IGF-I than after dephosphorylation (Jones *et al.*, 1991). Phosphorylated forms of IGFBP-1 are known that potentiate the mitogenic activity of IGF-I. The possible IGFBP-1 inhibition or stimulation of IGF mitogenic effect on fibroblasts depends on its phosphorylation degree (Russell and Van Wyk, 1995). On the other hand, it is not known if phosphorylated forms of IGFBP-3 influence the IGFs activity.

There is more and more information about possible mechanisms that explain how IGFBPs contact to cells. Then it leads to signal transmission either by means of IGF receptor type I or IGF receptor type I-independent pathways of mitogenic signal transduction (Hossner *et al.*, 1997). IGFBP-1 and -2 contain the integrin recognition sequence Arg-Gly-Asp (RGD) that causes their binding to $\alpha_5\beta_1$ integrin (fibronectin) receptor. Both these effects depend on the presence of RGD sequence but not IGF-I (Jones *et al.*, 1993).

On the other hand, IGFBP-3 and -5 do not contain the RGD sequence and are able to associate to cell surface. IGFBP-5 binds to extracellular matrix and IGFBP-3 interacts with membrane glycosaminoglycans. Cell-associated IGFBPs have lower affinity to IGFs and potentiate IGFs effects (Hossner *et al.*, 1997).

Proteolysis of IGFBP-3, -4 and -5 decreases their affinity to IGF-I for 50 to 100 times. IGFBP-3 protease present in chorion fluid and maternal serum, but not in amniotic fluid, cleaves IGFBP-3 into two fragments with molecular weight 18 and 15 kDa that regulate IGF-II amounts for target chorion and amnion cells (Nonoshita *et al.*, 1994). Heparin and serine endoproteases release IGFs from the IGFBPs binding. The increasing concentrations of these substances near the wounds can serve for increasing IGFs amounts which can enter into the extravascular spaces and interact with tissue receptors (Russell and Van Wyk, 1989; McCusker and Clemmons, 1992). IGFBPs proteolysis may be an important mechanism in a release of biologically active molecules of IGFs, which increases their ability of interaction with their receptors on cell surfaces.

Human amniotic fluid IGFBP-1 potentiates IGF-I growth effect. Coincubation with platelet-pure plasma

(PPP) is needed for this potentiation. PPP probably contains a thermostable and acidostable factor present not only in blood plasma but also in cerebrospinal fluid and not in amniotic fluid (Clemmons and Gardner, 1990).

De Mellow and Baxter (1988) described IGFBP-3 (53 kDa) inhibitory effect in the case of coincubation with IGF-I. On the other hand, preincubation of human fibroblasts with IGFBP-3 before IGF-I supplementation is followed by potentiation of the growth factor effect. The stimulatory effect was observed in IGFBP-1, -2, -3 and -5, contrary to IGFBP-4 which only inhibits the growth factor effects.

3.3. Inhibition of IGFs effects

Binding of IGFBPs to IGFs inhibits IGFs association to target cells and so IGFs effect is weak (Russell and Van Wyk, 1995). Burch *et al.* (1990) isolated a human amniotic fluid binding protein with molecular weight of 25 kDa and tested its effect on the growth of chicken embryo pelvic cartilage in comparison with IGF-I stimulated growth. They concluded that this BP has an inhibitory effect. By the way, they found two forms of IGFBP in a conditioned medium: the first one means to be a chicken equivalent to 25-kDa BP and the molecular weight of the second one is very similar to human 34-kDa IGFBP.

IGFBP-1 inhibits IGF effect on human osteosarcoma cells (Campbell and Novak, 1991). Binding of human IGF-I to recombinant human IGFBP-2 is followed by inhibition of free IGF-I effect on the replication of bone cells and synthesis of rat fetal matrix (Feyen *et al.*, 1991).

Rat serum IGFBP-3 has an inhibitory effect on the growth of chicken embryonal fibroblasts that was stimulated by IGF-I, IGF-II and whole human blood serum (Liu *et al.*, 1992).

A binding protein from Madin-Darby bovine kidney (MDBK) cells potently inhibits the ability of IGF-II and of IGF-I to a less extent to stimulate DNA synthesis, but does not influence the biological activity of des-(1-3)-IGF-I (it is IGF-I shorter form without amino terminal tripeptide) (Ross *et al.*, 1989).

Hyunh (1996) described an autocrine mode in the growth regulation of MCF-7 cells by the use of estradiol which inhibits IGFBP-3 and antiestrogen ICI 182780 production. Antiestrogen ICI 182780 stimulates IGFBP-3 production. Increased concentration of IGFBP-3 inhibits the proliferation of breast cancer cell line MCF-7 either directly or by competition with IGF receptors. Maybe IGFBPs work as antimitogens and IGFs are potential promotors of cancer growth (Zapf, 1995).

Koistinen *et al.* (1990) purified two peaks of IGFBP-1 with the same molecular weight of 30 kDa from the midtrimestral human amniotic fluid. This BP caused the inhibition of ^{125}I-IGF binding to fetal skin fibroblasts, on the other hand it increased IGF-I stimulated ^{3}H-thymidine incorporation into DNA of the same cells but not *membrana granulosa* cells. It means that DNA synthesis stimulation or inhibition by the same IGFBP also depends on target cells.

Exogenous IGFBP-3, -4 and -5 had an inhibitory action on IGF-II-dependent DNA synthesis in a monolayer culture of chondrocytes established from the proliferative zone of the growth plate of ovine fetus. IGFBP-2 had a biphasic effect, potentiating IGF-II action at low concentrations but inhibiting DNA synthesis at equimolar or greater concentrations relative to IGF-II (de los Rios and Hill, 1999).

4. DIAGNOSTIC AND THERAPEUTIC ASPECTS

Interest in the role of IGF axis in growth control and carcinogenesis has recently been increased by the finding of elevated serum IGF-I levels in association with three most prevalent human cancers: prostate cancer, colorectal cancer and lung cancer (Grimberg and Cohen, 2000).

RIA for IGFBP-1, -2 and -3 are currently commercially available and information is accumulating on their diagnostic usefulness. This includes some clinical situations, such as growth disorders, where serum IGFBP-3 is a highly specific screening tool for growth hormone deficiency, various malignancies in which serum IGFBP-2 levels are elevated and disorders of carbohydrate metabolism that display an inverse relationship between serum IGFBP-1 and insulin secretion (Cohen and Rosenfeld, 1994).

The study of IGFBPs levels is diagnostically important, for example in patients with total growth hormone deficiency, when IGFBP-3 levels are decreased and IGFBP-2 increased. Three GH injections increase the IGFBP-3 level, without restoring them to normal. This may be diagnostically helpful in doubtful cases (Gourmelen *et al.*, 1994). Patients with tumor-induced GH insufficiency and normal or reduced IGF-I levels have normal or slightly depressed IGFBP-3 levels. This supports the notion of IGF-I having greater control over IGFBP-3 synthesis than GH, which is confirmed by the gradual increase in IGFBP-3 levels following

IGF-I administration observed in Laron's syndrome patients. Patients with the Laron-type dwarfism lack functional growth hormone receptors and thus do not respond to GH; their IGF-I levels are very low and circulating GH levels are high because of decreased feedback suppression of GH by IGF-I (Underwood, 1994). An IGFBP profile similar to that in hypopituitarism is found in malnutrition such as in coeliac disease. It restores to normal when patients are on glutean-free diet (Gourmelen *et al*., 1994).

Inappropriate IGFBP synthesis may occur relative to the prevailing IGF levels like in the case of pygmies during puberty when the previously low levels of IGF-I begin to rise, but the IGFBP-3 levels are markedly increased (Hardouin *et al*., 1989). This might account for the lack of pubertal growth in these subjects.

Increased plasma concentrations of IGFBP-3 are described in chronically uremic patients. Elevated IGFBP-3 levels are believed to inhibit the activity of IGF-I by reducing its availability for biological actions (Kopple, 1992). IGFBP-3 and -1 abundance was determined in children with chronic renal insufficiency and so these patients have growth retardation (Zumkeller and Schofield, 1992).

IGF-I is potently antiapoptotic for neurons and oligodendrocytes *in vivo* injury responses, where postinjury intraventricular injection of IGF-I enhances neuronal survival by blocking apoptosis (Werther *et al*., 1998).

Elevated levels of IGFBP-2 may contribute to the diagnosis of highly malignant phenotype of adrenocortical cancer (Hoeflich, 2000).

San Roman *et al.* (1992) found 6 IGFBPs bands in polycystic ovary women. The dominant BP was IGFBP-3 but its concentration did not change in comparison with health individuals. On the other hand, IGFBP-2, -4 and BP with molecular weight of 29 kDa concentrations increase significantly in the follicular fluid of polycystic ovaries.

The IGF-independent action of IGFBP-3 requires interaction with cell-surface association proteins, presumably putative IGFBP-3 specific receptors, and is responsible for the growth inhibitory action of known growth suppressing factors such as TGF-beta, retinoic acid, and antiestrogens in human breast cancer cells (Oh, 1998). IGFBP-7 functions as a modulator of cell growth in an IGF-independent manner, similar to the action observed with IGFBP-3 in breast cancer cells (Oh, 1997). The breast cancer cell (MCF-7) growth that depends on estrogen presence can be regulated by endogene IGFBPs effects. Exogenously added IGFBP-1 blocks IGF-I effect and it should be used as a pharmacological inhibitor of IGF function (McGuire *et al*., 1992).

Coadministration of IGFBP-1 inhibits IGF-I-induced body growth of GH-deficient mice but significantly stimulates the growth promoting effects on the kidneys and the spleen (Van Buul-Offers *et al*., 2000).

IGFBP-6 expression is associated with the inhibition of growth of tumor cells *in vitro* and *in vivo*. The major function of IGFBP-6 appears to be the regulation of IGF-II action, which could be especially significant since IGF-II is implicated as an autocrine tumour growth factor (Bach, 1999).

The GH-IGF-IGFBP axis is complex and powerful. Future research on its physiology promises exciting insights into cell biology as well as therapies for diseases such as cancer, diabetes mellitus, vascular disease, asthma and growth disorders (Ferry Jr *et al*., 1999; Wetterau *et al*., 1999).

IGFBPs with enhanced affinity for insulin might contribute to the insulin resistance of pregnancy, type II diabetes mellitus and other pathological conditions.

5. CONCLUSION

Hormones, growth factors and their binding proteins and other substances that regulate the cell cycle play an important role in the control of cell growth and differentiation in animal organisms. The growth factors influence cells by binding to specific receptors on the cell surface and trigger transduction process of mitogenic signal from the cell membrane to the nucleus. The IGFs proliferative activity is modulated by insulin-like growth factors binding proteins (IGFBPs) which potentiate or inhibit the IGFs effects in cell proliferation. The results of present-day research in the field of growth factors and their binding proteins speak not only about the understanding of physiology of these substances in live organisms but also about possible utilization in medical practice in diagnostic determination and therapy of some disorders especially in the endocrine system.

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