STUDIES OF COMPLEXES FORMED IN BLOOD *IN VIVO* BETWEEN AN INSULIN-LIKE GROWTH FACTOR ANALOG AND BINDING PROTEINS

by

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ABSTRACT

Insulin-like growth factor-I (IGF-I) is a polypeptide growth factor with an insulin-like chemical structure and biological properties. IGF-I stimulates proliferation and differentiation of many cultured cells and also regulates prenatal and postnatal growth of animals. LR^{3}IGF-I is a synthetic analog of IGF-I that is biologically active *in vitro* and *in vivo*. Compared to IGF-I, LR^{3}IGF-I has much lower affinity for IGF-binding proteins and slightly lower affinity for the type-I IGF receptor. Treatment with LR^{3}IGF-I promotes liveweight gain in rats but not in non-rodents, e.g. pigs and sheep. Lord *et al.* (1994) showed that although [^{125}I]-LR^{3}IGF-I exhibits extremely weak binding to binding proteins in rat plasma, it binds significantly to proteins in plasma from sheep, pigs, humans and chickens. The *in vivo* formation of complexes between the different IGF-binding proteins and IGFs and the physiological functions of different types of complexes in blood are not well understood.

The aims of this study were to examine the molecular forms of circulating LR^{3}IGF-I during treatment of animals with biologically active doses of this analog and to identify factors which affect the *in vivo* formation of circulating complexes between LR^{3}IGF-I and IGF-binding protein(s).

In order to detect LR^{3}IGF-I in specimens obtained from animals treated with this analog, a simple assay for LR^{3}IGF-I was developed. Mouse monoclonal antibodies and rabbit polyclonal antisera against LR^{3}IGF-I were produced, their binding affinities and specificities were characterised and their binding complementarities were evaluated. Two antibodies were found to be suitable as the basis of an ELISA. The ELISA system was able to detect as little as 50 pg of LR^{3}IGF-I and the native peptides IGF-I and IGF-II have less than 0.01% cross

reactivity. Due to the high sensitivity of the ELISA, blood plasma from animals treated with pharmacologically active doses of this growth factor analog could be diluted between 20- and 100-fold before assay, at which concentrations plasma had no effect on the assay response to LR^3IGF-I. There was a positive correlation between results obtained by LR^3IGF-I ELISA of unextracted plasma and those obtained by LR^3IGF-I RIA. The advantages of the LR^3IGF-I ELISA compared to the LR^3IGF-I RIA include improved sensitivity, elimination of the need for radioiodination of LR^3IGF-I and elimination of the requirement for extraction of plasma before assay.

The molecular size classes of LR^3IGF-I in blood from animals treated with biologically active doses of this IGF analog were characterised by size exclusion high performance liquid chromatography of plasma at pH 7.4. Assays of LR^3IGF-I in the eluent by both ELISA and RIA showed that LR^3IGF-I was present in free (unbound) form in plasma from fasted cows intravenously infused with LR^3IGF-I for 7 hours at 12 $\mu g.kg^{-1}.h^{-1}$. This LR^3IGF-I treatment reduced blood levels of glucose and amino-acids in these heifers (Owens *et al.* 1994). However, in plasma from well-fed guinea pigs infused subcutaneously with this analog for 7 days at 14 $\mu g.kg^{-1}.h^{-1}$, the LR^3IGF-I activity detected by both RIA and ELISA was present in high molecular weight forms. LR^3IGF-I was also biologically active in this study, because treatment increased growth of several organs in these guinea pigs (Conlon *et al.* 1995*b*). In both of these *in vivo* experiments, treatment with LR^3IGF-I also reduced blood levels of IGF-I and IGF-II. *In vitro* addition of IGF-I and IGF-II to plasma from guinea pigs treated with LR^3IGF-I converted the high molecular weight LR^3IGF-I activity into the free form whereas addition of insulin did not. Therefore LR^3IGF-I was associated with IGF-binding protein(s) in blood during treatment of guinea pigs. The column fractions that contained the high molecular weight form of LR^3IGF-I also contained IGF-binding proteins of approximately

48, 43, 31 and 25 kDa. Complexes between LR^3IGF-I and guinea pig IGF-binding proteins could not be generated by *in vitro* addition of LR^3IGF-I to blood plasma from normal untreated guinea pigs. The mechanism responsible for the formation of IGF-binding protein/LR^3IGF-I complexes in guinea pigs is not clear.

Age and nutrition, which regulate the concentrations in blood of insulin-like growth factors and their binding proteins, were evaluated as determinants of the *in vivo* formation of circulating complexes between LR^3IGF-I and IGF-binding proteins. Male hooded Wistar rats aged either 5 weeks or 12 weeks were fed for 7 days either *ad libitum* or at one of three restricted rates, reduced either by 22%, by 44% or by 67%, compared to the *ad libitum* rate of feed intake. Rats were infused throughout this period either with LR^3IGF-I, at an average rate of 38 $\mu g.kg^{-1}.h^{-1}$, or with placebo (vehicle).

Undernutrition progressively decreased plasma concentrations of rat IGF-I in vehicle treated younger rats. Reduction of feed intake to 67% less than *ad libitum* in younger rats decreased plasma concentrations of IGF-binding proteins of ~40-50 kDa and ~24 kDa but increased concentrations of ~29 kDa IGF-binding protein. Reduction of feed intake to 44% less than *ad libitum* in younger rats decreased plasma concentrations of IGFBP of ~40-50 kDa but had no effect on other binding proteins. Treatment with LR^3IGF-I further reduced concentrations of rat IGF-I in plasma of undernourished younger rats by ~40-50% compared to vehicle treated animals. Treatment with LR^3IGF-I increased the concentrations of ~29 kDa IGF-binding protein in all feed-restricted groups of younger rats but not in *ad libitum* fed rats.

In older rats, only extreme undernutrition (reducing feed intake to 67% less than *ad libitum*) significantly decreased plasma levels of rat IGF-I in vehicle treated animals. Food

intake had no significant effect on plasma concentrations of IGF-binding proteins in older rats. Treatment with LR^3IGF-I did not affect plasma concentrations of rat IGF-I and IGF-binding proteins at any level of feed intake in older rats.

The concentrations of LR^3IGF-I in plasma after 7 days treatment were considerably lower than those of rat IGF-I in all nutrition groups at both ages. Plasma LR^3IGF-I was affected by the level of feed in younger but not older rats.

Size exclusion chromatography of plasma under non-dissociating conditions followed by LR^3IGF-I ELISA of the eluted fractions showed that all of the circulating LR^3IGF-I was in the free form in all groups of older rats and in the better fed groups of younger rats. However, about 30% of the LR^3IGF-I in blood plasma from the 44% underfed younger rats and ~100% in 67% underfed younger animals was present in plasma in a high molecular weight form(s). The high molecular weight form(s) of LR^3IGF-I in LR^3IGF-I treated 67% underfed young rats could be converted to free LR^3IGF-I by acidification to pH 2.5 indicating that LR^3IGF-I in rat plasma is associated with an IGF-binding protein under certain conditions. Moreover, fractions containing high molecular weight form(s) of LR^3IGF-I also contained ~29 kDa IGFBP.

Complex formation between LR^3IGF-I and a ~29 kDa IGFBP in undernourished young rats (44% and 67% underfed) may be promoted by the very low plasma concentrations of rat IGF-I and the high concentrations of ~29 kDa IGFBP which may result in high levels of unsaturated ~29 kDa IGFBP *in vivo*. Indirect evidence indicates that LR^3IGF-I forms complexes with a ~29 kDa IGF-binding protein and that this protein is IGFBP-1.

There were positive and negative associations between plasma concentrations of free LR^3IGF-I with several organ weights in young rats that were consistently stronger than those seen with total LR^3IGF-I levels. The association between levels of free LR^3IGF-I and kidney weight is an example of a negative association whereas the association between levels of free LR^3IGF-I and thymus weight is an example of a positive association. In all groups of animals in which circulating LR^3IGF-I was exclusively in the free form, treatment with this IGF-I analog increased thymus weight. In contrast, free LR^3IGF-I was associated with reduced growth response of the kidney.

In conclusion, this study shows that when LR^3IGF-I is administered to animals in pharmacologically active doses, it may be present in either the free form or bound to IGF-binding protein(s) in the circulation. Age and nutrition which are factors that regulate synthesis of endogenous IGF-I and IGF-binding proteins, affect the *in vivo* formation of complexes between the analog and IGFBP(s). This study also suggests that IGFBP-1 inhibits the pharmacological activity of circulating LR^3IGF-I on thymus whereas it appears to stimulate the pharmacological activity of LR^3IGF-I in kidneys.