International Journal of Pharmaceutics, 90 (1993) 81–86

IJP 02940

Note

Pharmacokinetics of a 22 kDa variant of unlabelled monomeric human growth hormone in rabbits

Sun Hwa Lee [a], Woo Hyun Yoon [a], Seong Hoon Jang [a], Kye Soo Han [a], Kyeong-Hoon Jeong [b], In Soo Kim [b] and Myung Gull Lee [a]

[a] *College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kawnak-Gu, Seoul 151-742 (South Korea)* and [b] *R / D Center, Biotechnology, Lucky Ltd, Science Town, P.O. Box 10, Dae-Jeon 305-343 (South Korea)*

(Received 28 May 1992)
(Accepted 23 June 1992)

Key words: Dose-dependent pharmacokinetics; Human growth hormone variant; Tissue distribution

Summary

The pharmacokinetic parameters of $MHGH_{22K}$, such as the mean values of $t_{1/2}$ (9.30, 16.8, 22.2 vs 25.8 min), MRT (11.6, 21.7, 26.7 vs 37.9 min) and V_{ss} (166, 218, 342 vs 556 ml kg^{-1}) tended to increase with increasing doses after i.v. administration of the hormone (0.2, 0.5, 2.0 and 5.0 U kg^{-1}) to rabbits. However, the mean values of CL, CL_R and CL_{NR} were dose-independent over the dose ranges studied, indicating that the dose-dependent pharmacokinetic parameters of $MHGH_{22K}$, such as $t_{1/2}$, MRT and V_{ss} may not be due to the saturable metabolism of the hormone. The extent of bioavailability after s.c. administration of $MHGH_{22K}$ (0.5 U kg^{-1}) to rabbits appeared to be complete when compared with the AUC values between i.v. and s.c. administration at the same dose. $MHGH_{22K}$ was highly concentrated in the kidney and liver (T/P ratio greater than unity), whereas lower levels were observed in the other tissues or organs studied at 2 h after s.c. administration of the hormone (0.5 U kg^{-1}) to rabbits.

Human growth hormone (HGH) consists of several molecular forms; heterogeneity of HGH has been demonstrated in pituitary extracts, pituitary culture media and plasma (Baumann and Abramson (1983) and references therein). The HGH forms that can be identified in pituitary extracts include the following: as the main component, the 22 kDa single-chain form (HGH-B); a 20 kDa single-chain form which lacks amino acid residues 32–46; three proteolytically cleaved, two-chain forms (HGH-C, -D and -E); the N^{α}-acetylated form; deaminated forms; slowly migrating forms; and oligomers of HGH (Baumann and Abramson (1983) and references therein). The principal form of HGH is a single-chain, 191-amino-acid protein; it lacks covalently bound carbohydrates, contains two intrachain disulfide bonds, and has an unblocked amino-terminus (Kuret and Murad, 1991). This form of HGH is produced biosynthetically by recombinant DNA technology and is marked as synthetic HGH (somatropin). The identical protein but containing an additional amino-terminal methionine residue is marketed as synthetic methionyl-HGH (somatrem; Kuret and Murad, 1991). Lucky Ltd

Correspondence to: M.G. Lee, College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea.

(Seoul, Korea) have recently manufactured 22 kDa, demethionylated monomeric HGH having 191 amino acids ($MHGH_{22K}$) by recombinant DNA technology.

Total body clearances of dimeric HGH (both 20 and 22 kDa forms) were significantly lower than those of the corresponding monomers, and both dimers were also degraded at slower rates than the respective monomers (Baumann et al., 1986). It has also been reported (Hendricks et al., 1985) that the larger the HGH component, the longer it takes to be cleared from the plasma. Although the structures and physiological actions of GH have been widely investigated (Fryklund et al., 1986), a thorough examination of the pharmacokinetics of HGH, especially at large dose intervals after i.v. administration, remains to be performed (Salmon et al., 1962; Laron et al., 1965; Frohman and Bernardis, 1970; Owens et al., 1973; Wallace and Stacy, 1975; Baumann and Abramson, 1983; Baumann et al., 1985, 1986; Hendriks et al., 1985).

The purpose of the present communication is to report the pharmacokinetics of $MHGH_{22K}$ after i.v. administration of the hormone (0.2, 0.5, 2.0 and 5.0 U kg^{-1}) to rabbits. The plasma level-time profiles and tissue distribution after s.c. administration of the hormone (0.5 U kg^{-1}) to rabbits are also reported.

47 healthy male New Zealand White rabbits (1.6–2.2 kg) were anesthetized with 30–50 mg of i.v. ketamine (kindly supplied by the Yuhan Research Center, Kunpo, Korea) via an ear vein. The left jugular vein (i.v. injection only) and carotid artery were catheterized with silastic tubing (Dow Corning Inc., Midland, MI). The animals were allowed to recover from anesthetization for 4–5 h before the study and fasted during the experiment.

$MHGH_{22K}$ (16 U per vial, kindly supplied by the Lucky Ltd; 394 μg of the hormone is equivalent to 1.0 U) was freshly reconstituted with distilled water before use. The hormone, 0.2, 0.5, 2.0 and 5.0 U kg^{-1} was injected (injection volume, approx. 2 ml) in 10 s through the cannula placed in the jugular vein into rabbits, 1–8, 9–16, 17–24 and 25–32, respectively. The midpoint of the injection was taken as time zero. Blood samples (0.5–2.5 ml, depending on blood sampling schedules and doses) were collected from the carotid artery and were centrifuged immediately to minimize the potential 'blood storage effect' (Lee et al., 1984; Shin et al., 1992) in the plasma concentrations of $MHGH_{22K}$. Urine samples were also collected for up to 24 h after the dose via a pediatric Foley catheter (Sewoon Medical Co., Seoul, Korea) which was introduced into the urinary bladder. $MHGH_{22K}$ (0.5 U kg^{-1}) was injected (injection volume, approx. 2 ml) subcutaneously into the dorsal side of the neck of rabbits 33–42, and blood and urine samples were similarly collected. The hormone (0.5 U kg^{-1}) was similarly injected subcutaneously into rabbits 43–47. 2 h after the injection, as much blood as possible was collected through the carotid artery, and the kidney, liver, stomach, heart, lung, spleen, large intestine, muscle and brain were excised. The liver was perfused with cold normal saline solution which was also used for washing the other organs or tissues to eliminate blood remaining in each organ or tissue. After blotting dry with paper tissue, exactly 1 g of each organ or tissue was measured. Each organ or tissue was minced into small pieces with scissors and then homogenized in 3 volumes of phosphate buffer of pH 7.4 in a tissuemizer (Ultra-Turrax T25, Janke & Kunkel, IKA-Labortechnit, Germany). Plasma was also mixed with 3 volumes of the buffer. Blood and urine collection methods were similar to those reported previously (Yoon et al., 1991).

The concentrations or amounts of $MHGH_{22K}$ in the above biological samples were determined by radioimmunoassay according to the double antibody method (Wroblewski et al., 1991). Radiolabelled hormone (^{125}I-$MHGH_{22K}$) as a tracer for $MHGH_{22K}$ was synthesized following a previously reported method (Salacinski et al., 1981).

The pharmacokinetic parameters, such as the area under the plasma concentration-time curve from time zero to time infinity (AUC), area under the first moment of the plasma concentration-time curve (AUMC), mean residence time (MRT), time-averaged total body (CL), renal (CL_R) and nonrenal (CL_{NR}) clearances, and apparent volume of distribution at steady state (V_{ss}),

were estimated based on standard methods (Gibaldi and Perrier, 1982; Yoon et al., 1991). The harmonic mean was employed for calculation of the mean values of the half-life, each clearance and V_{ss} (Chiou, 1979). The data were analyzed for statistical significance ($p < 0.05$) using analysis of variance.

After i.v. bolus administration of $MHGH_{22K}$, the plasma concentrations declined polyexponentially in all the rabbits studied, the concentrations also declining slowly with increasing doses (Fig. 1). It is of interest to note that some of the pharmacokinetic parameters of $MHGH_{22K}$ were dose-dependent within the dose ranges studied. For example, the mean values of $t_{1/2}$ (9.30, 16.8, 22.2 vs 25.8 min), MRT (11.6, 21.7, 26.7 vs 37.9 min) and V_{ss} (166, 218, 342 and 556 ml kg^{-1}) tended to increase with increasing doses, and at the doses of 0.5, 2.0 and 5.0 U kg^{-1} the mean values increased significantly compared with those at the dose of 0.2 U kg^{-1} (Table 1). Mean terminal half-lives of 21.5 min (Hendricks et al., 1985) and 19.0 min (Owens et al., 1973) have been reported previously when unradiolabelled HGH (9–14 IU) was administered intravenously in 2–4 min to six hypopituitary patients, and unradiolabelled HGH was infused to 11 normal subjects, reaching mean steady-state plasma concentrations of the hormone ranging from 5 to 50 ng ml^{-1}, respectively, as determined via RIA analysis. However, significantly increased values of $t_{1/2}$, namely, 165 min (Salmon et al., 1962) and 120–130 min (Laron et al., 1965), were reported when radiolabelled (^{131}I) HGH was injected intravenously into rabbits. The above might be due to the fact that the estimated total radioactivity

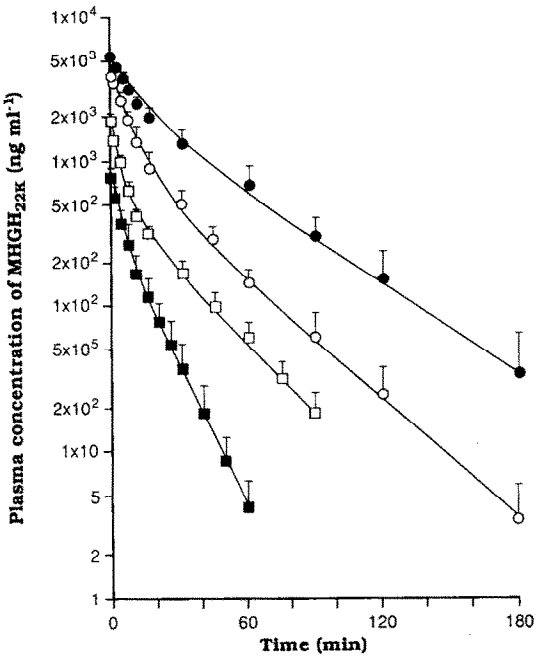

Fig. 1. Mean arterial plasma concentration-time profiles of $MHGH_{22K}$ after intravenous bolus administration of the hormone: 0.2 U kg^{-1} (■), 0.5 U kg^{-1} (□), 2.0 U kg^{-1} (○) and 5.0 U kg^{-1} (●) to rabbits 1–8, 9–16, 17–24 and 25–32, respectively. Bars represent standard deviation.

(Salmon et al., 1962; Laron et al., 1965) does not represent only ^{131}I-HGH, but rather, it corresponds to the sum of the radioactivities of ^{131}I-HGH, its metabolites and degraded ^{131}I. The plasma concentrations of total radioactivity were reported to be significantly higher and the plasma levels declined slower than those of immunoprecipitable radioactivity when rat ^{131}I-GH was infused into the rats (Frohman and Bernardis,

TABLE 1

Mean pharmacokinetic parameters of $MHGH_{22K}$ after intravenous administration of the hormone (0.2, 0.5, 1.0 and 5.0 U kg^{-1}) to rabbits

	0.2 U kg^{-1}	0.5 U kg^{-1}	2.0 U kg^{-1}	5.0 U kg^{-1}
$t_{1/2}$ (min)	9.30 ± 2.06	16.8 ± 4.75 [b]	22.2 ± 6.74 [b]	25.8 ± 6.78 [b]
MRT (min)	11.6 ± 1.74	21.7 ± 2.92 [a]	26.7 ± 7.32 [a]	37.9 ± 7.44 [b]
V_{ss} (ml kg^{-1})	166 ± 31.8	218 ± 27.4 [a]	342 ± 73.0 [b]	556 ± 46.0 [b]
CL (ml min^{-1} kg^{-1})	14.2 ± 5.61	10.2 ± 0.79	14.3 ± 0.788	14.7 ± 3.13
CL_R (ml min^{-1} kg^{-1})	0.0419 ± 0.0386	0.00929 ± 0.0483	0.00714 ± 0.143	0.0162 ± 0.0455
CL_{NR} (ml min^{-1} kg^{-1})	13.9 ± 6.62	10.1 ± 0.710	14.2 ± 0.781	14.2 ± 2.10

[a] $p < 0.01$, [b] $p < 0.001$ when compared to the value of 0.2 U kg^{-1}. Values are expressed as means ± S.D.

1970), and ^{125}I- or ^{131}I-HGH was administered into the rats (Baumann et al., 1985). On the other hand, a very short terminal half-life (4–5 min) was reported for the case where unlabelled and ^{125}I-HGH$_{20K}$ were injected into mice, and could be due to short (for up to 8 min) blood sampling schedules (Sigel et al., 1982).

The mean values of CL (14.2, 10.2, 14.3 vs 14.7 ml min^{-1} kg^{-1}), CL_R (0.0419, 0.00929, 0.00714 vs 0.0162 ml min^{-1} kg^{-1}) and CL_{NR} (13.9, 10.1, 14.2 vs 14.2 ml min^{-1} kg^{-1}) were not significantly different between the dose ranges studied (Table 1). A similar result was also reported that the CL of HGH was constant at serum concentrations of HGH ranging from 5 to 50 ng ml^{-1} when unlabelled HGH was infused to normal subjects and patients with liver disease, renal disease, thyroid disease and diabetes mellitus (Owens et al., 1973). The above data indicated that some of the dose-dependent pharmacokinetic parameters of MHGH$_{22K}$, such as $t_{1/2}$, MRT, and V_{ss} might not be due to the saturable metabolism of the hormone. The increased value of V_{ss} with increasing doses might be due to decreased plasma protein binding of the hormone with increasing doses. The possibility of a capacity-limited catabolic process of unlabelled HGH in rats was suggested (Baumann et al., 1985). It was reported (Frohman and Bernardis, 1970; Owens et al., 1973; Baumann et al., 1985, 1986) that the mean values of CL were variable depending upon the sources of HGH (radiolabelled or unlabelled), the specificity of HGH (monomer, dimer, 20 or 22 kDa) and the species (human or rats).

The amounts of unchanged MHGH$_{22K}$ excreted in 24 h urine were negligible; the mean percentages of the i.v. dose excreted in 24 h urine were 0.58, 0.43, 0.44 and 0.24% for doses of 0.2, 0.5, 2.0 and 5.0 U kg^{-1}, respectively. Similar results have also been reported elsewhere; small amounts of HGH are excreted in humans (Lowry et al., 1971); only a minute fraction (0.01%) of the HGH secreted reaches the final urine (Baumann and Abramson, 1983); the percentage of administered radioactivity excreted in 2 h urine is 2.4% when bovine ^{125}I-GH is administered to rats (Retegui-Sardou et al., 1977); and the ratio of CL_R to GFR is less than 1% in rats (Johnson and Maack, 1977). Baumann and Abramson (1983) obtained no evidence for HGH forms other than monomers in urine, and Hanssen (1972) also found no detectable large HGH in urine as assessed by gel chromatography. Since the amounts of unchanged MHGH$_{22K}$ excreted in urine are negligible, almost all of the intravenously administered MHGH$_{22K}$ could be metabolized. It has been reported that rat ^{125}I-GH is extensively filtered via the glomerulus (glomerular sieving coefficient, approx. 0.6), and subsequently almost completely absorbed by the proximal tubular epithelium (Rabkin et al., 1973; Johnson and Maack, 1977). The absorbed rat ^{125}I-GH is catabolized within renal cells (intracellularly) and a detectable product of catabolism is returned to its circulation (Johnson and Maack, 1977). Blood plasma does not degrade the injected hormone, but the kidney, liver and muscle rapidly produce radioactive fragments soluble in 10% trichloroacetic acid (Retegui-Sardou, 1977). Kidney (Wal-

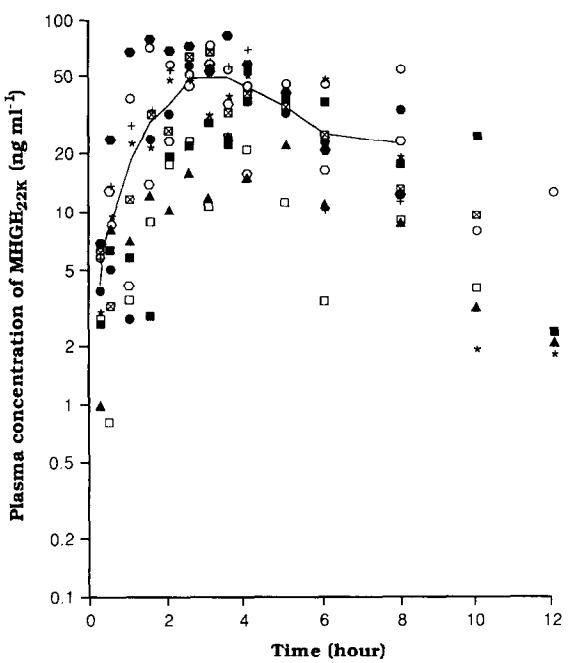

Fig. 2. Individual and mean (solid line) arterial plasma concentration-time profiles of MHGH$_{22K}$ after subcutaneous administration of the hormone (0.5 U kg^{-1}) to rabbits 33 (□), 34 (■), 35 (⊠), 36 (*), 37 (+), 38 (○), 39 (●), 40 (▲), 41 (○) and 42 (●).

TABLE 2

Amount (ng per g tissue) of $MHGH_{22K}$ remaining in each tissue at 2 h after s.c. administration of the hormone (0.5 U kg^{-1}) to rabbits 43–47

Rabbit	Kidney	Liver	Stomach	Heart	Lung	Spleen	L.I.	Muscle	Brain	Plasma
43	48.8	15.0	5.0	2.9	1.8	2.8	4.5	2.5	4.0	15.4
	(3.17) [a]	(0.974)	(0.325)	(0.188)	(0.117)	(0.182)	(0.292)	(0.162)	(0.260)	(1.00)
44	11.6	16.9	6.3	1.9	3.3	2.8	3.5	2.3	3.0	6.48
	(1.79)	(2.61)	(0.972)	(0.293)	(0.509)	(0.432)	(0.540)	(0.355)	(0.463)	(1.00)
45	18.1	15.4	1.2	3.3	4.7	1.8	2.9	3.0	2.9	21.5
	(0.842)	(0.716)	(0.195)	(0.116)	(0.219)	(0.0837)	(0.135)	(0.140)	(0.135)	(1.00)
46	5.8	18.3	4.2	2.5	5.5	2.0	3.1	2.5	3.1	9.45
	(0.614)	(1.94)	(0.444)	(0.265)	(0.582)	(0.212)	(0.328)	(0.265)	(0.328)	(1.00)
47	41.6	17.4	5.5	4.3	4.8	3.2	4.5	1.8	2.1	31.8
	(1.31)	(0.547)	(0.173)	(0.135)	(0.151)	(0.101)	(0.142)	(0.0566)	(0.0660)	(1.00)
Mean	25.2	16.6	5.04	2.82	4.02	2.52	3.7	2.42	3.02	16.9
	(1.54)	(1.36)	(0.422)	(0.200)	(0.316)	(0.202)	(0.287)	(0.200)	(0.250)	(1.00)
S.D.	19.0	1.38	0.896	0.901	1.48	0.593	0.761	0.432	0.676	10.1
	(1.01)	(0.882)	(0.326)	(0.0788)	(0.215)	(0.139)	(0.166)	(0.116)	(0.157)	(0.00)

[a] Tissue to plasma ratio

lace and Stacy, 1975) and liver (Owens et al., 1973) were also reported to be major sites of degradation of HGH.

Individual and mean arterial plasma concentration-time curves of $MHGH_{22K}$ after s.c. administration of the hormone (0.5 U kg^{-1}) to rabbits 33–42 are shown in Fig. 2. The extent of bioavailability of $MHGH_{22K}$ after s.c. administration appeared to be complete when compared with the values of AUC; the mean value of AUC from time 0 to 480 min after s.c. administration of the hormone (0.5 U kg^{-1}) was 14400 ng min ml^{-1} (the AUC from time 0 to infinity could be larger than 14400 ng min ml^{-1}, and that from time 480 min to infinity could not be estimated because the terminal phase in the plasma concentration-time profile could not be obtained after s.c. administration) and the AUC value from time 0 to infinity after i.v. administration of the hormone (0.5 U kg^{-1}) was 18400 ng min ml^{-1}. The percentages of the dose excreted in 24 h urine as unchanged $MHGH_{22K}$ after s.c. administration of the hormone (0.5 U kg^{-1}) were also negligible, the mean value being 0.55%.

The amounts (ng per g organ or tissue) of $MHGH_{22K}$ remaining in each organ or tissue at 2 h after s.c. administration of the hormone (0.5 U kg^{-1}) to rabbits 43–47 are listed in Table 2. $MHGH_{22K}$ was highly concentrated in the kidney, liver and plasma, however, lower levels were found in the other organs or tissues studied; the mean values of the tissue (or organ) to plasma ratio (T/P) were 1.54, 1.36, 0.422, 0.200, 0.316, 0.202, 0.287, 0.200 and 0.250 for the kidney, liver, stomach, heart, lung, spleen, large intestine, muscle and brain, respectively. A T/P ratio greater than unity in the kidney and liver was also reported (Salmon et al., 1962) for the case of ^{131}I-HGH being administered intravenously to rabbits.

Acknowledgement

This work was supported by the contract, 'Pharmacokinetics of LBD-009 in rabbits', from Lucky Ltd, Seoul, Korea.

References

Baumann, G. and Abramson, E.C., Urinary growth hormone in man: Evidence for multiple molecular forms. *J. Clin. Endocrinol. Metab.*, 56 (1983) 305–311.

Baumann, G., Stolar, M.W. and Buchanan, T.A., Slow

metabolic clearance rate of the 20.000-dalton variant of human growth hormone: Implications for biological activity. *Endocrinology*, 117 (1985) 1309–1313.

Baumann, G., Stolar, M.W. and Buchanan, T.A., The metabolic clearance, distribution and degradation of dimeric and monomeric growth hormone (GH): Implications for the pattern of circulating GH forms. *Endocrinology*, 119 (1986) 1497–1501.

Chiou, W.L., New calculation method for mean apparent drug volume of distribution to rational dosage regimens, *J. Pharm. Sci.*, 68 (1979) 1067–1069.

Frohman, L.A. and Bernardis, L.L., Growth hormone secretion in the rat: Metabolic clearance and secretion rates. *Endocrinology*, 86 (1970) 305–312.

Fryklund, L.M., Bierich, J.R. and Ranke, M.B., Recombinant human growth hormone, *Clin. Endocrinol. Metab.*, 15 (1986) 511–535.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd Edn, Dekker, New York 1982.

Hanssen, K.F., Immunoreactive growth hormone in human urine. *Acta Endocrinol.*, 71 (1972) 665–676.

Hendricks, C.M., Eastman, R.C., Takeda, S., Asakawa K. and Gorden, P., Plasma clearance of intravenously administered pituitary human growth hormone: Gel filtration studies of heterogenous components. *J. Clin. Endocrinol. Metab.*, 60 (1985) 864–867.

Johnson, V. and Maack, T., Renal extraction, filtration, absorption and catabolism of growth hormone, *Am. J. Physiol.*, 233 (1977) F185–F196.

Kuret, J.A. and Murad, F., Chapter 56, Adenohypophyseal hormone and related substances. In Gilman, G., Rall, T.W., Nies, A. and Taylor, P. (Eds), *The Pharmacological Basis of Therapeutics*, pp. 1334–1343, Maxwell MacMillan, 1991.

Laron, Z., Mannheimer, S. and Guttmann, S., Plasma disappearance of various ^{131}I-labelled growth hormone preparations in man and rabbit. *Nature*, 207 (1965) 298–299.

Lee, M.G., Lui, C.Y., Chen, M.L. and Chiou, W.L., Pharmacokinetics of drug in blood IV: Unusual distribution, storage effect and metabolism of methotrexate. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 22 (1984) 530–534.

Lowry, C., Wright, A.D., Fraser, T.R., Rubenstein, A.H. and Spitz, I., Urine excretion of insulin and growth hormone in subjects with renal failure. *Acta Endocrinol.*, 67 (1971) 85–96.

Owens, D., Srivastava, M.C., Tompkins, C.V., Nabarro, J.D.N. and Sönksen, P.H., Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled human growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus. *Eur. J. Clin. Invest.*, 3 (1973) 284–294.

Rabkin, R., Pimstone, B.L. and Eales, L., Autoradiographic determination of glomerular filtration and proximal tubular absorption of growth hormone ^{125}I in the mouse. *Hormone Metab. Res.*, 5 (1973) 172–175.

Retegui-Sardou, L.A., Scaramal, L.O., Dellacha, J.M. and Paladini, A.C., Rate of concentration and digestion of radioactive growth hormone preparations injected in rats, as measured by the amount and nature of radioactivity in the tissues. *Mol. Cell Biochem.*, 16 (1977) 87–96.

Salacinsky, P.R.P., McLean, C., Sykes, J.E.C., Clement-Jones, V.V. and Lowry, P.J., Iodination of proteins, glycoproteins and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3α, 6α-diphenyl glycoluril (Iodogen). *Anal. Biochem.*, 117 (1981) 136–146.

Salmon, S., Utiger, R., Parker, M. and Reichlin, S., The fate of I^{131}-labelled human growth hormone in the rabbit. *Endocrinology*, 70 (1962) 459–464.

Shin, W.G., Lee, M.G., Lee, M.H. and Kim, N.D., Pharmacokinetics of drugs in blood VII: Unusual distribution and blood storage effect of vancomycin. *Biopharm. Drug Dispos.*, 13 (1992) 305–310.

Sigel, M.B., VanderLaan, W.P., Kobrin, M.S., VanderLaan E.F. and Thorpe, N.A., The biological half-life of human growth hormone and a biologically active 20,000-dalton variant in mouse blood. *Endocr. Res. Commun.*, 9 (1982) 67–77.

Wallace, A.L.C. and Stacy, B.D., Disappearance of ^{125}I-labelled rat growth hormone in nephrectomized and sham operated rats. *Hormone Metab. Res.*, 7 (1975) 135–138.

Wroblewski, V.J., Masnyk, M. and Becker, G.W., Proteolytic cleavage of human growth hormone (hGH) by rat tissues in vitro: Influence on the kinetics of exogenously administered hGH. *Endocrinology*, 129 (1991) 465–474.

Yoon, E.J., Chang, H.W., Lee, M.G., Lee, H-j., Park, M.K. and Kim, C-K., Pharmacokinetics of methotrexate after intravenous infusion of methotrexate-rabbit serum albumin conjugate to rabbits. *Int. J. Pharm.*, 67 (1991) 174–184.