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**Notes**

# Dose-independent pharmacokinetics of recombinant human interferon-$\alpha$A in rabbits

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## Summary

The pharmacokinetic parameters of rHuIFN-$\alpha$A, such as $t_{1/2}$ (53.7–85.3 min), MRT (29.5–42.4 min), $V_{ss}$ (107–163 ml kg$^{-1}$) and CL (3.65–4.69 ml min$^{-1}$ kg$^{-1}$) were found to be dose-independent after intravenous administration to rabbits using a 1000-fold range of doses, i.e., $10^6$–$10^9$ U kg$^{-1}$. The extent of bioavailability was essentially complete when interferon at $10^7$ U kg$^{-1}$ was administered subcutaneously to rabbits. Radioactivity was highly concentrated in the kidney and plasma, and less concentrated in the other tissues or organs studied at 2 h after subcutaneous administration of the radiolabelled interferon, $^{125}$I-rHuIFN-$\alpha$A (100 000 cpm kg$^{-1}$) to rabbits.

Interferons (IFNs) are a family of functionally related proteins with potent antiviral, antiproliferative and immunomodulating effects (Gresser, 1977; Borden and Fall, 1981). They are divided into three major antigenic classes based on their biological and physicochemical properties (Gutterman et al., 1984): leukocytic interferon (IFN-$\alpha$), at least one fibroblast interferon (IFN-$\beta$), and immune interferon (IFN-$\gamma$). Recombinant DNA techniques have made available relatively large quantities and highly purified forms of interferons (Goeddel et al., 1980). Several recombinant human IFN-$\alpha$ (rHuIFN-$\alpha$) subtypes have been identified (Nagata et al., 1981). Among them, recombinant human leukocyte A interferon (rHuIFN-$\alpha$A) is a highly purified single protein moiety of human $\alpha$-interferon derived by recombinant DNA techniques (Levy et al., 1981). Lucky Ltd (Seoul, South Korea) has recently succeeded in manufacturing rHuIFN-$\alpha$A by recombinant DNA techniques, and its structure and physicochemical properties have been studied (unpublished data).

The pharmacokinetics of rHuIFN-$\alpha$A in mice (Bohoslawec et al., 1986), dogs (Gibson et al., 1985), monkeys (Wills et al., 1984b; Collins et al., 1985), cancer patients (Gutterman et al., 1982;

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Sherwin et al., 1982) and healthy subjects (Wills et al., 1984a) have been reported. However, the pharmacokinetics of the interferon after intravenous (i.v.) administration of wide dose ranges appeared not to be thoroughly studied.

The purpose of this Note is to report the pharmacokinetics of rHuIFN-$\alpha$A after i.v. administration of the interferon, $10^6$–$10^9$ U kg$^{-1}$ to rabbits. The extent of bioavailability ($F$) after subcutaneous (s.c.) administration of the interferon ($10^7$ U kg$^{-1}$) and the tissue distribution and renal excretion after s.c. administration of the radiolabelled interferon ($^{125}$I-rHuIFN-$\alpha$A) (100000 cpm kg$^{-1}$) to rabbits are also reported.

50, healthy, male New Zealand White rabbits (1.3–2.9 kg) were anesthetized with 30–50 mg of i.v. ketamine (kindly supplied by the Yu-Han Research Center, Kumpo, Seoul, South Korea) via an ear vein. The left jugular vein (i.v. study only) and carotid artery were catheterized with silastic tubing (Dow Corning Inc., Midland, MI) for drug administration and blood sampling, respectively. The animals were allowed to recover from anesthetization for 4–5 h before the study and fasted during the experiment.

rHuIFN-$\alpha$A ($2.88 \times 10^9$ U per vial dissolved in 10 mM PBS buffer, kindly supplied by Lucky Ltd) was freshly reconstituted with normal saline before use. The interferon, $10^6$, $10^7$, $10^8$ and $10^9$ U kg$^{-1}$, was injected (injection volume approx. 2 ml) during a period of 1 min through the cannula placed in the jugular vein into rabbits 1–5, 6–15, 16–25 and 26–35, respectively. The interferon, $10^7$ U kg$^{-1}$ was injected (injection volume approx. 2 ml) s.c. into the dorsal side of the neck of rabbits 36–40. $^{125}$I-rHuIFN-$\alpha$A (kindly supplied by Lucky Ltd), 100000 cpm kg$^{-1}$ was similarly injected (injection volume approx. 2 ml) s.c. into rabbits 41–45. 2 h after the injection, as much whole blood as possible was collected through the carotid artery. The animal was killed and the liver, kidney, spleen, heart, lung, brain, stomach, small intestine, large intestine and muscle were excised. $^{125}$I-rHuIFN-$\alpha$A, 100000 U kg$^{-1}$, was also injected s.c. into rabbits 46–50, and urine samples were collected for 4 days using pediatric Foley catheter (Dover, Searl Medical Products, U.S.A. Inc., Dallas, TX) which was introduced into the urinary bladder. Blood and urine collection methods were similar to those reported previously (Yoon et al., 1991).

Plasma concentrations of rHuIFN-$\alpha$A were evaluated by slight modification of the reported method (Yousefi et al., 1985); crystal violet and MDBK (kidney, bovine, Bostourus, ATTCC CCL 22) cells were used instead of methylene blue and WISH (amnion tissue): $HE_p$-2 (carcinoma of the larynx) cell, respectively, since MDBK was reported (Kramer et al., 1983) to have greater sensitivity. The total radioactivity was measured using a $\gamma$-counter.

The pharmacokinetic parameters, such as the area under the plasma concentration-time curve from time zero to time infinity (AUC), apparent volume of distribution at steady state ($V_{ss}$), time-averaged total body clearance (CL) and mean residence time (MRT) were estimated by the standard method (Gibaldi and Perrier, 1982). The data were analyzed for statistical significance ($p < 0.05$) using analysis of variance. The harmonic mean was employed for the calculation of the mean values of $t_{1/2}$, CL and $V_{ss}$ (Chiou, 1979).

After i.v. doses of $10^6$–$10^9$ U kg$^{-1}$, the mean arterial plasma concentrations of rHuIFN-$\alpha$A declined polyexponentially (Fig. 1) with mean terminal half-lives of 53.7 (ranging from 44.4 to 87.6 min), 57.0 (ranging from 31.9 to 130 min), 76.2 (ranging from 33.8 to 140 min) and 85.3 min (ranging from 59.6 to 126 min) for the doses of $10^6$, $10^7$, $10^8$ and $10^9$ U kg$^{-1}$, respectively (Table 1). The present mean terminal half-lives of 53.7–85.3 min are lower than 2.25 h in monkeys (Wills et al., 1984b; Collins et al., 1985), and 5.1 h in healthy subjects (Wills et al., 1984a). It might be due to the differences in blood sampling schedules and analytical assay method used, such as bioassay and ELISA. The values of AUC increased almost proportionally with the increasing doses and those of $t_{1/2}$, MRT, $V_{ss}$ and CL were not significantly different among the dose ranges studied (Table 1). The above data indicate that the pharmacokinetic parameters of rHuIFN-$\alpha$A are dose-independent over the 1000-fold range of doses, $10^6$–$10^9$ U kg$^{-1}$. It was reported that the maximum serum concentration and AUC of the interferon increased in proportion to the doses

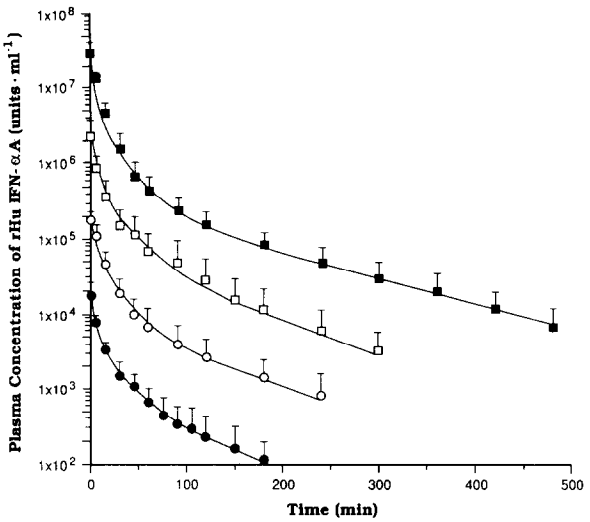


Fig. 1. Mean plasma concentration-time profiles of rHuIFN-$\alpha$A after intravenous administration of the interferon, $10^6$ (●), $10^7$ (○), $10^8$ (□), and $10^9$ U kg$^{-1}$ (■) to rabbits 1–5, 6–15, 16–25 and 26–35, respectively. Bars represent standard deviation.

when the interferons, 3–198 $\times$ $10^6$ U, were administered intramuscularly to cancer patients (Gutterman et al., 1982), and the concentration of the interferon in CSF increased in proportion to the doses and clearance was dose-independent when the interferons, 5000 and 120000 U kg$^{-1}$, were administered intraventricularly to monkeys (Collins et al., 1985). However, dose-dependent pharmacokinetics was reported (Sherwin et al., 1982) when the interferons, 3, 50 and 100 $\times$ $10^6$ U, were administered intramuscularly to cancer patients. The $V_{ss}$ ranged from 107 to 163 ml kg$^{-1}$ and CL from 3.65 to 4.69 ml min$^{-1}$ kg$^{-1}$ at the dose ranges studied and the comparable values of $V_{ss}$ in mice (Bohoslawec et al., 1986), dogs (Gibson et al., 1985) and monkeys (Wills et al., 1984b; Collins et al., 1985), and of CL in healthy subjects (Wills et al., 1984a) and monkeys (Wills et al., 1984b) were reported. It should be noted that renal clearance could not be estimated in the present study, since rHuIFN-$\alpha$A was not detected in urine even in the samples collected at 0–10 min after the dose. This result is consistent with the report that rHuIFN-$\alpha$A was undetectable in urine from rats and monkeys (Bino et al., 1982a) and cancer patients (Gutterman et al., 1982).

After s.c. administration of rHuIFN-$\alpha$A, $10^7$ U kg$^{-1}$, the peak plasma concentration reached at 4–8 h after dose (except at 1 h for rabbit 39) and declined (Fig. 2) with a mean terminal half-life of 219 min (ranging from 162 to 303 min), which is longer than that after i.v. dose of $10^7$ U kg$^{-1}$ (Table 1). The extent of bioavailability ($F$) after s.c. administration was essentially complete when compared with AUC values after i.v. and s.c. administration of the same dose, $10^7$ U kg$^{-1}$. A comparable value, 93%, was reported for the case where interferon was administered intramuscularly to four monkeys (Wills et al., 1984b), however, a lower value was observed when the interferon was administered s.c. to dogs (42%; Gibson

TABLE 1

*Mean pharmacokinetic parameters of rHuIFN-$\alpha$A after intravenous administration of the interferon, $10^6$, $10^7$, $10^8$ and $10^9$ U kg$^{-1}$ to rabbits 1–5, 6–15, 16–25 and 26–35, respectively, and subcutaneous administration of the interferon, $10^7$ U kg$^{-1}$ to rabbits 36–40*

| | Dose | | | | |
|---|---|---|---|---|---|
| | $10^6$ U kg$^{-1}$ (i.v.) | $10^7$ U kg$^{-1}$ (i.v.) | $10^8$ U kg$^{-1}$ (i.v.) | $10^9$ U kg$^{-1}$ (i.v.) | $10^7$ U kg$^{-1}$ (s.c.) |
| $t_{1/2}$ (min) | 53.7 ± 17.9[a] | 57.0 ± 30.1 | 76.2 ± 36.2 | 85.3 ± 22.8 | 219 ± 63.6 |
| MRT (min) | 35.8 ± 11.7 | 42.4 ± 25.2 | 41.2 ± 11.1 | 29.5 ± 5.90 | |
| AUC $\times$ $10^5$ (U min ml$^{-1}$) | 2.13 ± 0.707 | 27.3 ± 9.52 | 265 ± 109 | 2740 ± 715 | 31.2 ± 11.0 |
| $V_{ss}$ (ml kg$^{-1}$) | 163 ± 43.7 | 128 ± 83.7 | 149 ± 77.5 | 107 ± 23.1 | |
| CL (ml min$^{-1}$ kg$^{-1}$) | 4.69 ± 1.45 | 3.67 ± 1.83 | 3.78 ± 1.90 | 3.65 ± 1.04 | |

[a] SD.

TABLE 2

*Amount (cpm per g tissue) of total radioactivity remaining in each tissue at 2 h after subcutaneous administration of radiolabelled interferon, $^{125}$I-rHuIFN-α A, 100 000 cpm kg$^{-1}$ to rabbits 41–45*

| Rabbit | Kidney | Small intestine | Liver | Stomach | Heart | Lung | Spleen | Large intestine | Muscle | Brain | Plasma |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | 253 | 69 | 48 | 47 | 98 | 58 | 47 | 66 | 2 | 34 | 204 |
| (2.25 kg) | (1.24) [a] | (0.338) | (0.253) | (0.23) | (0.48) | (0.284) | (0.230) | (0.324) | (0.010) | (0.167) | (1.00) |
| 42 | 411 | 66 | 24 | 38 | 80 | 87 | 54 | 44 | 45 | 5 | 164 |
| (2.20 kg) | (2.51) | (0.402) | (0.146) | (0.232) | (0.488) | (0.530) | (0.329) | (0.286) | (0.274) | (0.031) | (1.00) |
| 43 | 486 | 78 | 48 | 85 | 52 | 45 | 51 | 75 | 19 | 22 | 151 |
| (2.25 kg) | (3.22) | (0.517) | (0.318) | (0.563) | (0.344) | (0.298) | (0.338) | (0.497) | (0.126) | (0.146) | (1.00) |
| 44 | 357 | 16 | 34 | 60 | 63 | 93 | 22 | 79 | 37 | 28 | 182 |
| (1.75 kg) | (2.06) | (0.088) | (0.187) | (0.330) | (0.346) | (0.511) | (0.121) | (0.434) | (0.203) | (0.154) | (1.00) |
| 45 | 216 | 38 | 36 | 21 | 47 | 76 | 49 | 38 | 40 | 45 | 101 |
| (2.00 kg) | (2.14) | (0.376) | (0.356) | (0.208) | (0.465) | (0.752) | (0.485) | (0.376) | (0.396) | (0.466) | (1.00) |
| Mean | 348 | 53.4 | 38.0 | 50.2 | 68.0 | 71.8 | 44.6 | 60.4 | 28.6 | 26.8 | 160 |
| | (2.23) | (0.344) | (0.252) | (0.313) | (0.425) | (0.475) | (0.301) | (0.380) | (0.202) | (0.189) | (1.00) |
| SD | 100 | 23.0 | 9.12 | 21.5 | 18.8 | 17.9 | 11.5 | 16.5 | 15.7 | 13.3 | 34.6 |
| | (0.644) | (0.141) | (0.078) | (0.132) | (0.065) | (0.173) | (0.121) | (0.080) | (0.131) | (0.138) | (0.00) |

[a] Tissue/plasma ratio.

et al., 1985) and monkeys (19–103%; Collins et al., 1985).

The radioactivity in the kidney was greater than that in the plasma (tissue-to-plasma ratio, *T/P* of 2.23), however, it was lower in the liver, stomach, heart, lung, spleen, small intestine, large intestine, muscle and brain as shown by the *T/P* ratios of less than unity (Table 2). A similar tissue distribution was reported when rHuIFN-αA was injected i.v. into mice (Bohoslawec et al., 1986), and rats and monkeys (Bino et al., 1982a), and $^{125}$I-rHuIFN-αA was administered i.v. to mice (Palleroni and Bohoslawec, 1984). It suggested that rHuIFN-αA has less affinity for the other

TABLE 3

*Cumulative amounts (cpm) of total radioactivity excreted in urine up to 96 h after subcutaneous administration of radiolabelled interferon, $^{125}$I-rHuIFN-α A, 100 000 cpm kg$^{-1}$ to rabbits 46–50*

| Rabbit | 0–24 h | 0–48 h | 0–72 h | 0–96 h | $X(u)_{0-96\,h}$/dose (%) |
|---|---|---|---|---|---|
| 46 | 109 000 | 122 000 | 126 000 | 128 000 | |
| (2.90 kg) | (85.0%) [a] | (95.6%) | (98.5%) | (100%) | 44.1 |
| 47 | 89 200 | 95 700 | 98 400 | 100 000 | |
| (2.35 kg) | (88.9%) | (95.4%) | (98.1%) | (100%) | 42.7 |
| 48 | 78 700 | 86 200 | 86 500 | 88 300 | |
| (2.15 kg) | (89.1%) | (97.6%) | (98.4%) | (100%) | 41.1 |
| 49 | 73 000 | 77 700 | 78 700 | 80 100 | |
| (2.35 kg) | (91.9%) | (97.0%) | (98.4%) | (100%) | 34.1 |
| 50 | 54 500 | 65 900 | 69 100 | 70 000 | |
| (2.35 kg) | (77.9%) | (94.2%) | (98.8%) | (100%) | 29.8 |
| Mean ± SD | 80 800 ± 18 000 | 89 600 ± 19 100 | 92 000 ± 19 600 | 93 300 ± 20 000 | |
| | (86.4 ± 4.69 [b]%) | (96.0 ± 1.21%) | (98.6 ± 0.314%) | (100 ± 0.00%) | 38.4 ± 5.49 |

$X(u)$: cumulative amounts of total radioactivity excreted in urine.

[a] $X(u)_{0-t\,h}/X(u)_{0-96\,h}$ ratio.

[b] SD.

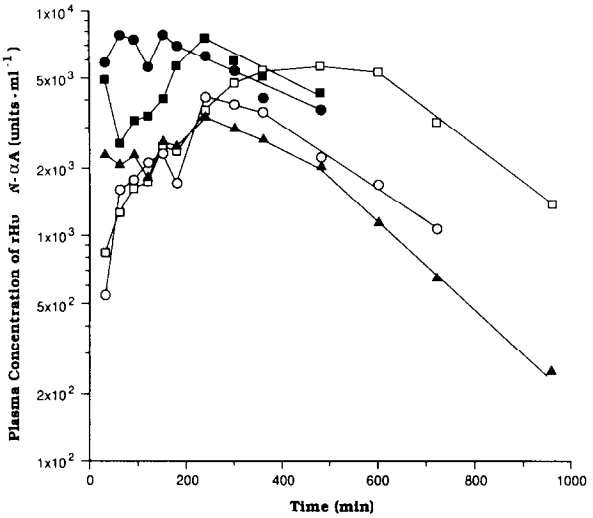


Fig. 2. Plasma concentration-time profiles of rHuIFN-$\alpha$A after subcutaneous administration of the interferon, $10^7$ U $kg^{-1}$ to rabbits 36 (■), 37 (□), 38 (▲), 39 (●) and 40 (○).

tissues studied except the kidney. The low affinity of rHuIFN-$\alpha$A for the tissues was expected on the basis of its high molecular weight (approx. 19000), considerable water solubility, and highly charged molecules. It was also proven that the value of $V_{ss}$ was small, 107–163 ml $kg^{-1}$, after i.v. doses of the interferon, $10^6$–$10^9$ U $kg^{-1}$ (Table 1). It should be noted that the total radioactivity was measured in tissue studies, therefore, it does not represent only unchanged rHuIFN-$\alpha$A, but also corresponds to the sum of both rHuIFN-$\alpha$A and its metabolites.

The urinary excretion of total radioactivity was low, 38.4% of the administered total radioactivity being excreted in 96-h urine (Table 3). This is consistent with the result (Bocci et al., 1983) that only small amounts of radiolabelled human interferon $\alpha$A appeared in the urine. It is well established (Bino et al., 1982a,b) that human IFNs-$\alpha$ are cleared through the kidney via glomerular filtration followed by rapid degradation by two types of proteinase formed in lysosomal fractions of rat, monkey and human kidneys during reabsorption, resulting in a negligible extent of reappearance of intact human IFNs-$\alpha$ in the systemic circulation. However, human $\alpha$-interferon was reported to be stable in isolated rabbit liver (Bocci, 1982). Therefore, the reabsorption of catabolites of the interferon from kidney can be anticipated from this study. Based on the above data, it could be concluded that the CL of the interferon in the present i.v. studies could be approximately equal to the renal clearance ($CL_R$). The CL values of 3.65–4.69 ml $min^{-1}$ $kg^{-1}$ after i.v. injection of the interferon, $10^6$–$10^9$ U $kg^{-1}$ (Table 1), were somewhat lower than the glomerular filtration rate based on inulin clearance in rabbits, 5 ml $min^{-1}$ $kg^{-1}$ (Chen and Chiou, 1983). This suggested that the tubular secretion could not play a significant role in the renal clearance of interferon in rabbits assuming that the interferon does not bind to plasma proteins in rabbits. It was also suggested that (Gibson et al., 1985) no tubular secretion or extrarenal clearance of the interferon occurred in the dog. It was reported that the CL value of the interferon was very close to that of inulin clearance (0.368 vs 0.38 ml $min^{-1}$) in mice (Bohoslawec et al., 1986), however, active secretion was reported in healthy subjects (Wills et al., 1984a).

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## References

Bino, T., Edery, H., Gertler A. and Rosenberg, H., Involvement of the kidney in catabolism of human leukocyte interferon. *J. Gen. Virol.*, 59 (1982a) 39–45.

Bino, T., Madar, Z., Gertler, A., and Rosenberg, H., The kidney is the main site of interferon degradation. *J. Interferon Res.*, 2 (1982b) 301–308.

Bocci, V., Pacini, A., Bandinelli, L., Pessina, G.P., Muscettola M. and Paulesu, L., The role of liver catabolism of human $\alpha$- and $\beta$-interferon. *J. Gen. Virol.*, 60 (1982) 397–400.

Bocci, V., Mogensen, K.E., Muscettola, M., Pacini, A., Paulesu, L., Pessina G.P. and Skiftas, S., Degradation of human $^{125}$I-interferon alpha by isolated perfused rabbit kidney and liver. *J. Lab. Clin. Med.*, 101 (1983) 857–863.

Bohoslawec, O., Trown, P.W. and Wills, R.J., Pharmacokinetics and tissue distribution of recombinant human alpha A,

D, A/D(Bgl), and I interferons and mouse alpha interferon in mice. *J. Interferon Res.*, 6 (1986) 207–213.

Borden, E.C. and Fall, L.A., Interferons; biochemical, cell growth, inhibitory, and immunological effects. *Prog. Hematol.*, 350 (1981) 1–641.

Chen, M.L. and Chiou, W.L., Clearance studies of methotrexate and 7-hydroxymethotrexate in rabbits after multiple-dose infusion. *J. Pharmacokinet. Biopharm.*, 11 (1983) 515–527.

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

Collins, J.M., Riccardi, R., Trown, P., O'Neill D. and Poplack, D.G., Plasma and cerebrospinal fluid pharmacokinetics of recombinant interferon alpha A in monkeys: Comparison of intravenous, intramuscular and intraventricular delivery. *Cancer Drug Deliv.*, 2 (1985) 247–253.

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

Gibson, D.M., Cotler, S., Spiegel H.E. and Colburn, W.A., Pharmacokinetics of recombinant leukocyte A interferon following various routes and modes of administration to the dogs. *J. Interferon Res.*, 5 (1985) 403–408.

Goeddel, D.V., Yelverton, E., Ullrich, A., Heyneker, H.L., Miozzari, G., Holmes, W., Seeburg, P.H., Dull, T., May, L., Stebbing, N., Crea, R., Maeda, S., McCandliss, R., Sloma, A., Tabor, J.M., Gross, M., Familletti, P.C. and Pestka, S. Human leukocyte interferon produced by *E. coli* is biologically active. *Nature*, 287 (1980) 411–416.

Gresser, I. On the varied biological effects of interferon. *Cell Immunol.*, 34 (1977) 406–415.

Gutterman, J.U., Fine, S., Quesada, J., Horning, S.J., Levine, J.F., Alexanian, R., Bernhardt, L., Kramer, M., Spiegel, H., Colburn, W., Trown, P., Merigan, T. and Dziewanowski, Z., Recombinant leukocyte A interferon: Pharmacokinetics, single dose tolerance, and biological effects in cancer patients. *Ann. Int. Med.*, 96 (1982) 549–556.

Gutterman, J.U., Rosenblum, M.G., Rios, A.A., Herbert, A.F. and Quesada, J. Pharmacokinetic study of partially pure gamma-interferon in cancer patients. *Cancer Res.*, 44 (1984) 4164–4171.

Kramer, M.J., Dennin, R., Kramer, C., Jones, G., Connell, E., Rolon, N., Gruarin, A., Kale, R. and Trown, P.W., Cell and virus sensitivity studies with recombinant human alpha interferons. *J. Interferon Res.*, 3 (1983) 425–435.

Levy, W.P., Rubinstein, M., Shively, S., Del Valle, U., Lai, C.Y., Moschera, J., Brink, L., Gerber, L., Stein S. and Pestka, S., Amino acid sequence of human leukocyte interferon. *Proc. Natl. Acad. Sci. USA*, 78 (1981) 6186–6190.

Nagata, S., Brack, C., Henco, K., Schamböck, A. and Weissmann, C., Partial mapping of ten genes of the human interferon-$\alpha$ family. *J. Interferon Res.*, 1 (1981) 333–336.

Palleroni, A.V. and Bohoslawec, O., Use of $^{125}$I-interferons in pharmacokinetic and tissue distribution studies. *J. Interferon Res.*, 4 (1984) 493–498.

Sherwin, S.A., Knost, J.A., Fein, S., Abrams, P.G., Foon, K.A., Ochs, J.J., Schoenberger, C., Maluish, A.E. and Oldham, R.K., A multiple-dose phase I trial of recombinant leukocyte A interferon in cancer patients. *J. Am. Med. Assoc.*, 248 (1982) 2461–2466.

Wills, R.J., Dennis, S., Spiegel, H.E., Gibson, D.M. and Nadler, P.I., Interferon kinetics and adverse reactions after iv infusion, im and sc routes of administration. *Clin. Pharmacol. Ther.*, 35 (1984a) 722–727.

Wills, R.J., Spiegel, H.E. and Soike, K.F., Pharmacokinetics of recombinant alpha A interferon following iv infusion and bolus, im and po administrations to african green monkeys. *J. Interferon Res.*, 4 (1984b) 399–409.

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.

Yousefi, S., Escobar, M.R. and Gouldin, C.W., A practical cytopathic effect/dye-uptake interferon assay for routine use in the clinical laboratory. *Am. J. Clin. Pathol.*, 83 (1985) 735–740.