ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1996, p. 2262–2265 Vol. 40, No. 10
0066-4804/96/$04.00+0

Pharmacokinetics of a Water-Soluble Fullerene in Rats

PRABHU RAJAGOPALAN,[1] FRED WUDL,[2] RAYMOND F. SCHINAZI,[3] AND F. DOUGLAS BOUDINOT[1]*

Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, Georgia 30602[1]; Institute for Polymers and Organic Solids and Departments of Chemistry and Physics, University of California, Santa Barbara, California 93106[2]; and Veterans Affairs Medical Center, Decatur, Georgia 30033, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322[3]

Received 15 February 1996/Returned for modification 15 June 1996/Accepted 14 July 1996

Fullerenes are the recently discovered third allotropic form of carbon. The biological activities of these compounds are being studied for various purposes. The bis(monosuccinimide) derivative of *p*,*p*′-bis(2-aminoethyl)-diphenyl-C_{60} (MSAD-C_{60}) is a water-soluble fullerene derivative. MSAD-C_{60} has been shown to have antiviral activity against human immunodeficiency virus types 1 and 2 in vitro and to have virucidal and anti-human immunodeficiency virus protease activities. Moreover, MSAD-C_{60} has been shown to be well tolerated in mice after intraperitoneal administration. The purpose of the present study was to develop a high-performance liquid chromatographic analytical methodology for MSAD-C_{60} and to characterize the preclinical pharmacokinetics of the compound in rats. Following intravenous administration of the fullerene derivative at a dose of 15 mg/kg of body weight, the concentrations of MSAD-C_{60} in plasma declined either bi- or triexponentially. The mean terminal-phase half-life of MSAD-C_{60} was 6.8 ± 1.1 h (mean ± standard deviation). Binding studies indicated that the compound is greater than 99% bound to plasma proteins. The average total clearance of the compound was 0.19 ± 0.06 liters/h/kg. Urine samples obtained 24 h after intravenous administration did not contain detectable levels of the compound, indicating the absence of a significant renal clearance mechanism. The steady-state volume of distribution of MSAD-C_{60} averaged 2.1 ± 0.8 liters/kg, indicating that the compound distributes into tissues. At a dose of 15 mg/kg, MSAD-C_{60} appeared to be well tolerated. However, a dose of 25 mg/kg resulted in shortness of breath and violent movement of the rats, followed by death within 5 min of dosing. Further controlled toxicity studies are needed to fully evaluate the toxicity of the compound.

Buckminsterfullerene (C_{60}) is a recently discovered third allotropic form of carbon. C_{60} and clusters with higher numbers of carbon atoms are commonly referred to as fullerenes. Fullerenes have been found to occur naturally in the geological environment of Shunga in Karwelia, Russia (5). These compounds have elicited intense interest among physicists and chemists for the past 10 years, and a recent report on the macroscopic production of C_{60} provided the impetus needed for increased research efforts (9). However, biological studies have been hindered because of the hydrophobic nature of fullerenes. In an effort to overcome problems associated with the insolubility of C_{60} in aqueous solutions, several strategies have been attempted. These strategies include derivatization with water-soluble moieties or the formation of complexes with cyclodextrin, liposomes, and polyvinylpyrrolidone (1, 3, 8, 22). Currently, water-soluble C_{60} and C_{70} are the only fullerenes being actively pursued in biological studies.

Friedman et al. (6), using molecular modeling, suggested that fullerene derivatives should inhibit human immunodeficiency virus (HIV) protease primarily because of steric interactions between C_{60} and hydrophobic active-site surfaces. The in vitro antiviral properties of fullerenes were verified by synthesizing a water-soluble fullerene derivative, a bis(monosuccinimide) derivative of *p*,*p*′-bis(2-aminoethyl)-diphenyl-C_{60} (MSAD-C_{60}) (15, 16). The structure of MSAD-C_{60} is illustrated in Fig. 1. MSAD-C_{60} possesses antiviral activity against HIV type 1 (HIV-1) and HIV-2 (50% effective concentration, 6 μM) in acutely and chronically infected cells and has similar activity against HIV-1 reverse transcriptase and DNA polymerase in a cell-free system. Its primary mechanism of action is inhibition of the HIV protease, with a 50% inhibitory concentration of 2.0 μM against recombinant HIV-1 protease (15). MSAD-C_{60} has also been shown to have virucidal properties (14, 15).

Investigations on the photophysical properties of fullerenes suggest that these compounds have a strong potential to cause photodynamic damage to biological systems (2). However, not all fullerenes have photochemical properties. Further studies by Tokuyama et al. (17) confirmed that a fullerene carboxylic acid exhibited cytotoxicity against HeLa S3 cells and cleaving activity against supercoiled pBR322 DNA when it was exposed to fluorescent light but not in the dark. Because of this property, certain fullerene derivatives may find a use in photodynamic therapy.

Yamago et al. (21) reported total radioactivity levels following the administration of ^{14}C-labeled C_{60} derivatives to rats. However, to date, no investigations concerning the disposition of intact C_{60} by analytical methods specific for the administered compound have been reported. Acute and subchronic toxicity studies with mice indicate that C_{60} does not have any acute toxic effects when it is applied to mouse skin (12). Furthermore, no apparent toxic effects were observed after intraperitoneal administration of 50 mg of MSAD-C_{60} per kg of body weight per day for 6 days to mice (14). These results were encouraging, so a specific analytical methodology for MSAD-C_{60} was developed, and the pharmacokinetics of this novel fullerene compound were characterized in rats.

* Corresponding author. Mailing address: Department of Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA 30602-2353. Phone: (706) 542-5335. Fax: (706) 542-5346. Electronic mail address: boudinot@rx.uga.edu.

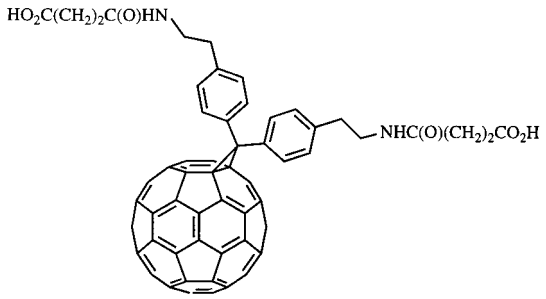

FIG. 1. Structure of MSAD-C_{60}.

MATERIALS AND METHODS

Chemicals. MSAD-C_{60} was synthesized as described previously (15, 16). Diethylstilbestrol dipropionate (DESD), used as an internal standard, was purchased from Sigma Chemical Co. (St. Louis, Mo.). Acetonitrile and methanol, high-performance liquid chromatography (HPLC) grade, and other chemicals, analytical grade, were purchased from J. T. Baker (Phillipsburg, N.J.).

Pharmacokinetic study design. Five adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, Ind.) with an average weight of 287 ± 12 g (mean ± standard deviation) were used for the pharmacokinetic studies. The rats were maintained at the University of Georgia College of Pharmacy Animal Care Facility, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. The animals were housed in an environment with a 12-h light and 12-h dark cycle and a constant temperature (22°C) and had free access to standard laboratory chow and water. Rats were acclimatized to this environment for 1 week prior to the experiments. The animal studies were approved by the University of Georgia Animal Care and Use Committee and were conducted in accordance with guidelines established by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (11).

On the day before the experiment, a cannula was surgically implanted in the right jugular vein under ketamine-acepromazine-xylazine (50:3.3:3.3 mg/kg) anesthesia. Rats were fasted overnight; however, water was available ad libitum. On the day of the experiment, the animals were placed in individual metabolic cages. MSAD-C_{60} (8.6 mg/ml), dissolved in 0.5 ml of dimethyl sulfoxide (DMSO), was administered intravenously at a dose of 15 mg/kg of body weight. The cannula was flushed with an additional 50 μl of DMSO and then saline. Food was withheld for the first 2 h after dosing, but water was available ad libitum. Blood samples (0.25 ml) were collected from the cannula into heparinized polypropylene microcentrifuge tubes prior to and at 0.08, 0.25, 0.50, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, and 24 h after drug administration. Preliminary studies demonstrated that MSAD-C_{60} did not adsorb to the cannulas. Blood was replaced with an equal volume of normal saline. The blood samples were immediately centrifuged at 9,000 × *g* for 10 min, and the plasma was separated. Urine samples were obtained 24 h after dosing, and the urine volume was recorded. Plasma and urine samples were stored at −20°C until analysis.

Protein binding. The plasma protein binding of MSAD-C_{60} was determined by ultrafiltration. To 500 μl of blank plasma, 6 to 10 μl of MSAD-C_{60} in DMSO was added to yield concentrations of 10 and 100 μg of MSAD-C_{60} per ml. The plasma samples were placed into a Centrifree micropartition system (Amicon, Beverly, Mass.) and were centrifuged at 1,500 rpm for 5 min. Preliminary studies conducted with buffer indicated about 15% adsorption to the membrane. The ultrafiltrate was injected directly onto the HPLC column.

Analytical methodology. Standard solutions of MSAD-C_{60} were prepared in DMSO. The standard curve concentration of MSAD-C_{60} prepared in blank rat plasma ranged from 1 to 15 μg/ml (0.86 to 12.9 μM). Plasma samples (100 μl) were placed in 15-ml glass extraction tubes to which 200 μl of ethyl acetate was added. This was followed by the addition of 100 μl of internal standard (5 μg/ml), and the contents of the tube were vortexed briefly. One hundred microliters of 10 N hydrochloric acid was added; this was followed by vigorous mixing for 30 s. MSAD-C_{60} was then extracted twice with 10 ml of ethyl acetate. The extraction tubes were shaken for 10 min at low speed; this was followed by centrifugation at 2,000 × *g* for 10 min. The organic layer was removed and evaporated under a gentle stream of nitrogen gas at ambient temperature. The residue was reconstituted in 5 mM phosphate buffer–acetonitrile–triethylamine (1:4:0.125). Since the drug is photosensitive, exposure to white light was avoided and the assay was performed under yellow light.

Standard curves for the analysis of urine samples were prepared with the analyte in the solvent mixture used for reconstitution. The standard curve concentrations for the determination of the MSAD-C_{60} concentration in urine ranged from 0.5 to 15 μg/ml (0.43 to 12.9 μM). To 300 μl of the diluted urine sample, 100 μl of internal standard (5 μg/ml) was added, the contents were vortexed, and 200 μl was injected onto the HPLC column.

A Hypersil octyldecyl silane (C_{18}) reversed-phase column (15 by 0.45 cm) packed with 5-μm particles (Alltech, Deerfield, Ill.) with a C_{18} guard column was used for the separation of the compounds. Chromatography was performed at ambient temperature. The mobile phase consisted of 5 mM dibasic sodium phosphate–methanol–acetonitrile (35:4:61; vol/vol). The pH of the phosphate buffer was adjusted to 7.5 with 10% phosphoric acid prior to preparation of the mobile phase. The flow rate of the mobile phase was 1.7 ml/min. The wavelength of detection was 270 nm, and the detector was set at 0.005 absorbance unit, full scale.

Standard curves were prepared by plotting the peak area ratio (drug/internal standard) versus the standard drug concentration. Slope values were determined by linear regression analysis with a weighting factor of $1/x^2$. Use of this weighing factor generated a normal distribution of weighted residuals around the standard curve over the entire range of drug concentrations.

Pharmacokinetic analysis. Area-moment analysis was used to calculate the pharmacokinetic parameters for MSAD-C_{60}. The area under the plasma concentration-versus-time curve (AUC) and the area under the first moment curve (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time (13), with extrapolation to time infinity by using the terminal slope (λ_z) generated by weighted (1/*C*) NONLIN least-squares regression (10). Total clearance (CL) was calculated from dose/AUC, steady-state volume of distribution (V_{ss}) was calculated from dose × AUMC/AUC^2, and half-life ($t_{1/2}$) was calculated from $0.693/\lambda_z$. Renal clearance (CL_R) was calculated as A_u/AUC, where A_u is the amount of MSAD-C_{60} excreted unchanged in the urine. Nonrenal clearance (CL_{NR}) was determined as the difference between CL and CL_R. The mean residence time in the body (MRT) was calculated as AUMC/AUC. Furthermore, the mean residence time in the central compartment (MRT_C) was calculated by AUC/C_p^0, where C_p^0 is the plasma MSAD-C_{60} concentration at time zero. The mean residence time in the peripheral compartment (MRT_P) was calculated as the difference between MRT and MRT_C (20).

RESULTS AND DISCUSSION

Fullerenes are being evaluated for a variety of biological activities. Toniolo et al. (18) reported the synthesis and evaluation of a hydrophilic fullerene peptide conjugate which exhibited potent chemotactic activity in a human monocyte chemotaxis assay. This compound also weakly inhibited HIV-1 protease. The fullerene was made water soluble by the covalent linking of a fullerene derivative to a peptide. Another study illustrated the promoting action of fullerene in chondrogene-

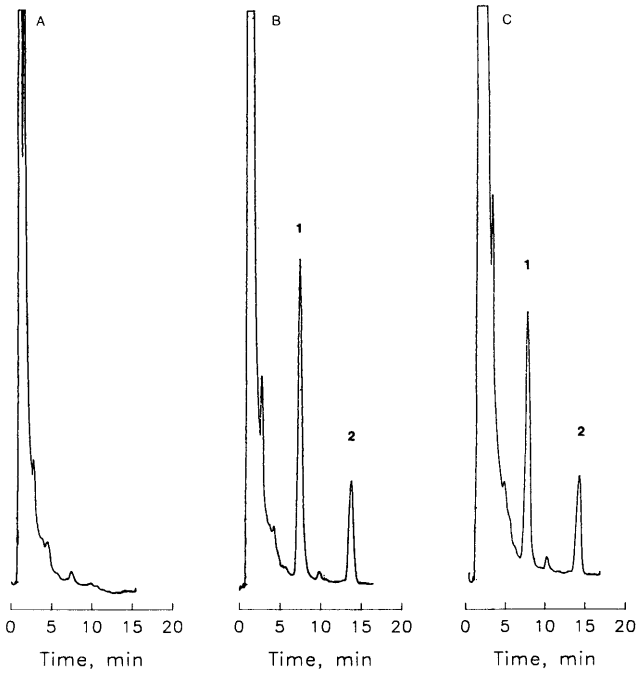

FIG. 2. Chromatograms obtained from blank rat plasma (A), blank rat plasma to which MSAD-C_{60} (curve 1) and the internal standard, DESD, were added (curve 2) (B), and a rat plasma sample obtained after the intravenous bolus administration of 15 mg of MSAD-C_{60} per kg (C).

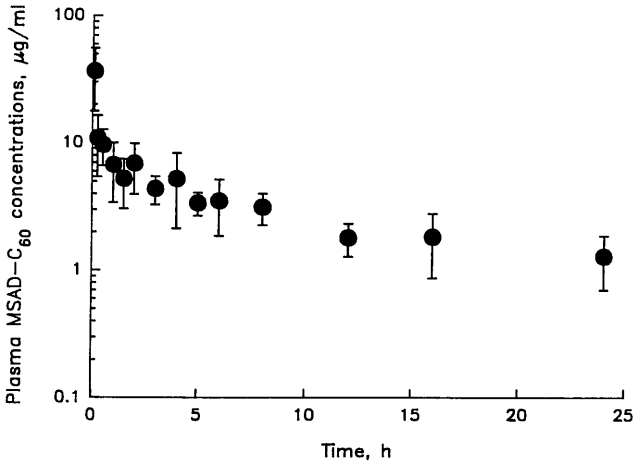

FIG. 3. Mean ± standard deviation concentrations of MSAD-C_{60} in plasma as a function of time following the intravenous bolus administration of 15 mg of MSAD-C_{60} per kg to rats.

sis. In that study, C_{60} solubilized with polyvinylpyrrolidone showed a promoting action in a rat embryonic limb bud cell differentiation system (19).

MSAD-C_{60}, a water-soluble fullerene derivative, has substantial activity against HIV-1 and HIV-2 in acutely and chronically infected cells. A direct interaction between the hydrophobic fullerene and the hydrophobic cleft of HIV-1 protease has been suggested as a mechanism for the virucidal properties of MSAD-C_{60}. This compound also inhibits HIV-1 reverse transcriptase and DNA polymerase α, and MSAD-C_{60} selectively inhibits HIV-1 protease in cell-free systems (15). Furthermore, toxicity studies after intraperitoneal administration of MSAD-C_{60} to mice (50 mg/kg) did not indicate any apparent toxicities (14). The purpose of the present study was to characterize the preclinical pharmacokinetics of this fullerene derivative in rats.

Chromatograms obtained from blank rat plasma (Fig. 2A), blank rat plasma to which MSAD-C_{60} and internal standard were added (Fig. 2B), and a rat plasma sample obtained after the intravenous bolus administration of 15 mg of MSAD-C60 per kg (Fig. 2C) are depicted in Fig. 2. The retention times of MSAD-C_{60} and the internal standard, DESD, were 7.6 and 14.0 min, respectively. There were no interfering peaks from endogenous compounds. Standard curves in plasma were linear over the range from 1 to 15 μg/ml. The extraction recovery of MSAD-C_{60} ranged from 73% at 15 μg/ml to 100% at 1 μg/ml. The extraction recovery of the internal standard was 99%. The intraday relative standard deviations were between 9 and 11% over the concentration range of 1 to 15 μg/ml. The interday relative standard deviations ranged from 14.5% at the lowest concentration (1 μg/ml) to 8.5% at the highest concentration (15 μg/ml).

The mean concentrations of MSAD-C_{60} in plasma as a function of time following the intravenous administration of 15 mg of MSAD-C_{60} per kg to rats are illustrated in Fig. 3. The concentrations of the fullerene derivative in plasma declined rapidly in a bi- or triexponential manner, as determined by the F test (4). Plasma protein binding studies indicated that the compound was highly protein bound (>99% bound). The concentrations of the fullerene derivative in plasma were greater than the in vitro median effective concentration (6 μM; 7 μg/ml) for 2 to 3 h following the intravenous administration of 15 mg/kg; however, its effectiveness might be limited by the high degree of protein binding.

The values of the pharmacokinetic parameters of MSAD-C_{60} are listed in Table 1. The mean CL of MSAD-C_{60} was 0.19 ± 0.06 liter/h/kg. Urine samples collected 24 h after administration of the fullerene derivative did not contain any detectable levels of the compound, indicating the absence of significant CL_R mechanisms for MSAD-C_{60}. Thus, the CL of MSAD-C_{60} reflected the CL_{NR} of the compound. The CL of MSAD-C_{60} was low compared with the hepatic blood flow rate (2.9 liters/h/kg) in rats (7). The V_{ss} of MSAD-C_{60} in rats was 2.1 ± 0.8 liters/kg. This value is nearly threefold greater than the total body water content (0.70 liter/kg) in rats (7), indicating an extensive distribution of MSAD-C_{60} in tissue. Furthermore, the MRT_C and MRT_p were 1.1 ± 0.5 and 10.3 ± 1.8 h, respectively, indicating that an average MSAD-C_{60} molecule spends approximately 10-fold more time in the peripheral tissue compartment than in the central compartment. Thus, tissues possess a greater affinity for MSAD-C_{60} than do plasma proteins.

The mean values of the pharmacokinetic parameters are reported in Table 1; however, there appeared to be a dichotomy in CL and V values. In two rats (rats 3 and 5) CL and V values were nearly twofold lower than those in the other three rats (rats 1, 2, and 4). No differences in $t_{1/2}$s or MRTs were evident. With highly protein bound drugs, small differences in the degree of binding can result in relatively large differences in pharmacokinetic parameters. Since MSAD-C_{60} is highly bound to plasma proteins, this dichotomy is probably due to slight differences in protein binding of the fullerene.

In a previous study, Yamago et al. (21) examined the disposition of total radioactivity following the intravenous and oral administration of another water-soluble fullerene derivative to rats. Similar to the results presented here for MSAD-C_{60}, the compound distributed rapidly and was eliminated slowly. High concentrations of the fullerene were observed in various tissues, especially the liver. The levels of the fullerene derivative

TABLE 1. Values of pharmacokinetic parameters of MSAD-C_{60} following intravenous administration of 15 mg/kg to rats

Rat no.	AUC (mg · h/liter)	CL (liters/h/kg)	V_{ss} (liters/kg)	$t_{1/2}$ (h)	MRT (h)	MRT_C (h)	MRT_P (h)
1	69.3	0.22	2.2	6.7	10.1	1.1	9.0
2	68.2	0.22	2.8	7.1	12.8	1.6	11.2
3	123.4	0.12	1.7	8.0	13.9	1.6	12.3
4	59.3	0.25	3.0	7.0	11.8	0.7	11.1
5	124.1	0.12	1.0	5.0	8.6	0.7	7.9
Mean	88.9	0.19	2.1	6.8	11.4	1.1	10.3
SD	32.1	0.06	0.8	1.1	2.1	0.5	1.8

in tissue declined slowly, consistent with the relatively long MRT_P of MSAD-C_{60} compared with the MRT_C.

Plasma samples obtained until 4 h after the administration of 15 mg of MSAD-C_{60} per kg appeared reddish in color, suggesting hemolysis of erythrocytes. Plasma samples obtained after 4 h appeared normal. Similar hemolysis was also noted in control animals receiving the same volume of DMSO alone; thus, this hemolysis can likely be attributed to DMSO. Previous studies have shown that C_{60} does not cause hemolysis (22). No other overt toxicities were noted at this dose. A higher dose of 25 mg of MSAD-C_{60} per kg administered by bolus intravenous injection to two rats resulted in shortness of breath and violent movement of the rats; this was followed by death within 5 min of dosing. When the rats were dosed with an equal amount of DMSO, no toxicities were observed. Thus, it appears that the toxicity was due to the compound and that it is dose dependent. The possibility that the toxicity was due to an interaction between MSAD-C_{60} and DMSO, however, cannot be ruled out. No toxicity was reported after intraperitoneal administration of 50 mg of MSAD-C_{60} per kg per day for 6 days to mice (14). Yamago et al. (21) also did not note any severe toxicities following the administration of a water-soluble fullerene to mice and rats. However, they did observe a transient withering and stretching of the trunk immediately following intraperitoneal administration of 200 to 500 mg/kg to mice. Further controlled toxicity studies are needed to fully evaluate the toxicities of fullerene derivatives.

ACKNOWLEDGMENTS

This work was supported in part by grants AI-27196 and AI-25899 from the National Institutes of Health, grants DMR-88-20933, DMR-91-11097, and CHE-89-08323 from the National Science Foundation, and grants from the U.S. Department of Veterans Affairs and the Georgia Veterans Administration Research Center for AIDS and HIV Infections.

REFERENCES

1. **Andersson, T., K. Nilsson, M. Sundahl, G. Westman, and O. Wennerstrom.** 1992. C_{60} embedded in γ-cyclodextrin: a water soluble fullerene. J. Chem. Soc. Chem. Commun. **1992:**604–606.
2. **Arbogast, J. W., A. P. Darmanyan, C. S. Foote, Y. Rubin, F. N. Diederich, M. M. Alvarez, S. J. Anz, and R. L. Whetten.** 1991. Photophysical properties of C_{60}. J. Phys. Chem. **95:**11–12.
3. **Bensasson, R. V., E. Bienvenue, M. Dellinger, S. Leach, and P. Seta.** 1994. C_{60} in model biological systems. A visible-UV absorption study of solvent-dependent parameters and solute aggregation. J. Phys. Chem. **98:**3492–3500.
4. **Boxenbaum, H. G., S. Riegelman, and R. M. Elashoff.** 1974. Statistical estimation in pharmacokinetics. J. Pharmacokinet. Biopharm. **2:**123–148.
5. **Buseck, P. R., S. J. Tsipursky, and R. Hettich.** 1992. Fullerenes from the geological environment. Science **257:**215–217.
6. **Friedman, S. H., D. L. DeCamp, R. P. Sijbesma, G. Srdanov, F. Wudl, and G. L. Kenyon.** 1993. Inhibition of the HIV-1 protease by fullerene derivatives: model building studies and experimental verification. J. Am. Chem. Soc. **115:**6506–6509.
7. **Gerlowski, L. E., and R. K. Jain.** 1983. Physiologically based pharmacokinetic modeling: principles and applications. J. Pharm. Sci. **72:**1103–1126.
8. **Hungerbuhler, H., D. M. Guldi, and K.-D. Asmus.** 1993. Incorporation of C_{60} into artificial lipid membranes. J. Am. Chem. Soc. **115:**3386–3387.
9. **Kratschmer, W., L. D. Lamb, K. Fostiropoulos, and D. R. Huffman.** 1990. Solid C_{60}: a new form of carbon. Nature (London) **347:**354–358.
10. **Metzler, D. M., G. L. Elfring, and A. J. McEwen.** 1974. NONLIN, a computer program for nonlinear least-squares regression. Biometrics **30:**562–563.
11. **National Institutes of Health.** 1986. Guide for the care and use of laboratory animals. National Institutes of Health, Washington, D.C.
12. **Nelson, M. A., F. E. Domann, G. T. Bowden, S. B. Hooser, Q. Fernando, and D. E. Carter.** 1993. Effects of acute and subchronic exposure of topically applied fullerene extracts on the mouse skin. Toxicol. Ind. Health **9:**623–630.
13. **Rocci, M. L., and W. J. Jusko.** 1983. LAGRAN program for area and moments in pharmacokinetic analysis. Comp. Prog. Biomed. **16:**203–216.
14. **Schinazi, R. F., A. McMillan, A. S. Juodawlkis, J. Pharr, R. Sijbesma, G. Srdanov, J. C. Hummelen, F. D. Boudinot, C. L. Hill, and R. Wudl.** 1994. Anti-human immunodeficiency virus, toxicity in a cell culture, and tolerance in mammals of a water-soluble fullerene, p. 689–696. *In* K. M. Kadish and R. S. Ruoff (ed.), Recent advances in the chemistry and physics of fullerenes and related materials. The Electrochemical Society, Pennington, N.J.
15. **Schinazi, R. F., R. Sijbesma, G. Srdanov, C. G. Hill, and F. Wudl.** 1993. Synthesis and virucidal activity of a water-soluble, configurationally stable, derivatized C_{60} fullerene. Antimicrob. Agents Chemother. **37:**1707–1710.
16. **Sijbesma, R., G. Srdanov, F. Wudl, J. A. Castoro, C. Wilkins, S. H. Friedman, D. L. DeCamp, and G. L. Kenyon.** 1993. Synthesis of a fullerene derivative for the inhibition of HIV enzymes. J. Am. Chem. Soc. **115:**6510–6512.
17. **Tokuyama, H., S. Yamago, and E. Nakamura.** 1993. Photoinduced biochemical activity of fullerene carboxylic acid. J. Am. Chem. Soc. **115:**7918–7919.
18. **Toniolo, C., A. Bianco, M. Maggini, G. Scorrana, M. Prato, M. Marastoni, R. Tomatis, S. Spisani, G. Palu, and E. D. Blair.** 1994. A bioactive fullerene peptide. J. Med. Chem. **37:**4558–4562.
19. **Tsuchiya, T., Y. N. Yamakoshi, and N. Miyata.** 1995. A novel promoting action of fullerene C60 on the chondrogenesis in rat embryonic limb bud cell culture system. Biochem. Biophys. Res. Commun. **206:**885–894.
20. **Veng-Pedersen, P.** 1989. Mean time parameters in pharmacokinetics. Definition, computation and clinical implications. I. Clin. Pharmacokinet. **17:** 345–366.
21. **Yamago, S., H. Tokuyama, E. Nakamura, K. Kikuchi, S. Kananishi, K. Sucki, H. Nakahara, S. Enomoto, and F. Ambe.** 1995. In-vivo biological behavior of a water-miscible fullerene—C-14 labeling, absorption, distribution, excretion and acute toxicity. Chem. Biol. **2:**385–389.
22. **Yamakoshi, Y. N., T. Yagami, K. Fuhuhara, S. Sueyoshi, and N. Miyata.** 1994. Solubilization of fullerenes into water with polyvinylpyrrolidone applicable to biological tests. J. Chem. Soc. Chem. Commun. **1994:**517–518.