ANTICANCER RESEARCH *24*: 1699-1704 (2004)

Stealth Liposomal 5-Fluorouracil With or Without Degradable Starch Microspheres for Hepatic Arterial Infusion in the Treatment of Liver Metastases. An Animal Study in VX-2 Liver Tumor-bearing Rabbits

U. POHLEN[1], R. RESZKA[2], P. SCHNEIDER[1], H.J. BUHR[1] and G. BERGER[1]

[1]*Department of Surgery, Charité, Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin;*
[2]*Max Delbrück Center for Molecular Medicine, Berlin, Germany*

Abstract. *Purpose: Regional application of cytostatics in liver metastases leads to increased concentrations in tumor tissue. Flow retardation by temporary occlusion and drug targeting via liposome encapsulation (PEG liposomes) will further increase tumor concentrations. Materials and Methods: Liver tumor-bearing rabbits were submitted to i.v. or i.a. therapy with or without liposome-encapsulated 5-fluorouracil (5-FU). I.a. groups were additionally treated with or without degradable starch microspheres. Tumor concentrations were calculated by HPLC as area under the curve (AUC). Results: A comparison with i.v.-applied 5-FU yielded the following increasing concentrations: 5-FU-PEG liposomes i.v. 6-fold, 5-FU i.a. 20-fold, 5-FU i.a. + DSM 226-fold, 5-FU-PEG liposomes i.a. 319-fold, 5.FU-PEG liposomes i.a. + DSM 2203-fold. Conclusion: The intratumoral concentration of 5-FU was increased up to 22O3 times the intravenous dose by combination of regional application via the hepatic artery with temporary embolization by degradable starch microspheres and drug targeting by liposome encapsulated 5-FU.*

The cytostatic level in tumor tissue is the decisive parameter for successful chemotherapy (1,2). The increase of the cytostatic concentration in the tumor necessitates a higher response rate (3). A promising approach is regional chemotherapy with intraarterial application of the cytostatic into the target region. Additional applications of starch microspheres slow down the blood flow in the unaffected residual liver in favor of the liver tumor. Moreover the blood flow rate is reduced by a concomitant increase of the cytostatic tumor contact time (4,5). A further increase of the cytostatic concentration can be achieved by applying liposomes as a drug carrier (6-9). Here, liposome-encapsulated cytostatics were therapeutically more effective in experimental tumors, since they can overcome both systemic toxicity and drug resistance (10-14). Furthermore, a number of authors (11,15-19) and our group (20) have shown that liposome-encapsulated cytostatics change the pharmacokinetic behavior and accumulation of the active substance in the tumor and influence the dose-limiting toxicity.

Liposomes are lipid vesicles from natural and synthetic phospholipids of different size, load and composition (15). They are defined as vesicular structures consisting mainly of amphiphilic, biologically degradable phospholipids and can thus encapsulate both water-soluble and lipid-soluble effective agents. A greater or lower affinity for RES can be observed depending on the size, composition, fluidity and load of liposomes. Basically, SUV (small unilamellar vesicles), REV (reversed-phase-evaporated vesicles) and MLV (multilamellar vesicles) liposomes are involved in cytostatic encapsulation. However, Papahadjopoulos *et al.* have demonstrated that modifying the SUV liposome membrane by adding polyethylene glycol can markedly reduce the interaction of the vesicles with the stationary macrophages in the liver and spleen after *i.v.* application (21). This increases the circulation half-time of so-called stealth liposomes. To achieve increased accumulation in tumor tissue, superparamagnetic iron oxide particles were enclosed in polyethylene glycol-modified liposomes as contrast medium and accumulation in the tumor was examined by magnetic resonance imaging (MRI). The best tumor accumulation was achieved with SUV-PEG liposomes (20). This liposome preparation was used for the following experiments.

The aim of our study was to measure 5-FU concentrations by HPLC in various tissues and the liver tumor. The concentration of PEG-5-FU liposomes was compared to

Correspondence to: Dr. med. U. Pohlen, Charité, Universitaetsmedizin Berlin, Campus Benjamin Franklin, Chirurgische Klinik, Hindenburgdamm 30, D-12200 Berlin, Germany. Tel: +49 30 8445-0, Fax: +49 30 8445 2740

Key Words: Stealth liposomes, PEG liposomes, 5-FU, locoregional chemotherapy.

Table I. *Experimental groups I - VI, HAI (hepatic arterial infusion) DSM (degradable starch microspheres Spherex® PharmaCept Berlin, Germany).*

Experimental groups:	
Group I	(n=25): 50 mg 5-FU systemic (*i.v.*)
Group II	(n=25): 50 mg 5-FU-PEG liposomes systemic (*i.v.*)
Group III	(n=30): 50 mg 5-FU regional HAI (*i.a.*)
Group IV	(n=35): 50 mg 5-FU plus DSM regional HAI (*i.a.*)
Group V	(n=35): 50 mg 5-FU-PEG liposomes HAI (*i.a.*)
Group VI	(n=45): 50 mg 5-FU-PEG liposomes plus DSM regional HAI (*i.a.*)

In groups I and II, 5 animals each were killed 15, 30, 60, 90 and 120 min after therapy was started, and the 5-FU concentrations in the different organs were determined by HPLC. An additional time point (240 min) was selected in group III, two additional time points (240 and 480 min) in groups IV and V, and four additional time points (240 and 480 min, 12 and 24h).

nonencapsulated 5-FU in systemic and regional applications with and without starch microspheres. We also examined how the concentration is changed by regional application, the addition of starch microspheres and liposomes.

Materials and Methods

5-FU SUV-PEG liposomes. 5-FU (Lederle, Germany, 50 mg/ml) was encapsulated in SUV-PEG liposomes in a composition of hydrated soy phosphatidylcholine (HSCP, 50 mg/ml, Nattermann Phosphilipid GmbH, Cologne, Germany), cholesterol (CH, 24.8 mg/ml, Merck, Darmstadt, Germany), dicetylphosphate (DCP)(Serva, Heidelberg, Germany) and polyethylene glycol (MPEG-DSPE, 3000. 5.4 mg/ml, Sygena, Liestal, Switzerland), molecular ratio (1:1:0, 1:0,1). Preparation was done by joining the lipids dissolved in chloroform (round-bottom flask) and subsequent creation of a lipid film by evaporating the solvent under vacuum (rotation evaporator). The lipid film is dispersed at room temperature by adding 5-FU (Lederle, Germany, 50 mg/ml) dissolved in phosphate buffer (PBS), pH 7.4, and by subsequent shaking (24). Subsequent intermittent application of ultrasound (10x4 min) to the multilayer liposome suspension leads to the development of small unilamellar vesicles (SUV). Separation of the nonencapsulated 5-FU component was dispensed with in this experimental approach and the cytostatic concentration was determined by HPLC. The size of these vesicles was done on the basis of quasi-elastic light scattering in the Coulter counter N 4MD (Coulter Electronics, Hialeah, Florida, USA). The liposomes measured 113nm±36nm.

Experimental animals. The experimental animals were 195 male chinchilla bastard rabbits (breeder: Charles River, Extertal, Germany). When the experiments were started, the animals were 126 to 210-days-old and weighed 2.4 - 3.3 kg. The animals were housed individually in rooms maintained at 21° ± 1°C with a 12-hour dark cycle. They were fed a standard rat chow with free access to water. Care was provided in accordance with the national guidelines for the care and use of laboratory animals. The study was approved by the local ethics committee.

Tumor model and transplantation of VX-2 tumors in the rabbit livers. A VX-2 tumor was used in the experiments, which is histomorphologically a squamous cell carcinoma. 0.2 ml of a tumor suspension (2 - 4 x 10^6 live tumor cells) was injected into the left liver lobe under general anesthesia by intramuscular bolus injection of 5 mg/kg xylazine (Rompun®, Bayer, Leverkusen, Germany) and 50 mg/kg ketamine (Ketavet, Bayer).

MR imaging. The tumor size and position was determined by MRI using a 1.5 Tesla Magnetom (Siemens Co.). A T1-weighted spin-echo sequence was used with a slice thickness of 5 mm, a repetition time of 350 ms, an echo time of 15 min and a total measuring time of 3 min.

Determination of 5-FU concentration by HPLC. A new procedure was established to determine the 5-FU concentration in the various organs (22). The blood was centrifuged to obtain serum and the individual organs were homogenized (tumor, liver, spleen, kidneys, stomach, pancreas, peritoneum and lymph nodes). After adding 5-bromouracil (Sigma, Deideshofen, Germany) as an internal standard, the proteins in serum and the homogenate were precipitated by 10% HCl and centrifuged. Ten µl of supernatant were injected into the HPLC device. The HPLC device consists of an HPLC pump (Gyntek, High Precision Pump, Model 300 C), a UV-Vis spectrophotometric detector (Shimadzu, SPD-6AV) and an autosampler (LKB, bromma 2157). An ODS hypersil column, 5 µm, 250 x 4.6 mm (VDS Optilap) was used as HPCL column. Data transmission was done by D2500 cromoto-integrators (Merck, Hatachi). The flow was 1.0 ml/min, the solvent mixture contained 3% methanol + 0.05% acidic acid and water (Merck, Darmstadt, Germany). The wavelength was 254 nm. HPLC was performed at room temperature.

Preparation of the experimental animals. The animals were randomized into the experimental groups when the tumors reached a size of 1.5-2 cm. In all rabbits undergoing systemic therapy in the further course, the cytostatic agent was applied *via* the ear vein. Rabbits randomized into regional therapy groups were submitted to general anesthesia with Rompun® and Ketanest® for implantation of a port system (Intraport, Braun-Melsungen, Germany) into the hepatic artery *via* the gastroduodenal artery.

Statistics. The mean values ± SD of the 5-FU concentration in the control and therapy groups were calculated for each group. The difference between the 5-FU concentration in the groups were determined using the global Kruskal-Wallis test. p values were adjusted for multiple comparison according to Bonferroni. A probability value of < 0.05 was considered significant.

Results

All four experimental groups (III, IV, V, VI) evidenced significantly higher 5-FU concentrations ($p<0.01$) than the systemic therapy groups (I, II). The following concentration increases in tumor tissue were observed when comparing the

Table II. *Area under the curve (AUC) of 5-FU in various tissues and serum.*

	Group I 5-FU *i.v.*	Group II 5-FU-PEG *i.v.*	Group III 5-FU *i.a.*	Group IV 5-FU + DSM *i.a.*	Group V 5-FU-PEG *i.a.*	Group VI 5-FU-PEG *i.a.* + DSM
μg/g	AUC15-60 min	AUC15-240 min	AUC15-240 min	AUC15-480 min	AUC15-480 min	AUC15-24h
Tumor	60.80	387.46	1,161.22	13,565.72	19,199.44	132,200.68
Liver	77.26	5,923.4	1,839.16	3,780.74	7,323.68	7119.1
Kidneys	216.27	5,276.08	5,242.6	240.5	5,043.8	9,988.88
Spleen	116.31	225.07	101.28	106.98	99.12	118.39
Stomach	118.89	401.17	880.21	2,057.43	1,539.85	3,999.81
Peritoneum	42.85	60.212	35.31	33.93	172.6	258.07
Pancreas	165.59	73.77	1007.17	2,017.95	3,055.97	6,698.3
Serum	342.805	1,052.51	309.22	297.16	330.07	314.93

individual therapy groups with group I (5-FU *i.v.*): after applying 50 mg of 5-FU *i.v.*, the area under the curve (AUC) measured at the time points from 15-60 min was 60.80 μg/g (5-FU was no longer detected at later time points). These concentration increases also apply to group I. The 5-FU concentration increased 6-fold after *i.v.* application of 5-FU-PEG liposomes (AUC 15-240 min, 387.46 μg/g). Intraarterial application of 5-FU increased the application 20-fold to 1,161.22 μg/g. A further 226-fold increase in concentrations was seen after *i.a.* application of 5-FU combined with DSM (AUC 15-480 min, 13,565.72 μg/g). After *i.a.* application of 5-FU-PEG liposomes, the concentration increased 319-fold to 19,199.44 μg/g (AUC 15-480 min). The highest concentrations were measured after applying 5-FU-PEG liposomes combined with DSM (2203-fold increase to 132,200.68 μg/g). Here, 5-FU was still detected in the tumor tissue 24 h after application (AUC 15min-24h) (Table II and Figures 1,3,4,5). These differences were significant ($p<0.01$ - 0.0001). The following concentration increases were observed when comparing the 5-FU concentrations in the liver parenchyma of group I with the other groups: 76-fold in 5-FU-PEG liposomes *i.v.* (77.26μg/g *vs*. 5,923.4 μg/g), 23-fold in 5-FU *i.a.* (189.16 μg/g), 49-fold in 5-FU/DSM *i.a.* (3,780.74 μg/g), 95-fold in 5-FU-PEG liposomes *i.a.* (7,323.68 μg/g), 92-fold in 5-FU-PEG liposomes/DSM *i.a.* (7,119.1 μg/g). These differences were significant ($p<0.01$) (Table II and Figure 2).

Liposome encapsulation changes the pharmacokinetics of 5-FU. When 5-FU was applied *i.v.*, the maximal tumor concentration was reached after 15 min compared to 30 min after *i.v.* application of liposomal 5-FU (Figures 3 and 4). Maximal tumor concentrations were reached after 2 h by infusing 5-FU-PEG liposomes and degradable starch microspheres into the hepatic artery (Figure 5).

Discussion

High cytostatic concentrations in tumor tissue are the key to effective therapy. Some authors assume that increasing the concentrations of active substances counteracts resistance. High concentrations in the tumor are achieved by a concomitant reduction of systemic toxicity (3). Based on our 5-FU tumor concentration after *i.v.* application, the 5-FU concentration is increased 20-fold after *i.v.* application of 5-FU-PEG liposomes and 226-fold after *i.a.* application of the monosubstance combined with DSM. Locoregional *i.a.* application of combined 5-FU-PEG liposomes and DSM led to a 2203-fold increase of the concentration time curve (AUC) compared to *i.v.* application of the monosubstance (group 1). Furthermore, the pharmacokinetics of 5-FU are changed so that the concentration maximum after *i.v.* application of 5-FU is reached after 15 minutes, after *i.v.* application of liposomal 5-FU after 30 minutes, and after HAI application of liposomal 5-FU and DSM after 2 hours. Regional *i.a.* application leads to cytostatic accumulation in the tumor. There are a number of experimental and clinical studies on the pharmacokinetics of regionally applied 5-FU and the increased concentration in tumor tissue (23-30).

An added advantage of regional application can be achieved by reducing the blood flow. Degradable microspheres slow down the blood flow for approximately

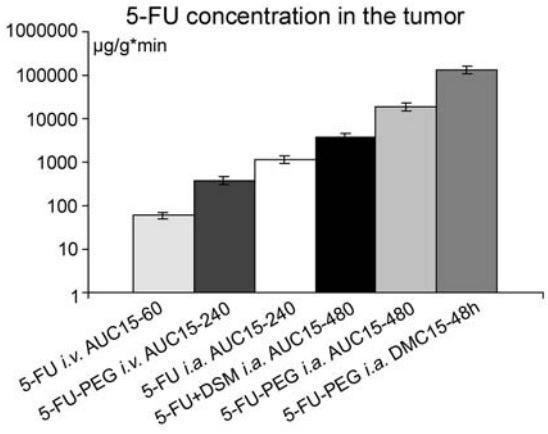

Figure 1. *Logarithm of 5-FU tumor concentration (AUC) in the different therapy groups. 5-FU-PEG = 5-FU-PEG liposomes, DSM = degradable starch microspheres.*

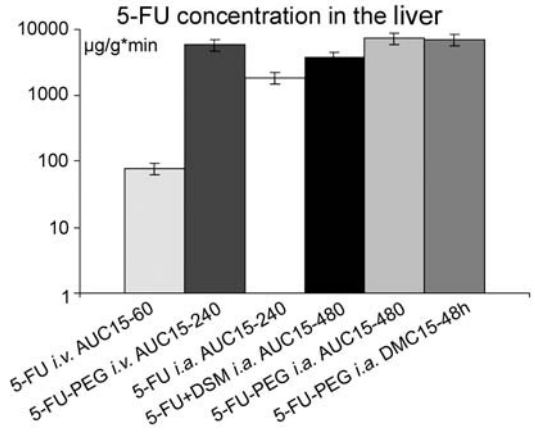

Figure 2. *Logarithm of the liver parenchymal concentration (AUC) of 5-FU in the different therapy groups. 5-FU-PEG = 5-FU-PEG liposomes, DSM = degradable starch microspheres.*

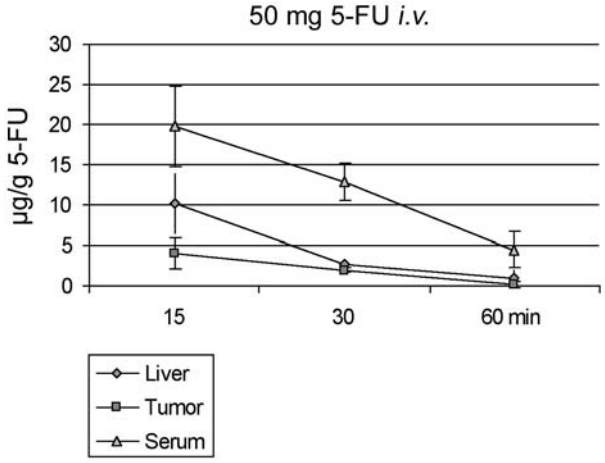

Figure 3. *Concentration time course after i.v. application of 50 mg of 5-FU.*

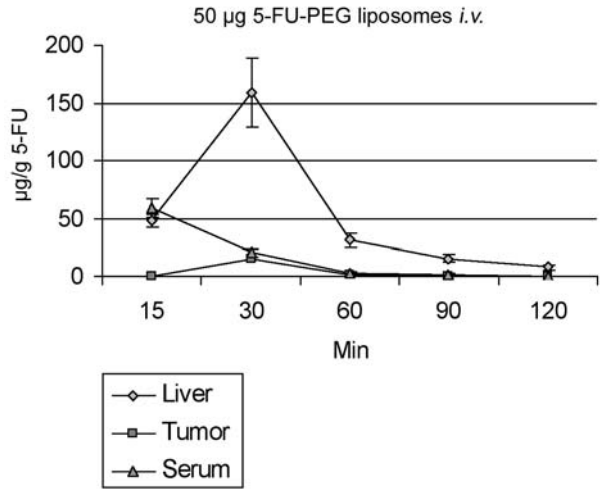

Figure 4. *Concentration time course after i.v. application of 50 mg of 5-FU -PEG liposomes.*

20 minutes. Furthermore, starch microspheres have a target effect on the tumor (31-33).

Clinical studies with PEG liposomes, also called stealth liposomes, have reported reduced toxicity with prolongation of the plasma half-life in liposome-encapsulated cisplatin and doxorubicin (33-35). This was also observed in our experiments but was considerably increased by DSM. Markedly increased tumor concentrations after *i.v.* application of PEG-cytostatic liposomes were measured in experimental and clinical pharmacokinetic studies. Thus, the concentration in tumor tissue was increased 6- to 30-fold compared to *i.v.* application of the monosubstance (36-38). This is in agreement with our results in which the tumor concentration increased 6-fold after *i.v.* application of liposomal 5-FU compared to *i.v.* application of the monosubstance. Some authors have therefore concluded that active substances encapsulated in stealth liposomes show better accumulation in tumor tissue than the monosubstance or other liposome preparations (39,40).

While different cytostatics like epirubicin, doxorubicin, cisplatin and topoisomerase inhibitors have been clinically and experimentally liposome-encapsulated, there is only one experimental study on 5-FU describing this drug delivery model *in vitro* (41). Also, liposomes have not yet been applied in regional therapy. However, what explains the relatively selective tumor accumulation?

According to one hypothesis, the EPR effect (enhanced permeability retention), tumor vessels have defects with endothelial gaps of up to 100 nm. Globulin and vesicular structures can accumulate in those gaps. These polymeric

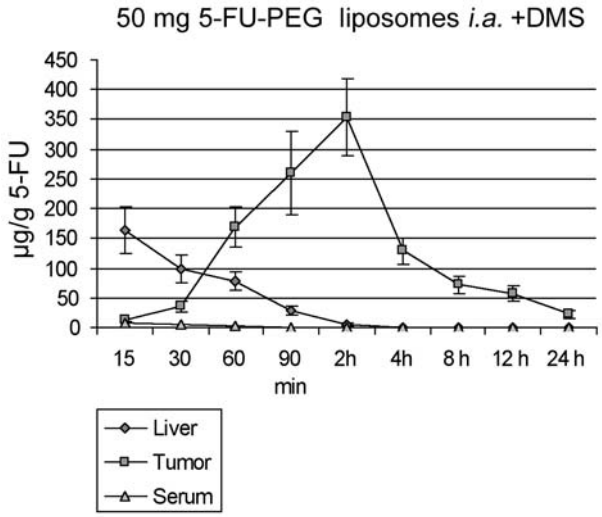

Figure 5. *Concentration time course after i.v. hepatic arterial infusion (HAI) of 50 mg of 5-FU-PEG liposomes combined with degradable starch microspheres (DSM).*

conjugates release their drugs intracellularly *via* endocytosis (42-46). Intravital microscopic examinations underscore this hypothesis. According to these examinations, PEG liposomes accumulate in the tumor interstitium and move in an intracellular direction due to increased vascular permeability (47). The affinity of liposomes to RES organs explains the high concentrations in the liver parenchyma when applying liposomal 5-FU (48).

Conclusion

Combined regional applications of 5-FU *via* the hepatic artery with temporary embolization by degradable starch microspheres and drug targeting by liposome-encapsulated 5-FU increases the intratumoral concentration 2203-fold compared to *i.v.* application.

References

1 Link KH, Kornmann M, Leder GH, Butzer U, Pillasch J, Staib L, Gansauge F and Beger HG: Regional chemotherapy directed by individual chemosensitivity testing *in vitro*: a prospective decision-aiding trial. Clin Cancer Res *2*: 1469-74, 1996.

2 Gieseler F: The dilemma of gastroenterological oncology: we know a lot but we still achieve too little. Int J Colorectal Dis *15*: 112-3, 2000.

3 Kerr DJ and Los G: Pharmacokinetic principles of locoregional chemotherapy. Cancer Surv *17*: 105-122, 1993.

4 Civalleri D, Esposito M, Fulco RA and Vannozzi M: Liver and tumor uptake and plasma pharmacokinetic of arterial cisplatin administered with or without starch microspheres in patients with liver metastases. Cancer *68*: 988-994, 1991.

5 Hemingway DM, Crooke TG, Chang D, Grime SJ and Jenkins SA: The effects of intra- arterial vasoconstrictors on the distribution of a radiolabelled low molecular weight marker in an experimental model of liver tumour. Br J Cancer *63*: 495-498, 1991.

6 Delgado G, Potkul RK, Treat JA, Lewandowski GS, Barter JF, Forst D and Rahman A: A phase I/II study of intraperitoneally administered doxorubicin entrapped in cardiolipin liposomes in patients with ovarian cancer. Am J Obst Gynecol *160*: 812-817, 1989.

7 Frangos DN, Killion JJ, Fan D, Fishbeck R, von Eschenbach AC and Fidler IJ: The development of liposomes containing interferon alpha for the intravesical therapy of human superficial bladder cancer. J Urol *143*: 1252-1255, 1990.

8 Lopez-Berestein G, Fainstein V, Hopfer R, Metha K, Sullivan MP, Keating M, Rosenblum MG, Mehta R, Luna M and Hersh EM: Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer – a preliminary study. J Infect Dis *151*: 704-710, 1985.

9 Treat J, Greenspan A, Forst D, Sanchez JA, Ferrans VJ, Potkul LA, Woolley PV and Rahman A: Antitumor activity of liposome-encapsulated doxorubicin in advanced breast cancer phase II study. J Natl Cancer Inst *82*: 1706-1710, 1990.

10 Balazsovits JA, Mayer, LD, Bally MB, Cullis PR, Mc Donell M, Ginsberg RS and Falk RE: Analysis of the effect of liposome encapsulation of the vesicant properties, acute and cardiac toxicities and antitumor efficacy of doxorubicin. Cancer Chemother Pharmacol *23*: 81-86, 1989.

11 Fichtner I, Reszka R, Elbe B and Arndt D: Therapeutic evaluation of liposome-encapsuslated Daunorblastin in murine tumor models. Neoplasma *28*: 141-149, 1981.

12 Forssen EA and Tokes ZA: Attentuation of dermal toxicity of doxorubicin by liposome encapsulation. Cancer Treat Rep *67*: 481-484, 1983.

13 Rahman A, More N and Schein PS: Doxorubicin-induced chronic cardiotoxicity and its protection by liposomal administration. Cancer Res *42*: 1817-25, 1982.

14 Yatvin MB and Lelkes PI: Clinical prospects for liposomes. Med Phys *9(2)*: 149-175, 1982.

15 Ryman BE and Tyrrell DA: Lipsomes-bags of potential. *In*: Essays Biochem (Edited by Cambell PN and Marshal RD), 16 p. 49-98 Academic Press, New York, 1980.

16 Gabizon A, Catane R and Uziely B: Prolonged circulation time and enhanced accumulation in malignant exudates of doxirubicin encapsulated in polyethylene-glycol coated liposomes (DOXIL) Cancer Res *54*: 987-992, 1994.

17 Mayer LD, Bally MB, Cullis PR, Wilson SL and Emermann JT: Comparison of free and liposomal encapsulated doxorubicin tumor drug uptake and antitumor efficacy in the SC 115 murine mammary tumor. Cancer Lett *53*: 183-190, 1990.

18 Mayer LD, Bally MB, Loughrey H, Masin D and Cullis PR: Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumors. Cancer Res *50*: 575-579, 1990.

19 Mayer LD, Tai LC, Bally MB, Mitilenes GN, Ginsberg RS and Cullis PR: Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. Biochim Biophys Acta *1025*: 143-151, 1990.

20 Päuser S, Reszka R, Wagner S, Wolf KJ, Buhr HJ and Berger G: Liposome encapsulates superparamagnetic iron oxide particles as markers in an MRT-guides search for tumor-specific drug carriers. Anticancer Drug Des *12*: 125-135, 1997.

21 Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Huang SK, Lee KD, Woodle MC and Lasic DD: Sterically stabilized liposomes: improvements in pharmacokinetics and anti-tumor therapeutic efficiency. Proc Natl Acad Sci USA *88*: 11460-11464, 1991.

22 Jung M, Berger G, Pohlen U, Päuser S, Reszka R and Buhr HJ: Simultanous determination of 5-fluoroucil and its active metabolites in serum and tissue by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl *702*: 193-202, 1997.

23 Sullivan RD, Miller E and Skyes MP: A clinical-pharmacological evaluation of hepatic arterial infusion of 5-flor0-2-deoxyuridin and 5-fluorouracil. Cancer Res *38*: 3784-3792, 1959.

24 Ackermann NB, Lien WM, Kondi ES and Silverman NA: The blood supply of experimental liver metastases: I. The distribution of hepatic artery and portal vein blood to "small" and "large" tumors. Surgery *66*: 1067-1072, 1969.

25 Kusunoki M, Yanagi H, Noda M, Yoshikawa R and Yamamura T: Results of pharmacokinetic modulating chemotherapy in combination with hepatic arterial 5-fluorouracil infusion and oral UFT after resection of hepatic colorectal metastases. Cancer *89*: 1228-1235, 2000.

26 de Brauw LM, Marinelli A, van de Velde CJ, Hermans J, Tjaden UR, Erkelens C and de Bruijn EA: Pharmacological evaluation of experimental isolated liver perfusion and hepatic infusion with 5-fluorouracil. Cancer Res *51*: 1694 1700, 1991.

27 Kaneko A, Naomoto Y, Aoyama M and Tanaka N: Tissue levels of chemotherapeutic agents for hepatic metastasis during hepatic arterial and portal injection. In Vivo *13*: 195-198, 1999.

28 Pahlman L: Neoadjuvant and adjuvant radio- and radio-chemotherapy of rectal carcinoma. Int J Colrectal Dis *15*: 1-8, 2000.

29 Fahlke J, Ridwelski K and Lippert H: High-dose therapy with combined 5-fluorouracil and folonic acid with and without amifostine in the treatment of patients with metastatic colorectal carcinoma. Int J Colorectal Dis *14*: 128-130, 1999.

30 Nott DM, Yates J, Grime SJ, Maltby M, Cook TG and Jenkins SA: The effect of portal venous flow on the washout of regionally injected marker substance 99mTC-methylene diphosphonate after hepatic arterial blockade with degradable starch microspheres. Eur J Surg Oncol *18*: 347-352,1992.

31 Hakansson L, Hakansson A, Morales O, Thorelius L and Warfving T: Spherex (degradable starch microsphers) chemo-occlusion-enhancement of tumor drug concentration and therapeutic efficacy: an overview. Semin Oncol *24*: 100-109, 1997.

32 Pohlen U, Berger G, Binnenhei M, Reszka R and Buhr HJ: Increased carboplatin concentration in liver tumors through temporary flow retardation with starch microspheres (Spherex) and gelatin powder (Gelfoam). An experimental study in liver tumor-bearing rabbits. J Surg Res *92*: 165-170, 2000.

33 Veal GJ, Griffin MJ, Price E, Parry A, Dick GS, Little MA, Yule SM, Morland B, Estlin EJ, Hale JP, Pearson AD, Welbank H and Boddy AV: A phase I study in paediatric patients to evaluate the safety and pharmacokinetics of SPI-77, a liposome encapsulated formulation of cisplatin. Br J Cancer *84*: 1029-1035, 2001.

34 Rivera E, Valero V, Syrewicz L, Rahman Z, Esteva FL, Theriault RL, Rosales MM, Booser D, Murray JL, Bast RC Jr and Hortobagyi GN: Phase I study of stealth liposomal doxorubicin in combination with gemcitabine in the treatment of patients with metastatic breast cancer. J Clin Oncol *19*: 1716-1722, 2001.

35 Symon Z, Peyser A, Tzemach D, Lyass O, Sucher E, Shezen E and Gabizon A: Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. Cancer *86*: 72-78, 1999.

36 Newman MS, Colbern GT, Working PK, Engbers C and Amantea MA: Comparative pharmacokinetics, tissue distribution, and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposomes (SPI-077) in tumor-bearing mice. Cancer Chemother Pharmacol *43*: 1-7, 1999.

37 Colbern GT, Dykes DJ, Engbers C, Musterer R, Hiller A, Pegg E, Saville R, Weng S, Luzzio M, Uster P, Amantea M and Working PK: Encapsulation of the topoisomerase I inhibitor GL147211C in pegylated (STEALTH) liposomes: pharmacokinetics and antitumor activity in HT29 colon tumor xenografts. Clin Cancer Res *4*: 3077-3082, 1998.

38 Siegal T, Horowitz A and Gabizon A: Doxorubicin encapsulated in sterically stabilized liposomes for the treatment of a brain tumor model: biodistribution and therapeutic efficacy. J Neurosurg *83*: 1029-1037, 1995.

39 Shimizu H, Kumai K, Uyama I, Shibata S, Tagawa T, Nagaike K, Yasuda T, Kitajima M and Tadakuma T: Improvement of pharmacokinetics and antitumor activity against human hepatoma cell line by using adriamycin-entrapped stealth liposomes. J Surg Oncol *62*: 186-193, 1996.

40 Mayhew EG, Lasic D, Babbar S and Martin FJ: Pharmacokinetics and antitumor activity of epirubicin encapsulated in long-circulating liposomes incorporating a polyethylene glycol-derivatized phospholipid. Int J Cancer *51*: 302-309, 1992.

41 Nagaich S, Khopade AJ and Jain NK: Lipid grafts of egg-box complex: a new supramolecular biovector for 5-fluorouracil delivery. Pharm Acta Helv *73*: 227-36, 1999.

42 Jain RK: Barriers to drug delivery in solid tumors. Sci Am *271*: 58-65, 1994.

43 Jain RK: The next frontier of molecular medicine: delivery of therapeutics. Nat Med *4*: 655-657, 1998.

44 Jain RK: Understanding barriers to drug delivery: high resolution *in vivo* imaging is key. Clin Cancer Res *7*: 1605-1606, 1999.

45 Seymour LW, Ulbrich K, Steyger PS, Brereton M, Subr V, Strohalm J and Duncan R: Tumor tropism and anti-cancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. Br J Cancer *70*: 636-641, 1994.

46 Seymour LW: Passive tumor targeting of soluble macromolecules and drug conjugates. Crit Rev Ther Drug Carrier Sys *9*: 135-137, 1992.

47 Wu NZ, Da D, Rudoll TL, Needham D, Whorton AR and Dewhirst MW: Increased microvascular permeability contributes to preferential accumulation of stealth liposomes in tumor tissue. Cancer Res *53*: 3765-3770, 1993.

48 van der Veen AH, Eggermont AM, Seynhaeve AL, van Tiel and ten Hagen TL: Biodistribution and tumor localization of stealth liposomal tumor necrosis factor-alpha in soft tissue sarcoma bearing rats. Int J Cancer *77*: 901-906, 1998.

Received February 12, 2004
Accepted April 14, 2004