Published in final edited form as:
J Vasc Interv Radiol. 2008 July ; 19(7): 1065–1069. doi:10.1016/j.jvir.2008.02.023.

Evaluation of Different Calibrated Spherical Polyvinyl Alcohol Microspheres in Transcatheter Arterial Chemoembolization: VX2 Tumor Model in Rabbit Liver

Kwang-Hun Lee, MD, **Eleni Liapi, MD**, **Veronica Prieto Ventura, MD**, **Manon Buijs, MD**, **Josephina A. Vossen, MD**, **Mustafa Vali, MD**, and **Jean-Francois H. Geschwind, MD**
From the Division of Vascular and Interventional Radiology, The Russell H. Morgan Department of Radiology, The Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287 (K.H.L., E.L., V.P.V., M.B., J.A.V., M.V., J.F.H.G.); and Division of Interventional Radiology, Department of Radiology & Research Institute of Radiological Science, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (K.H.L.).

Abstract

PURPOSE—To assess whether porosity and compressibility of calibrated spherical polyvinyl alcohol (PVA) microspheres affect doxorubicin plasma and tumor concentrations after transcatheter arterial chemoembolization (TACE) in a VX2 rabbit model.

MATERIALS AND METHODS—Fifteen rabbits were divided into three groups of five rabbits each. Three different types of calibrated spherical PVA microspheres with variable levels of porosity and compressibility were blindly evaluated. TACE was performed by injecting a mixture of doxorubicin (5 mg) and iodized oil (0.5 mL) followed by injection of the embolic material (0.3–0.5 mL). Plasma concentrations of doxorubicin and doxorubicinol were analyzed 20, 40, 60, and 120 minutes and 2 days after TACE, and liver tissue and tumor doxorubicin concentrations were measured 2 days after TACE.

RESULTS—All calibrated spherical PVA microspheres showed similar patterns of plasma doxorubicin and doxorubicinol release and tumor concentration of doxorubicin. There were no significant differences of drug levels in either plasma or tumor in each group ($P > .05$).

CONCLUSIONS—After TACE in a rabbit model of liver cancer, testing of three different types of spherical PVA microspheres with varying degrees of porosity and compressibility showed no significant differences in the plasma doxorubicin release pattern and tumor doxorubicin uptake.

Transcatheter arterial chemo-embolization (TACE) has become the mainstay of palliative therapy for most primary and secondary unresectable hepatic malignancies (1–3). The technique consists of delivering high concentrations of chemotherapeutic agents emulsified in iodized oil to the tumor bed followed by embolization. The blockage of arterial inflow by using embolic material is an important step in TACE because it allows the prolonged stasis of chemotherapeutic agents inside the tumor, which may then decay according to their half-life. Currently, the most commonly used embolic agents are gelatin absorbable sponge pledgets (4,5), a temporary embolic material, and polyvinyl alcohol (PVA) particles (6) and tris-acryl gelatin microspheres (7,8), both of which are permanent embolic agents. A recent study testing

Address correspondence to J.F.H.G.; jfg@jhmi.edu.

From the 2008 SIR annual meeting.

None of the authors have identified a conflict of interest.

for the arterial distribution of calibrated tris-acryl gelatin and PVA microspheres showed that the repartition of a spherical embolic agent in an arterial lumen could be influenced by its size as well as by its deformation within the arterial lumen (9). That is, the degree of porosity and compressibility that is directly related to the deformation of microspheres and their distribution within the arterial lumen seems to be an important character of microspheres.

In this study, we are focusing on one spherical microsphere composition and one size range with three different degrees of porosity and compressibility and their performance in affecting the stasis of a chemotherapeutic agent within the VX2 liver tumor model.

MATERIALS AND METHODS

Our institution′s Animal Care and Use Committee approved the study, and all animal care and procedures were performed under institutional guidelines.

Animal and Tumor Model

Adult New Zealand White male rabbits weighing 3.8–4.3 kg (Myrtle′s Rabbitry, Thompson Station, Tennessee) were used. Fifteen rabbits were divided into three groups of five rabbits each for the study of three different types of spherical PVA microspheres. A commercially available PVA microsphere was used as an embolic material in one group, and two different prototypes were used in the others. Although the VX2 tumor is of non-hepatic origin, it has proved to be a convenient animal model of liver cancer due to its rapid growth and similarity in blood supply to that of human hepatocellular carcinoma.

Anesthesia and Analgesia

A mixture of 2.5 mg/kg acepromazine (Phoenix, St Joseph, Missouri) and 44 mg/kg ketamine hydrochloride (Phoenix) was injected intramuscularly before TACE. Intravenous access was secured via a marginal ear vein, and 0.1–0.2 mL (2.5–5 mg) of sodium pentobarbital (Hospira, Lake Forest, Illinois) was administered intravenously periodically to maintain anesthesia. When performing TACE, endotracheal intubation with a 3.0-mm endotracheal tube (Mallinkrodt Medical, St Louis, Missouri) was performed to monitor end-tidal CO_2. After the surgery, analgesic buprenorphine (0.02–0.05 mg/kg) was injected intramuscularly for pain control.

Tumor Implantation

To obtain solid tumors for implantation, VX2 tumor cell suspension (~1×10^7 cells, 200 μL) was injected into both thigh muscles of a carrier rabbit. Two weeks later, the bulk of solid tumors were harvested from the carrier rabbit by means of surgical excision of tumor-bearing muscle and put into 0.9% sodium chloride. The abdomen of each recipient rabbit was shaved and prepared in a sterile fashion. The liver was exposed with a midline subxiphoid incision and the left lateral lobe pulled out. Then, a small incision of the liver capsule and parenchyma at the implantation site was made and adequate space created to insert an approximately 3-mm^3 single piece of harvested tumor. The incised capsule was approximated and compressed manually. After the absence of bleeding was confirmed, the exposed liver was repositioned into its original peritoneal space. The abdomen was closed in two layers with aseptic technique. The tumors were allowed to grow for another 13–15 days, at which time they were sufficiently large and well demarcated.

Embolic Material

Three different spherical PVA microspheres (Contour SE, Boston Scientific, Natick, Massachusetts; and prototypes A and B, Boston Scientific/Oncology Division, Marlborough,

Massachusetts) were labeled with different codes and tested blindly. The microspheres were produced by means of a previously published process (10). The characteristics of the microspheres and scanning electron microscope (SEM) images are shown in the Table and Figure 1, respectively. These spherical PVA microspheres were of the same size range (100–300 μm) and chemical composition and produced with the same technological process. The manufacturing process was modified to introduce various levels of porosity to the microspheres in each group. This was accomplished by varying the ratio of PVA to gelling agent. Although it was not possible to quantify the differences in compressibility for microspheres of this size, the correlation between porosity and compressibility has been described previously (9), where it was reported that more porous embolic microspheres compressed more and penetrated to more distal locations compared with nonporous materials of equivalent size. We therefore presumed that the differences in porosity, as measured with SEM imaging, resulted in different levels of compressibility for each group. The amount of particles was 1 mL in 5 mL of solution. This volume was then diluted with an equal volume of contrast medium (Omnipaque; Amersham, Piscataway, New Jersey).

TACE

Access to the right common femoral artery was performed by means of surgical cut-down. A 3.5-F sheath (Cook, Bloomington, Indiana) was then slowly inserted, followed by a 2-F catheter with a tip in the shape of a hockey stick (JB1 catheter; Cook), which was first advanced into the aorta and then into the celiac trunk and common hepatic artery. Arteriography of the common hepatic artery demonstrated the hepatic arterial anatomy and the location, size, and vascularity of the tumor. Then, the 2-F JB1 catheter was advanced over the 0.014-inch guide wire (Transend; Boston Scientific/Medi-tech, Miami, Florida) and into the tumor feeding artery. The mixture of doxorubicin (Bedford Laboratories, Bedford, Ohio; 5 mg) and iodized oil (Ethiodol, 0.5 mL; Savage Laboratories, Melville, New York) was injected, followed by embolization with spherical PVA microspheres (0.3–0.5 mL) until flow reduction was observed. After the embolic material was injected, the catheter and sheath were removed and the common femoral artery was ligated with absorbable sutures.

Plasma Concentrations of Doxorubicin/Doxorubicinol

After TACE, whole blood samples (5 mL) were collected to assay plasma concentrations of doxorubicin and doxorubicinol (a metabolite of doxorubicin) at various time points (20, 40, 60, and 120 minutes and 2 days). Whole blood samples were placed on ice and centrifuged within 3.5 hours with 2,000 rpm for 10 minutes at room temperature. Isolated plasma was frozen at −20°C until the time of analysis.

Euthanasia and Tumor Doxorubicin Concentration

Two days after TACE, rabbits were euthanized under deep anesthesia by means of slow intravenous injection of a lethal dose (100 mg per 5 mL) of sodium pentobarbital. Liver tissue was obtained immediately after euthanasia. Careful dissection of tumor was performed from explanted liver, and two tumor pieces were cut—one from the tumor core and one from the tumor periphery. Nontumorous liver tissue from the left medial and right lobes was also obtained to check for nontargeted drug deposition. All tissue samples were immediately placed on dry ice and frozen at −80°C until the time of analysis. Analysis was performed with atomic absorption spectroscopy.

Statistics

The plasma and tissue doxorubicin concentrations for all groups were compared with the Student *t* test. Differences were considered statistically significant at $P < .05$.

RESULTS

Plasma Doxorubicin/Doxorubicinol

Plasma levels of doxorubicin and doxorubicinol in each group measured at 20, 40, 60, and 120 minutes and 2 days after TACE are shown in Figure 2 and Figure 3. Drug levels were undetectable 2 days after TACE. All spherical PVA microspheres showed similar patterns of plasma doxorubicin and doxorubicinol release without statistically significant differences ($P > .05$).

Tumor Doxorubicin Concentrations

The intratumoral doxorubicin concentrations in each group measured at euthanasia are shown in Figure 4. All spherical PVA microspheres showed a similar pattern of doxorubicin concentration inside the tumor without statistically significant differences ($P > .05$). Doxorubicin concentration at the nontargeted left medial lobe and the right lobe of the liver was undetectable (<0.6 nmol/g).

DISCUSSION

The goal of TACE is to deliver large amounts of chemotherapeutic agents to the tumor via the tumor feeding artery. Apparently, additional embolization after the administration of the mixture of chemotherapeutic agent and iodized oil is desirable in most clinical practices. To that end, several types of embolic materials are used to reduce arterial inflow, which subsequently decelerates drug washout and maximizes contact time between the drug and the tumor cells. The first embolic agent to be employed for this purpose was gelatin absorbable sponge pledgets. Raoul et al (4) first showed that additional embolization with gelatin absorbable sponge particles, after the injection of the mixture of doxorubicin and iodized oil, reduced doxorubicin plasma concentration and significantly increased its intratumoral concentration. However, the problem with gelatin absorbable sponge pledgets is that their size, which is made by hand cut, is large and unpredictable, most likely leading to proximal obstruction and the development of revascularization of treated lesions through aberrant collateral vessels and, therefore, limiting the efficacy of further embolization. Nonspherical PVA particles were next introduced into clinical practice (11). PVA particles seem to have a more controllable effect than gelatin sponge, as their smaller size allows deeper penetration and more permanent vessel occlusion. Their irregular shape, however, does not ensure product uniformity and may result in unpredictable catheter or vessel occlusion (12–15). In a study comparing nonspherical PVA and one type of spherical microspheres (Embosphere, Biosphere Medical, Rockland, Massachusetts) (8), doxo-rubicin concentration in the tumor was found to be greater in the Embosphere group than in the nonspherical PVA group. Although the clinical evidence of such results remains to be supported, the authors suggest that TACE with calibrated spherical microspheres may be more beneficial than nonspherical PVA because of the greater concentration of drug present within the tumor.

The aim of our study was to assess for differences in doxorubicin plasma and intratumoral concentrations among calibrated spherical microspheres of a similar size range. Because the arterial distribution of microspheres of the same size may depend on porosity and compressibility (9), we assumed that more distal embolization that may be achieved with more compressible microspheres may impede collateral circulation more efficiently and allow less washout of the chemotherapeutic agent. We therefore expected to see differences in plasma and intratumoral doxorubicin concentrations among the three tested types of spherical PVA microspheres. Our results, however, did not show any significant differences in plasma and intratumoral drug concentrations among the tested microspheres. These results could be explained due to the special inherent characteristics of iodized oil. Iodized oil may act as both

an embolic agent and a drug carrier, leading to embolization and drug deposition at the level of the intratumoral capillaries. Moreover, the injected volume of iodized oil has been shown to affect the total amount of injected chemotherapy (6,16). Our results could also be explained due to the endothelial damage and subsequent arterial spasm generated by the injected toxic chemotherapeutic agent. It has been demonstrated that porosity and compressibility affect the injectability and injectable amount of spherical microspheres through a catheter; however, in our study, it was hard to detect differences in injectability among the three tested types of spherical PVA microspheres because the preceding iodized oil injection and the arterial spasm caused by the toxic chemotherapeutic agent had already created a significant reduction in arterial inflow.

In conclusion, after TACE in the VX2 liver rabbit model, there were no significant differences in the doxorubicin plasma and intratumoral concentrations among three different PVA microspheres with a similar size range, despite their varying degrees of porosity and compressibility.

Abbreviations

PVA, polyvinyl alcohol; TACE, transcatheter arterial chemoembolization.

Acknowledgments

Supported partially by a grant from Boston Scientific Oncology. J.F.G. and K.L. supported by the Charles W. Pratt Fund for Liver Cancer Research.

References

1. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35:1164–1171. [PubMed: 11981766]
2. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002;359:1734–1739. [PubMed: 12049862]
3. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology 2003;37:429–442. [PubMed: 12540794]
4. Raoul JL, Heresbach D, Bretagne JF, et al. Chemoembolization of hepatocellular carcinomas: a study of the biodistribution and pharmacokinetics of doxorubicin. Cancer 1992;70:585–590. [PubMed: 1320447]
5. Nakao N, Uchida H, Kamino K, et al. Effectiveness of Lipiodol in transcatheter arterial embolization of hepatocellular carcinoma. Cancer Chemother Pharmacol 1992;31:S72–S76. [PubMed: 1333913]
6. Geschwind JF, Ramsey DE, Cleffken B, et al. Transcatheter arterial chemoembolization of liver tumors: effects of embolization protocol on injectable volume of chemotherapy and subsequent arterial patency. Cardiovasc Intervent Radiol 2003;26:111–117. [PubMed: 12616414]
7. Ball DS, Heckman R, Olenick SW, Folander HL, Reed J III. In vitro stability of tris-acryl gelatin microspheres in a multipharmaceutical chemoembolization solution. J Vasc Interv Radiol 2003;14:83–88. [PubMed: 12525591]
8. Hong K, Kobeiter H, Georgiades CS, Torbenson MS, Geschwind JF. Effects of the type of embolization particles on carboplatin concentration in liver tumors after transcatheter arterial chemoembolization in a rabbit model of liver cancer. J Vasc Interv Radiol 2005;16:1711–1717. [PubMed: 16371540]
9. Laurent A, Wassef M, Saint Maurice JP, et al. Arterial distribution of calibrated tris-acryl gelatin and polyvinyl alcohol microspheres in a sheep kidney model. Invest Radiol 2006;41:8–14. [PubMed: 16355034]
10. Janel, BM.; Casey; Thomas, V. inventors; Boston Scientific Scimed, assignee. Lanphere. US patent. 7311861. 2004 Jun 1. Filed

11. Martinelli DJ, Wadler S, Bakal CW, et al. Utility of embolization or chemoembolization as second-line treatment in patients with advanced or recurrent colorectal carcinoma. Cancer 1994;74:1706–1712. [PubMed: 8082071]

12. Choe DH, Han MH, Kang GH, Yeon KM, Han MC. An experimental study of embolic effect according to infusion rate and concentration of suspension in transarterial particulate embolization. Invest Radiol 1997;32:260–267. [PubMed: 9140745]

13. Bendszus M, Klein R, Burger R, Warmuth-Metz M, Hofmann E, Solymosi L. Efficacy of trisacryl gelatin microspheres versus polyvinyl alcohol particles in the preoperative embolization of meningiomas. AJNR Am J Neuroradiol 2000;21:255–261. [PubMed: 10696005]

14. Andrews RT, Binkert CA. Relative rates of blood flow reduction during transcatheter arterial embolization with tris-acryl gelatin microspheres or polyvinyl alcohol: quantitative comparison in a swine model. J Vasc Interv Radiol 2003;14:1311–1316. [PubMed: 14551279]

15. Yamamoto A, Imai S, Kobatake M, Yamashita T, Tamada T, Umetani K. Evaluation of tris-acryl gelatin microsphere embolization with monochromatic X rays: comparison with polyvinyl alcohol particles. J Vasc Interv Radiol 2006;17:1797–1802. [PubMed: 17142710]

16. Matsuo N, Uchida H, Sakaguchi H, et al. Optimal lipiodol volume in transcatheter arterial chemoembolotherapy for hepatocellular carcinoma: study based on lipiodol accumulation patterns and histopathologic findings. Semin Oncol 1997;24(2 suppl 6):S6-61–S6-70. [PubMed: 9151919]

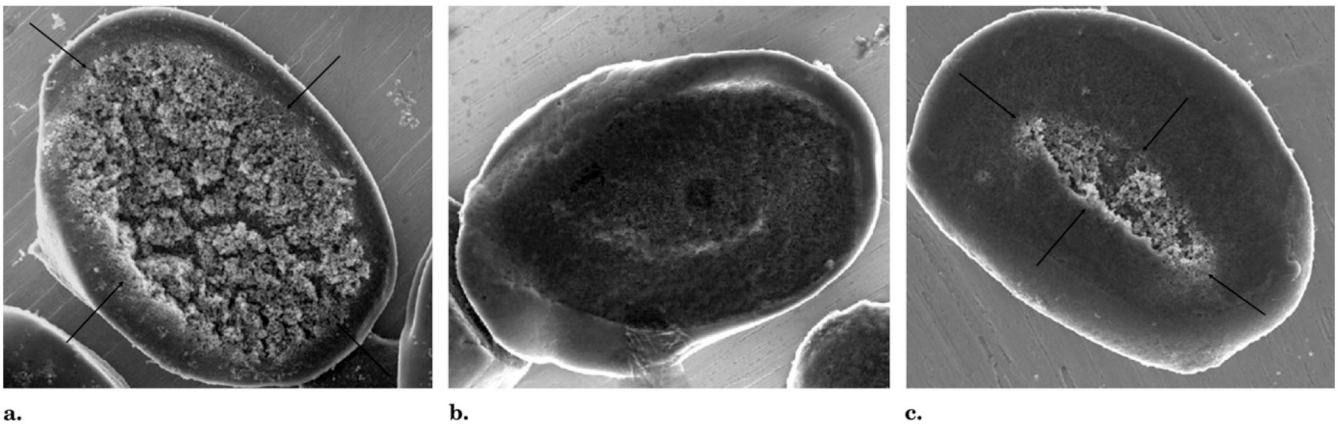

Figure 1.

Images from cross-section scanning electron microscopy of Contour SE **(a)**, prototype A **(b)**, and prototype B **(c)** microspheres. Contour SE is the most compressible microsphere, followed by prototype B and prototype A. The Contour SE microsphere exhibits large macropores distributed in the microsphere center surrounded by a microporous structure toward the exterior surface (arrows in **a**). The number and size of macropores decreases in prototype B (arrows in **c**), whereas prototype A (**b**) shows no macropores.

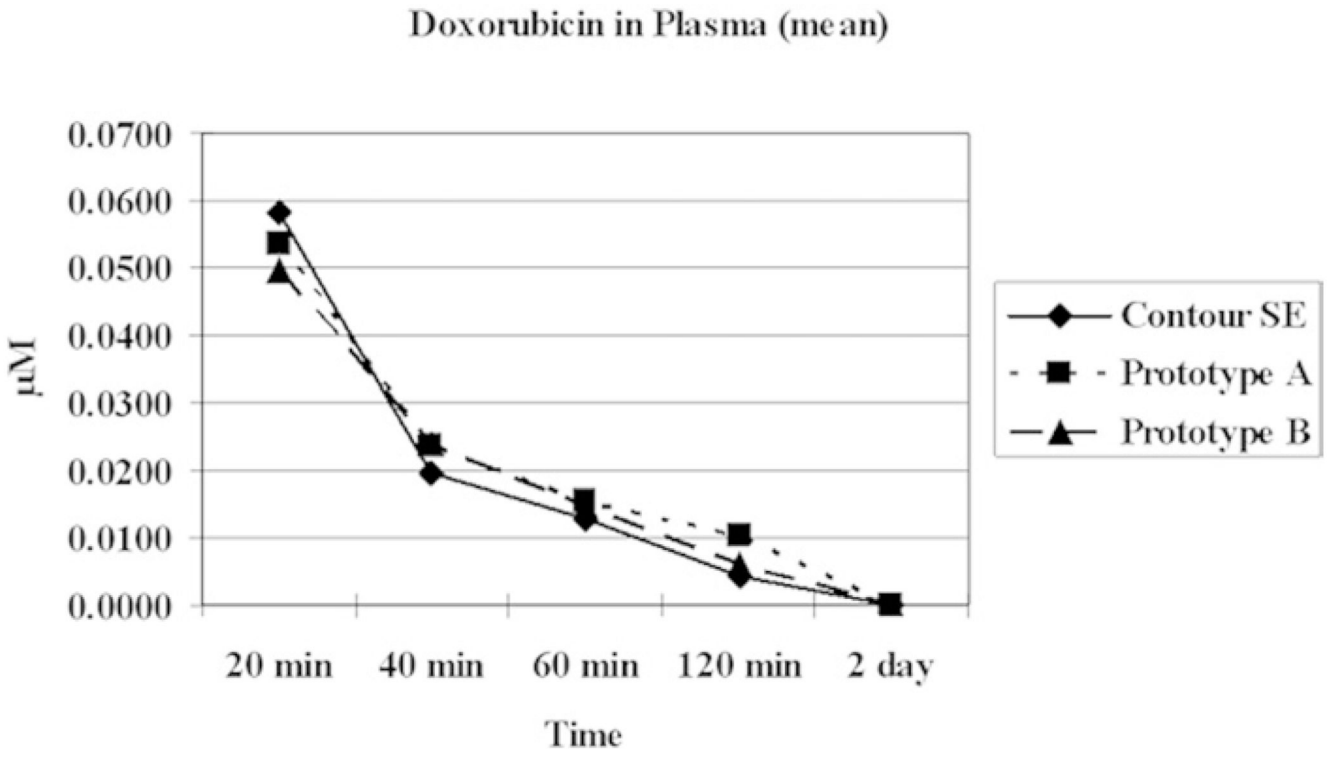

Figure 2.
Graph shows plasma doxorubicin levels at sequential time points after TACE.

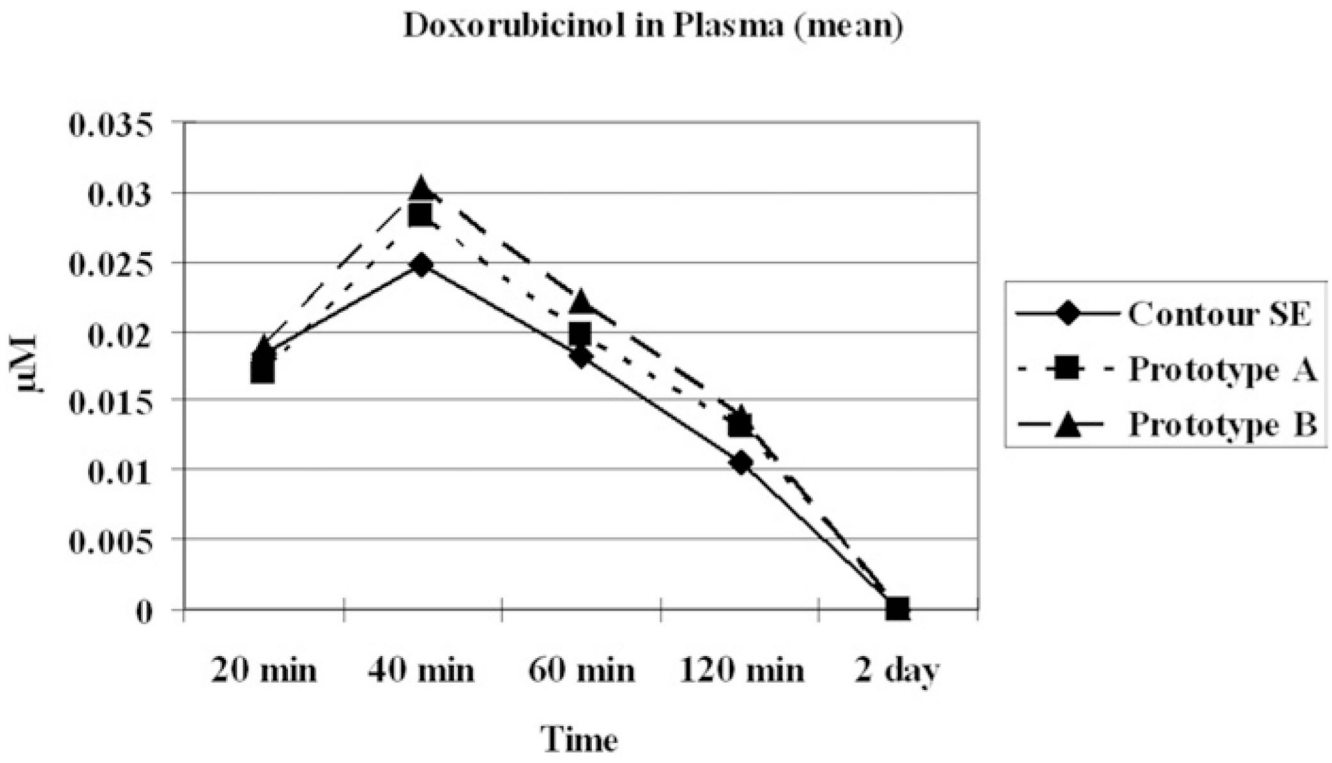

Figure 3.
Graph shows plasma doxorubicinol levels at sequential time points after TACE.

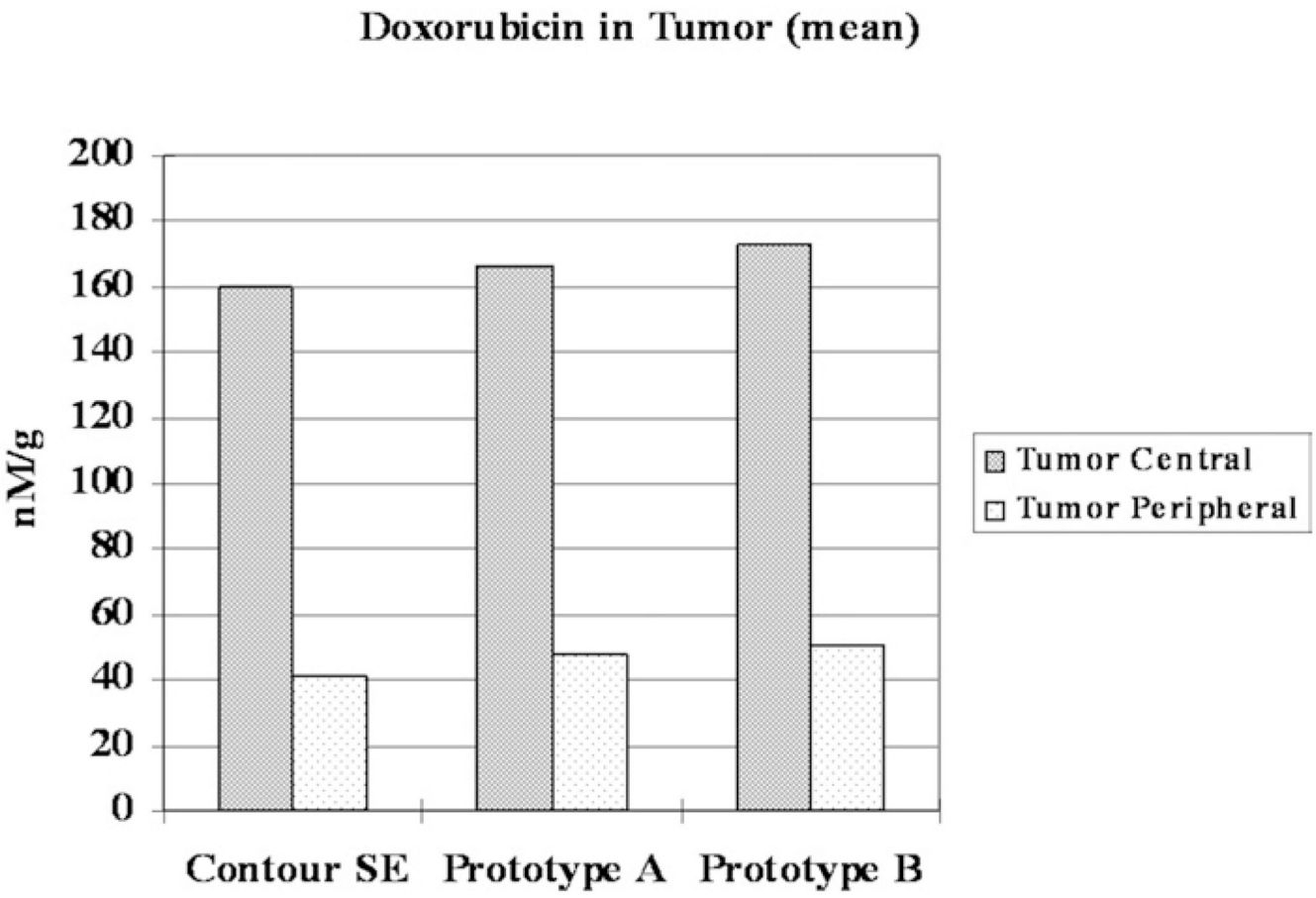

Figure 4.
Bar chart shows doxorubicin concentration in tumor 2 days after TACE.

Characteristics of Three Spherical PVA Microspheres

Characteristics	Group 1 (Contour SE)	Group 2 (Prototype A)	Group 3 (Prototype B)
Mean particle diameter (μm)	275 ± 90	269 ± 58	278 ± 66
Sphericity	~0.90	0.93	0.94
Porosity/compressibility	Most	Least	Intermediate