Does Microparticle Size Affect Bland Embolization Outcomes of Local Treatment for Liver Malignancies?

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Introduction

Treatment options for primary liver tumors (i.e., hepatocellular carcinoma or HCC) and metastatic liver neoplasms have increased in the past decade in response to the incidence of these tumors; HCC is the most common primary liver cancer, while liver tumors are the sixth most common cancer and the third most common cause of cancer-related death globally.1,2 The incidence of liver cancer in the United States and Western Europe is increasing.3,4 Liver metastases from colorectal cancer (CRC) develop in 50% of patients.5

While liver resection remains the gold standard in curative local treatment, several promising local treatments have been developed in the past three decades (e.g., intra-arterial treatments, percutaneous thermal ablation, stereotactic radiotherapy, and high intensity-focused ultrasound).

There is no consensus on the best local therapy. However, intra-arterial therapies offer great promise based upon the premise that hepatic tumors are fed mainly, if not exclusively, by arteries. Using this theoretical approach, investigators have tested multiple local treatments for liver tumors, such as chemoembolization (TACE), bland embolization (TAE), intra-arterial chemotherapy (HIAC), and selective internal radiation therapy (SIRT). TACE and TAE are the most common endovascular approaches for the local treatment of liver tumors, and several embolic agents have developed for that purpose.

Two early embolic agents have proven to be less successful. Gelfoam sponge powder was one of the first embolic agents used, but the efficacy was reported to be low because it stayed only temporarily within the tumor vascular mesh. Polyvinyl-alcoholic foam (PVA) is reported to be too heterogeneous in shape and size to be effective.6,7 PVA performance of this material can be unpredictable, primarily because the particles clump and aggregate within the vessel lumen, thereby causing occlusion of larger peripheral vessels. This may allow for the development of new feeding arteries distal to the target lesion, leading to poor clinical outcomes.

In the past two decades, several spherical embolic agents have been developed, including trisacryl gelatin microspheres,8 collagen-coated microspheres,9 dextran microspheres,10 and PVA microspheres.11 The development and refinement of spherical embolic particles has dramatically increased the treatment armamentarium for liver tumors, especially HCC or “hypervascular” liver lesions.12 Spherical embolics help to reduce or avoid particle clusters within peripheral vessels and allow for a deeper penetration in the neoplasm vasculature, with permanent and effective staining. More recently, several different embolic agents were developed, some of them with promising new features such as drug elution.13,14

The question remains: What feature of spherical embolics can increase clinical outcomes? According to the literature,15 optimal microsphere calibration should allow the clinician to match the most appropriately sized microsphere to the size of the vessels to be occluded for better targeting. Moreover, the use of well-calibrated microspheres should permit better control of the extent of occlusion, which depends on the number of injected particles and the penetration of the embolic agent within the tissue.

Until now, there has been no evidence to determine the most important feature an embolic agent should have for effective local treatment. The dimension and shape of embolic particles, however, seem to be the most important characteristics for this aim.

Background

By means of injection experiments, it was shown that malignant neoplasms growing in the liver tend to acquire an exclusively arterial blood supply.16 This is mainly true for HCC in which an aberrant inner arterial network is present, defined by a surrounding capsule, and generally fed by arteries coming directly from the main branches. In patients affected by HCC who are not candidates for liver surgery or ablation, TACE and TAE are the most common approaches, with...
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a proven improvement of survival in selected patients with well-preserved liver function.17

However, liver metastases show different vascularization compared with primary liver tumors, as well demonstrated by Breedis and Young in 1954. During an investigation of the hepatic circulation in animals, they noted ink stains within liver metastases when it was only injected via the hepatic arterial branches, compared to when it was injected via the portal vein.18 The authors observed in microscopic sections that both the portal vein and the hepatic vein, even if connected to the tumor, were invaded and/or occluded by tumor cells, preventing ink perfusion through those vessels. From the same authors, microscopic studies in humans on 13 metastatic livers16 revealed occlusion of portal branches due to the invasion by growing tumor cells, similar to what is seen in the experimental tumors. Occlusion of arterial branches by tumor invasion was never observed in experimental or human studies. They concluded that liver tumors are mainly, if not exclusively, supplied by arterial blood flow and that the occlusion of main proximal liver arteries does not result in tumor reduction, due to the presence of many peripheral connections. Much, if not all, of the failure of portal blood to supply tumors growing in the liver is due to progressive invasion and occlusion of portal branches by tumor cells. Based upon many other similar published studies, the hepatic artery has long been considered the predominant source of blood supply for colorectal liver metastases.19–22 However, targeted therapies based on arterial blood supply to the tumor have been usually disappointing. Local tumor control is rare, and the impact on survival is minimal.12,24

Discussion

TAE, TACE, and radio-embolization of liver tumors are some of the most commonly performed local treatments for patients with liver cancers.25,26 Both TACE and TAE shut down the arterial blood flow to the tumor, leading to tumor ischemia and eventually tumor cell death, if anoxia is induced. Herman et al described cell-surface bleb formation occurring in three stages. These cell wall damages were classified in 3 stages that were strictly related to the anoxic time exposure. In stage 1 and 2 if cells came back to normoxic ambient, the blebs disappeared. They concluded that the onset of cell death due to anoxia is a rapid event initiated by an increase of cell membrane permeability caused by rupture of stage-III blebs. Anoxia injury and cell death is irreversible when this event occurs.27

Because there are multiple arterial feeders to liver tumors, complete tumor death may be obtained only if the entire vascular network within and surrounding the tumor can be completely stopped. Only then will it cause anoxia, cell death, and irreversible necrosis.

At least two main principles must be carefully followed in order to obtain the best results for embolization: 1) select the right vascular target, and 2) use appropriate particle size to reach the small and deep vessels feeding the whole tumor. The practitioner must embolize all of the right vessels feeding the tumor. This is mandatory to avoid incomplete and/or non-target embolization. And the physician must select the optimal size of particles to be used in order to achieve the right penetration depth of the feeding tumor vasculature.

In order to enhance and maximize the outcome of arterial embolization, many clinical techniques have been proposed and many different sizes and types of embolic materials have been engineered.

Pillai et al28 used 27 µm-sized particles in microscopic examination of random liver and tumor samples and revealed that 6–12 times as many microspheres were found within the tumor than in the normal liver when injected through the main hepatic artery. This was mainly related to the dosage and the size of particles delivered; the smallest particles were needed for higher tumor concentration than in normal surrounding liver tissue.29

The optimal size range for selective arterial embolization particles is 10–140 µm. Both dye tracer studies and in vivo microscopy (experimental models) show sinusoidal connections between host and peripheral tumor vessels. The range size of these feeders has been estimated between 10 and 140µm.30 This micro-quantitative analysis provides a fundamental description of how regional intra-arterial microsphere therapy allows the targeted delivery of microspheres to neoplastic tissue to potentially improve the therapeutic index in the treatment of hepatic metastases.

Embolic agents with different morphologic, chemical, and physio-mechanical properties are currently employed for intra-arterial procedures.30 The level of their vascular, peripheral, and intra-tumoral distribution has been confirmed most frequently by invasive histopathology.15,24 Lee et al17 determined spatial distribution with MRI and histopathologic analysis of two different size ranges of iron oxide-containing microspheres injected intra-arterially in an animal model of liver cancer. The 100–300 µm and 300–500 µm sized microparticles were used by the authors for embolizing two groups of animals. Signal void due to the paramagnetic effect of iron oxide at MR imaging was detected at the periphery and within the lesion of the group of animals embolized with smallest particles (100–300 µm). Meanwhile, in the group of animals treated with bigger particles, the signal void was observed only within the main feeding arteries, but not within the tumor and only around 60% of the lesion. At the histopathology analysis, microparticles were detected inside the tumor in 70% of all lesions treated with the smallest size. Conversely, intra-tumoral deposition of microparticles was
not detected in any of the tumors embolized with 300–500 µm spheres and they were found at the periphery in only 40% of cases. The authors concluded that small microspheres were lodged inside the tumor, whereas their larger counterparts remained outside the tumor. The authors even stated that if the purpose of embolization is to deliver microspheres as close to the tumor as possible or within the tumor bed, the small particles measuring 100–300 µm should be used.

Two other key points were underlined in this paper: 1) the dilution/concentration of microparticles during the injection; and 2) the speed of injection, in accordance with blood pulsatility within arteries feeding the tumor. Both MR imaging and histopathologic analysis showed the presence of microparticle clusters within the tumor and the larger peripheral tumor feeding arteries.

Pillai et al., using 27 µm blue-dyed polystyrene micro-spheres in rabbit livers implanted with VX2 carcinoma, reported the presence of microparticles clusters of various sizes both within the tumor microcirculation and the normal liver parenchyma. The microsphere concentrations in the tumor were significantly greater than in the liver. Approximately eight times as many clusters were observed within the tumor compared to the normal liver. By doubling the concentration of injected microspheres, the tumor contained twice the number of spheres per cubic millimeter, but the average cluster concentration did not significantly increase. In conclusion, the authors reported that a concentration of 15 x 10^6 per milliliter was sufficient to saturate tumor vasculature. The intercluster distances were also evaluated in order to detect different distribution within the tumor and the liver. The tumors had approximately a five-fold decrease of intercluster distances compared to the liver. The average distance between the spheres to the target cell was significantly reduced within the tumor.

In another study, trisacryl gelatin microspheres (TGMS) clusters or chains were observed in large vessels only when smallest microspheres (40–120 µm) were used. The authors hypothesized that this event could be related to the very high number of small particles present in each milliliter of microsphere sediment in the commercial vial. If there was insufficient dilution with a quick injection rate, small microspheres could form clusters in large vessels proximal to the site of the occlusion. They suggested that it is important to carefully and accurately determine the size, amount, and dilution of microspheres to precisely fill the pathologic vascular mesh. It has also been shown that deeper penetration of TGMS can be advantageous and can lead to more effective devascularization of hypervascularized lesions. In conclusion, successful embolization of these tumors without proximal occlusion can only be obtained with the used of small, calibrated TGMS.

Most of these experimental studies were related to the so called “hypervascular” primary liver lesions. For liver metastases, embolization, chemotherapy and radio-embolization via the hepatic artery only occasionally provide local tumor control. Recurrences eventually occur in more than 80% of patients and usually at the tumor periphery, (Figure 1).

Nikfarjam et al., using electron microscopy, observed...
the presence of multiple direct sinusoidal connections between CRC liver metastases and host vessels in 61% of examined specimens. In a study in which liver tumors (metastases from CRC and primary tumors) were examined after 5-bromo-2′deoxyuridine infusion, both via the portal vein and hepatic artery, cells at the periphery of lesions were equally stained when injections were administered through either the portal or arterial supplies. This means that the periphery of these tumors even had a sinusoidal blood supply. In vivo microscopy in experimental models also consistently showed sinusoidal connections between host and tumor vessels.

It is our experience that computerized tomography (CT) is useful to precisely locate all tumor-feeding arteries. When angio-CT was performed by injecting contrast media (CM) via the intra-arterial microcatheter within the tumor feeders, lesions were entirely enhanced as in primary tumors (Figure 2). This is probably because the CM can flow through the arterial branches to the tumor and because the CM can bypass the hepatic sinusoids at the periphery of the tumor. Hence, it can be supposed that microparticles should be small enough to have the same performance and to embolize the surrounding sinusoidal pathological connections to the tumor. Regarding these target vessels, microparticles for embolization should be as small as possible, in order to flow within the deeper portion of the tissue for filling the vascular space and then in the more peripheral space, if the goal is maximizing vascular shut down to the tumor and achieving permanent anoxia rather than hypoxia of tumor cells. For this reason, as elsewhere reported, we use 40 μm hydrogel microspheres coated by a polymer, Polyzene-F (Embozene Microspheres, CeloNova BioSciences, Newnan, Georgia).

Moreover, embolic particles should be size-calibrated with a small bandwidth in diameter variations because during administration, larger particles within the same vial or syringe may occlude micro-vessels more proximally and prevent a deeper penetration of the smaller ones. Based on this background, we started our experience in bland embolization for liver malignancies by using Embozene microspheres which, due to the construction technology, are size-calibrated with a range of ± 10 for 40 μm, and a range of ± 25 for 100 μm. This should guarantee a more homogenous distribution of the particles deeply in the tumor (unpublished data).

It is important to underline that the use of small microparticles for intra-arterial treatments (TACE, TAE, and SIRT) may lead to two major side effects, which have been previously reported in literature: inflammation with foreign body reaction and the risk of non-target embolization (NTE).

Inflammatory reaction is an important issue due to the associated production of high rates of vascular growth stimulating factors that oncologists must take into account.

Microparticles as foreign bodies stimulate an inflammatory reaction with the production of giant cells. This outcome varies, depending on the biocompatibility and dimension of embolics. Giant cells have been demonstrated to be a substrate-specific cellular response representing a complex interaction between cellular reactions and cytokine or chemokine production. A recent study showed differences between four spherical embolic agents of different sizes used in embolization of pig livers. It has been reported that the 40–120 Embosphere microspheres (BioSphere Medical, Rock-
land, Massachusetts) resulted in a high inflammatory reaction at four weeks follow up. Microscopy showed that particles were surrounded by giant cells infiltrating the gelatin-coated layer. The authors concluded that this reaction was probably due to the acrylic polymer matrix of the particle’s core, which cross-linked with the allogenic gelatin shell.8 Embozene microspheres of 100 µm size were the second group of small microparticles used in this study. They are composed of a polymethylmethacrylate hydrogel core with a shell of Polyzene-F and a biostable and biocompatible polymer with a thickness of approximately 30 nm. The authors observed low inflammatory reactions without giant cells when 100µm Embozene microspheres were used, compared to Embosphere microparticles. No significant inflammatory reaction was observed when larger particles of the four different embolic agents were used. They concluded that all embolic materials triggered a mild inflammatory reaction after embolization. Small Embosphere microspheres caused a pronounced inflammatory reaction after four weeks, while the counterpart Embozene microspheres resulted in a very mild inflammatory reaction. This suggests that there would be a lower incidence of revascularization from cytokine or chemokine production associated with the Embozene microspheres.

A high risk of pulmonary embolism has been reported when very small microparticles are used for liver embolization, especially in primary liver lesions associated with cirrhosis or in liver neoplasms close to the hepatic veins.15 This complication might be due to the presence of arteriovenous shunting for small straight intra-lesional shortcuts (mainly in HCC) or for peripheral tumor infiltration of hepatic vein walls (mainly in metastases), with the direct passage of arterial blood to the hepatic vein circulation. The percentage of pulmonary shunting is variable and the size of these connections is unpredictable. For these reasons, whole-body scintigraphy after arterial injection of albumin Tc99 labeled macro-aggregates (MAA) is mandatory when SIRT is indicated, because of the very small size of labeled particles used.18

In our experience with using 40 µm Embozene microspheres for primary and metastatic liver lesions, patients were screened as if for SIRT therapy, with the injection of 99mTc-MAA just before embolization, in order to reduce the risk of pulmonary embolism. If lung shunting is detected, microparticles for embolization are up-sized from 40 µm to 100 µm, or more (Figure 3). In very few selected cases where the shunting is more than 20%, embolization with microparticles should be considered contra-indicated.

A different mechanism is conversely advocated for NTE of non-target organs such as pancreas, spleen or bowel (i.e., stomach and duodenum), which is mainly due to particles reflux during embolization. It has been recently reported to be mainly related to the use of larger particles because it is faster and there is more peripheral embolization.19
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Conclusions

Advances in cancer research promoted an increase of different local treatments for liver tumors and TACE and TAE with embolic agents are the most common techniques routinely employed. Up to now, many different embolic agents were investigated for this purpose, with no evidence about which feature is mainly responsible for local effect. Because tumor cell anoxia and consequent death represent the only aim of embolization, a complete and long-lasting shutdown of tumor blood supply must be achieved. Liver distribution of microspheres differs greatly by their size and 100–300 µm microspheres have been reported as the biggest ones to be used for achieving a deeper tumor penetration. Dimensions of target vessels feeding the tumor are sized between 10 and 140 µm. Hence, the literature suggests that the use of an accurately calibrated microsphere with a smaller size bandwidth within the range of the target should be used for a more homogeneous and deeper distribution inside the tumor vascular net, in order to maximize local effect. Caution must be observed when using very small microspheres, in order to avoid major complications such as pulmonary embolism.

References