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RESEARCH PAPER

Formulation of a Charcoal Suspension for Intratumoral Injection. Study of Galenical Excipients

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ABSTRACT

To tattoo human breast cancer prior to chemotherapy, radiotherapy, or surgery, thus allowing a better localization of the remaining tumor by the surgeon, we developed a formulation containing 10% charcoal suspended in water for parenteral preparations. The present study concerns a new step in the development of the charcoal suspension. We sought to determine whether the addition of various excipients could improve the formulation properties and affect the labeling of tumor by the suspension. We have tested surfactants (egg lecithin, polysorbate 80, Cremophor EL, and Pluronic F68), isotonisants (sugars such as glucose and mannitol), polysaccharides (dextrans 20 and 40), and Cabosil, a pyrogenated silica. Except for glucose and mannitol, which were added at a 5% concentration, the other excipients were

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added at a 0.1% concentration. they were dissolved in water for parenteral injection and sterilized at 120°C for 20 min. We then measured diffusion in vivo in mammary tumor. In vivo, when injected intratumorally in mice, a greater diffusion of charcoal particles was noted within the tumor (in the case of egg lecithin, polysorbate 80, dextran 20 and 40, and glucose) and sometimes in some organs (e.g., Cremophor EL and mannitol). Pluronic F68 slightly improved the stability of the suspension and did not lead to marked diffusion at the injection site, but it showed slight toxicity and cannot be used in the formulation. We concluded that the best formulation was an aqueous 10% micronized peat charcoal suspension.
Key Words: *Charcoal; Excipients; Intratumoral; Mammary tumors; Suspension.*

INTRODUCTION

A main goal of neoadjuvant chemotherapy (CT) in locally advanced breast carcinomas (clinical size $>$ 3 cm) is to decrease the tumor volume in order to permit conservative surgery. In about 10% of the cases, the tumor nodule is not clinically palpable after CT, and it is necessary to tattoo the initial site in order to guide the surgeon for the resection of any residual tumor.

In a previous study concerning the development of a charcoal suspension to label human breast tumors, we defined a preparation containing 10% peat charcoal in water for parenteral injection (1); 50% of the particles measured, on average, between 2 and 5 μm. Pharmacological and toxicological studies in animals indicate that the suspension is well tolerated with minimal diffusion into surrounding tissues, thus aiding histological examination (2,3). In an attempt to improve its formulation performances and the ease with which the suspension may be injected, we added various excipients to the suspension to modify either the viscosity of the dispersing phase or the charge of the particles to improve the ability to administer it by syringe. Those two characteristics that have an influence on physical properties of suspensions consequently have a major role on diffusion. Optimization of the formulation was achieved gradually, with diffusion assays in mouse tumors at each phase. The present paper reports how formulation properties and in vivo diffusion were modified and the impact it had on the use of the preparation as a consequence.

MATERIAL AND METHODS

Charcoal

Peat charcoal SX4 (Norit, Centre d'affaires, Blanc Mesnil, Paris Nord, 95153). We defined a preparation containing 10% peat charcoal in water for parenteral injection (1) with 50% of the particles measuring, on average, between 2 and 5 μm.

Surfactants

Surfactants used were egg lecithin (phosphatidylcholine), polysorbate 80 (Tween 80) [sorbitan mono-9-octodecenoate poly(oxy-1,2-ethanediethyl)] derivative, Cremophor EL (hydrogenated polyoxyethylenated ricin oil), and Pluronic F68 (polyoxyethylene-polyoxypropylene copolymer). The egg lecithin and polysorbate 80 derivative were obtained from Cooper, Melun, France. Cremophor EL and Pluronic F68 were purchased from BASF, Ludwigshafen, Germany.

Isotonisants

For isotonisants, sugars (glucose and mannitol), polysaccharides (dextran 20 and 40) were used. Glucose and mannitol were obtained from Pharmacie centrale des Hôpitaux de Paris, France. Dextran 20 and 40 were supplied by Fluka, Switzerland.

Other

Cabosil, a pyrogenated silica obtained by hydrolysis of silicium tetrachloride at 1100°C and composed of particles with an average diameter between 500 Å and 700 Å linked in ramified chains, was obtained from Cabot, Neuilly-sur-Seine, France. It is a pyrogenation silica used both for manufacturing pellets and as an emulsifier for suspensions.

Experimental Protocol

Charcoal was crushed in a stainless steel micronizer (Jet O'Mizer) with a compressed filtered air jet (7 bars). The excipients were dissolved or dispersed in water and were added to the 10% charcoal suspension in water for parenteral injection. The excipients were added at 0.1%

Table 1

The Formulation Characteristics of the 10% Charcoal Suspension by Addition of Emulsifiers

	Additives				
Formulation Properties	None (1)	Egg Lecithin 0.1% (2)	Cremophor EL 0.1% (3)	Polysorbate 80 0.1% (4)	Pluronic F68 0.1% (5)
Granulometry: mean diameter (μm)	7.6 ± 0.2 (A–D)	5.2 ± 0.0 (E, G)	6.7 ± 0.5 (H)	5.0 ± 0.3 (J)	6.4 ± 0.3
	% of Each Size				
1.0–2.4 μm	21.4 ± 3.2 (C)	30.6 ± 1.1 (E, G)	22.2 ± 2.3 (H)	34.2 ± 0.8 (J)	18.4 ± 1.7
2.4–5.4 μm	55.3 ± 4.5 (B, D)	65.8 ± 0.6 (E, G)	70.9 ± 0.1 (H, I)	63.5 ± 0.5 (J)	68.7 ± 0.7
5.4–10.8 μm	23.2 ± 1.2 (A–D)	3.5 ± 0.4 (G)	6.8 ± 2.5	2.3 ± 0.2 (J)	12.7 ± 2.4
Sedimentation (hu/ho %) at 20 min and at 7 months	87/46	66/45	18/14	84/36	91/46
Resuspension at 48 hr and at 7 months (sec)	10/13	9/12	4/6	9/8	6/7
pH	5.28 ± 0.02	4.97 ± 0.03	5.67 ± 0.02	5.1 ± 0.02	4.84 ± 0.03
Zeta potential RAM (mm)/Vs × 10^{-12}	−244	−264	−161	−82	1.26
	Apparent Viscosity (Pa/Sec)				
Shear rate (S −1)					
10	0.058	0.059	0.041	0.053	0.040
150	0.011	0.012	0.014	0.010	0.010
500	0.007	0.008	0.006	0.007	0.007

A = 1 versus 2; B = 1 versus 3; C = 1 versus 4; D = 1 versus 5; E = 2 versus 3; F = 2 versus 4; G = 2 versus 5; H = 3 versus 4; I = 3 versus 5; J = 4 versus 5.
Statistical comparisons, $p < .05$ between.

concentration, except for glucose and mannitol, which were added at a concentration of 5%.

Dispersion was obtained in a turbine mixer at a shirring rate of 200 rpm for 10 min at room temperature. The preparation was distributed in penicillin-type bottles, sealed, and sterilized at 120°C for 20 min. Sterility was verified for all preparations.

Animals

C3H female mice aged 6–8 weeks were bred at the Institut Gustave Roussy animal experimentation department. The studies were carried out on animals weighing 20–25 g, 21 days after implantation of tumor cells of C3H mouse mammary adenocarcinoma into the hind leg. This is a syngenetic implantable tumor obtained from solid tissue transplants. A 0.5-ml volume of filtered tumoral cell (5×10^5 cells) suspension was injected subcutaneously.

The tumor reached a size of 1 to 2 cm diameter 3 weeks after the injection, and 50 μl of the charcoal suspension were injected directly into the tumor mass at that time. The animals underwent an autopsy 10 days later.

Description of the Assays

Suspensions

Granulometry

Measurements were performed in a Coulter Counter (Model TA 11, Coultronics France SA 95 Andilly). The average granulometry and percentages of particles of the following sizes were determined: 1–2 μm, 2–5 μm, and 5–10 μm. Results were analyzed statistically with the

Table 2

The Formulation Characteristics of the 10% Charcoal Suspension by Addition of Polymers and Isotonisants

	Additives					
Formulation Properties	None (1)	Dextran 20 0.1% (2)	Dextran 40 0.1% (3)	Mannitol 5% (4)	Glucose 5% (5)	Cabosil 0.1% (6)
Granulometry: mean diameter (μm)	7.6 ± 0.2 (A, B, E)	4.8 ± 0.1 (I)	5.6 ± 0.4 (L)	8.4 ± 2.5	9.3 ± 5.2	6.8 ± 0.1
	% of Each Size					
1.0–2.1 μm	21.4 ± 3.2 (A)	32.2 ± 0.2 (F)	18.3 ± 2.0	31.7 ± 4.5	30.0 ± 1.1	23.6 ± 8.3
2.1–5.4 μm	55.3 ± 4.5 (B)	65.9 ± 0.1 (F, I)	73.2 ± 0.8 (K, L)	65.9 ± 0.1 (N)	67.1 ± 0.6 (O)	46.2 ± 0.4
5.4–10.8 μm	23.2 ± 1.2 (A–D)	1.6 ± 0.1 (I)	8.2 ± 2.8	2.1 ± 0.8 (N)	3.0 ± 0.6 (O)	24.8 ± 1.1
Sedimentation: hu/ho % at 20 min and at 7 months	87/46	86/43	86/50	86/48	84/45	89/54
Resuspension: seconds at 48 hr and at 7 months	10/13	10/14	9/20	10/20	16/22	4/6
pH	5.28 ± 0.02	4.96 ± 0.03	4.99 ± 0.01	4.75 ± 0.02	4.77 ± 0.04	4.83 ± 0.02
Zeta potential RAM (mm)/Vs × 10^{-12}	−244	−15	−111	−119	ND	−1.56
	Apparent Viscosity (Pa/Sec)					
Shear rate (S −1)						
10	0.058	0.027	0.057	0.065	0.069	0.024
150	0.011	0.008	0.011	0.012	0.012	0.008
500	0.007	0.006	0.007	0.008	0.008	0.006

A = 1 versus 2; B = 1 versus 3; C = 1 versus 4; D = 1 versus 5; E = 1 versus 6; F = 2 versus 3; G = 2 versus 4; H = 2 versus 5; I = 2 versus 6; J = 3 versus 4; K = 3 versus 5; L = 3 versus 6; M = 4 versus 5; N = 4 versus 6; O = 5 versus 6; ND = not done.
Statistical comparisons, $p < .05$ between.

nonpaired Student's *t* test. A *p* value less than .05 was considered significant.

Sedimentation and Resuspension

A 50-ml suspension was placed in a 50-ml graduated test tube. Spontaneous sedimentation was measured at room temperature at 5, 10, 15, 20, 30, 40, 60, and 120 min, then at 24 hr and 48 hr, 1 week, and 1 and 7 months. Sedimentation is expressed by the ratio of the height of the sediment at time *x* to the height of the suspension at time 0.

Resuspension, the time required to resuspend the preparation, was obtained by turning the tube upside down and was expressed in seconds. The suspension was considered homogeneous as soon as the sediment was no longer visible.

pH

The pH was measured with a pH meter analyzer, multiparameter P 407 MCNS II, calibrated between each measurement with pH 4 and pH 7 buffers.

Rheology

Measurements for rheology were performed with a CSL 100 rheometer (Carri-med Rheo, 91 Champlan), with a shear rate given by a geometric cone/plate (diameter 4 cm, angle 2°).

Zeta Potential

A 250-ml sample was placed in the cell of an acoustophoretic analyzer Pen Kem 7000 (Noviprofibre, 38 Eybens). The zeta potential was expressed as the relative

Table 3

In Vivo Influence of the Addition of Emulsifiers on the Diffusion of the 10% Charcoal Suspension in the Organs and in the Tumors

Additive	None	Egg Lecithin 0.1%	Cremophor EL 0.1%	Polysorbate 80 0.1%	Pluronic F68 0.1%
Immediate death	0/5	0/5	1/4	0/4	0/4
Delayed death	0	0	0	1	1
Macroscopic presence	0	+	+	+	0
In the organs:					
Lungs	0	4/5	1/4	3/4	0
Liver	0	0/5	1/4	1/4	0
Spleen	0	3/5	0/4	1/4	0
Histologic presence	0	+	+	+	+
In the organs:					
Lungs	0/5	2/5	3/4	3/4	1/4
Liver	0/5	2/5	2/4	2/4	1/4
Spleen	0/5	1/5	2/4	2/4	0/4
Kidney	0/5	0/5	0/5	0/4	0/4
Heart	0/5	0/5	0/5	0/4	0/4
Histology of tumors	Charcoal seen on the tumor periphery in necrosis and in macrophages	Diffuse aspect of the distribution; scarce charcoal on the slides	Localized as nodulary spots in the periphery of the tumor; scarce charcoal in macrophages	Diffuse aspect in many places; scarce charcoal visible	Localized in well-defined nodules and in histiocytes
Diffusion in the tumor	Very weak	Diffuse	Weak	Diffuse	Very weak

acoustophoretic mobility (RAM) in millimeters per volt per second.

In Vivo Diffusion

The diffusion of the suspensions was checked at the time of tumor excision by macroscopic examination of the tumor, kidney, liver, spleen, lungs, and heart. Sections of each organ were then fixed for histology and were examined to determine whether the charcoal particles had diffused toward the edges of the tumor and had been taken up by histiocytes in the tumor or organs.

RESULTS

Formulation Studies

Granulometry

With the addition of all the excipients studied (except for glucose 5% and mannitol 5%), a diminution of the mean diameter of the charcoal particles was observed (Tables 1 and 2).

Sedimentation and Resuspension

Control values of the sedimentation (Hu/Ho) were at 20 min and 7 months (87%/46%). They were decreased by the addition of egg lecithin (66%/45%) and Cremophor EL (18%/14%) and increased with Pluronic F68 (Hu/Ho 91%/48%).

For isotonisants and polymers, except for Cabosil, for which the sedimentation rate increased (89%/54%), the values observed with the other additives are close to those of the original suspension without additive.

The times required to resuspend the original suspension without additive were 10 and 13 sec after 48 hr and 7 months sedimentation, respectively.

When Pluronic F68 was added to the charcoal suspension, resuspension times were decreased (6/11 sec), which is associated with the slightly longer sedimentation rate. When Cremophor EL was added, resuspension times were 4 and 6 sec after 48 hr and 7 months, respectively, and this mixture had the lowest sedimentation rate.

With isotonisants and stabilizers, resuspension times were significantly lower with Cabosil and higher with

Table 4

In Vivo Influence of the Addition of Polymers and Isotonisants of the 10% Charcoal Suspension in the Organs and in the Tumors

Additive	None	Dextran 20 (0.1%)	Dextran 40 (0.1%)	Glucose (5%)	Mannitol (5%)	Cabosil (0.1%)
Immediate death	0/5	1/5	1/5	0/5	3/6	0/5
Delayed death	0	2	1	0	0	0
Macroscopic presence	0	0	+	+	0	0
In the organs:						
Lungs	0/5	0/2	3/3	1/5	0/5	0/5
Liver	0/5	0/2	3/3	0/5	0/5	0/5
Spleen	0/5	0/2	3/3	0/5	0/5	0/5
Histologic presence	0	+	+	+	+	+
In the organs:						
Lung	0/5	1/2	3/3	4/5	0/3	1/5
Liver	0/5	1/2	3/3	5/5	2/3	1/5
Spleen	0/5	0/2	1/3	2/5	1/3	0/5
Kidney	0/5	0/2	1/3	2/5	1/3	0/5
Heart	0/5	0/2	0/3	0/5	0/3	0/5
Histology of tumors	Charcoal in the periphery in the necrosis, and in the histiocytes		Diffuse spots	Large charcoal spots; diffuses within the tumor	Nodular in the periphery; slightly diffuse	Nodular in macrophages in the necrosis; extracellular
Diffusion in the tumor	Very weak	Diffuse	Diffuse	Diffuse	Very weak	Weak

glucose 5% (16 and 22 sec, respectively), with no evident modification of sedimentation rate.

Rheology

The fluidifying character was not modified for any excipient studied. Viscosity values were reduced by the addition of Pluronic F68, Cabosil, Cremophor EL, polysorbate 80, and dextran 20, while no modification was observed with egg lecithin, dextran 40, and sugars.

pH

The pH was slightly increased by the addition of Cremophor EL. All the other additives decreased it, mannitol in particular.

Zeta Potential

The 10% peat charcoal suspension was very viscous due to an electroviscous effect, with electrostatic repulsion of the particles; as a consequence, it had an important negative RAM (−244).

The addition of egg lecithin slightly increased the zeta potential of the suspension. This may be attributed to ionizable phospholipids present in minor compounds (4).

Hydrophilic polymers decreased the RAM values; molecular mass plays an important role in this decrease. Addition of dextran 20 led to the formation of a less negatively charged suspension (−15) than addition of dextran 40 (−111); this was as if more dextran 20 molecules were adsorbed on charcoal, which could explain the viscosity results.

Variations of zeta potential, in most cases, were accompanied by a viscosity decrease, an interesting property for amelioration of the ease with which the suspension can be injected. This was observed for Pluronic F68, Cabosil, Cremophor EL, and dextran 20.

Zeta potential variations result from different configurations of additive adsorption on the charcoal surface. Charcoal is a heterogeneous substance with hydrophobic sites on the organic components and hydrophilic sites on the minerals where the materials are disseminated. The apolar part of the additives would be physically adsorbed on hydrophobic sites by hydrophobic-type links, while on hydrophilic sites, additives would be adsorbed through

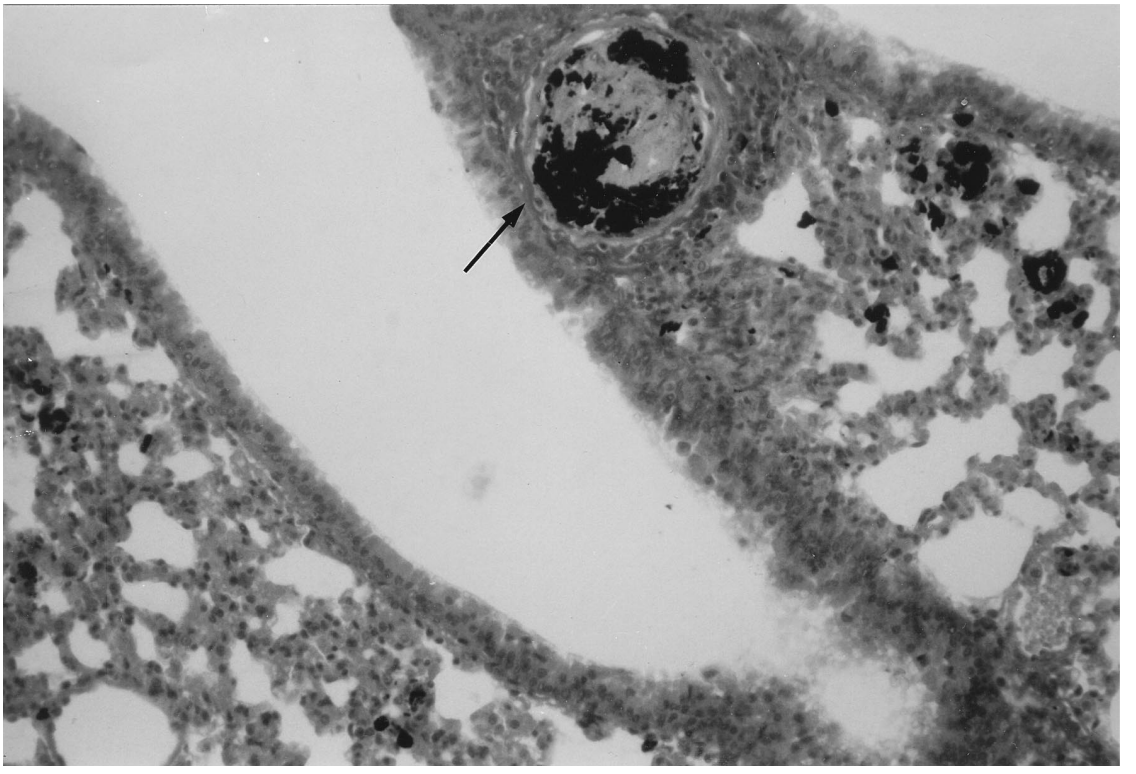

Figure 1. Lung of a mouse intratumorally injected with a charcoal suspension containing Cremophor EL. Charcoal was seen within alveolar macrophages and diffusely distributed. A thrombosis was made of histiocytes loaded with charcoal particles in the lumen of a vein (→) (HEX × 200A).

physical bonds, such as hydrogen bonds in the case of nonionic agents.

Pluronic F68, a copolymer with hydrophilic ethylene oxide segments, hydrophobic propylene oxide segments, and hydrophilic ethylene oxide segments, would be fixed as a comb or a ring structure, while for polysorbate 80, made of ethylene oxide hydrophilic segments and fatty acid hydrophobic segments, other types of configurations could be suggested, such as parallel structures.

In Vivo Diffusion

As reported in Tables 3 and 4, some of the mice died immediately after the intratumoral injection of the supplemented charcoal suspensions, an indication of intravenous passage of the suspension. Some delayed deaths were also observed.

Death observed as soon as injection or soon after the injection of the suspension with additives was likely to be due to the fact that charcoal passed into the bloodstream and induced microthrombosis, which would lead to death. Charcoal particles could also have adsorbed platelets after passage into the bloodstream as previously described (5).

Away from the injection site, except for suspensions with Pluronic F68 and Cabosil, charcoal was macroscopically visible in all the organs.

After the injection of suspension supplemented with Cremophor EL, charcoal was observed in the lungs and in a thrombosis containing histiocytes in the lumen of a vein (Fig. 1). Charcoal was found in the renal glomerules when injected with mannitol, dextran 40, and glucose (Fig. 2). Macroscopic and histologic charcoal diffusion in the tumors (Figs. 3–6) decreases in the following order: Pluronic F68, mannitol, Cabosil, and Cremophor EL.

After Cabosil injection, an inflammatory reaction was observed histologically. Pluronic F68 appeared to be the additive best able to limit diffusion, but a few days after the injection at a concentration of 0.1%, one mouse died. Histological study of the tumor after charcoal injection

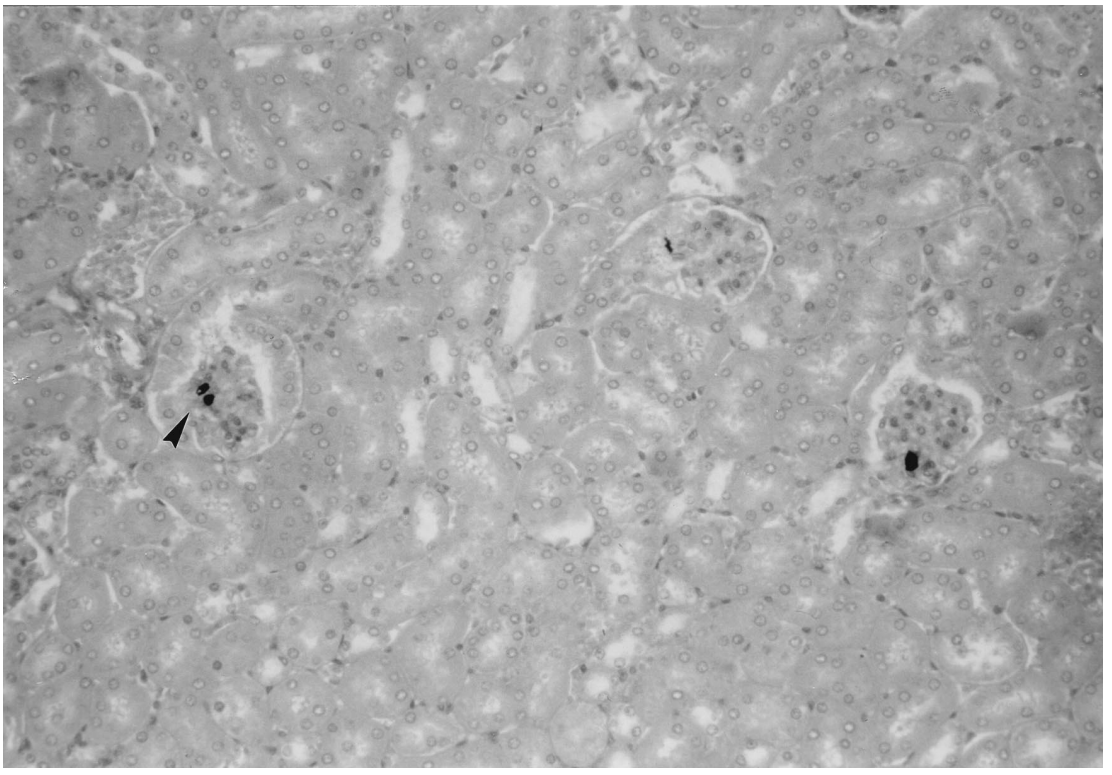

Figure 2. Histology of a mouse kidney 10 days after intratumoral injection of a charcoal suspension containing 5% mannitol. Charcoal particles were observed in glomerules (➤) (HES × 200).

without additive showed that particles had limited diffusion and were seen as a spot at the tumor periphery.

When emulsifiers were added, macroscopic charcoal was more abundant in organs when the mean diameter of the particle decreased. With polymers and isotonisants, there was no correlation.

The pH decrease by the additives was not accompanied by less charcoal particle diffusion away from the injection site, except for Pluronic F68, Cabosil, and mannitol 5%, as far as macroscopic examination of the tumor was concerned.

No significant correlation was noted between rheological properties and charcoal particle migration. For additives that did not modify rheology of the suspensions, charcoal particles, however, have diffused in the organs. Additives such as Pluronic F68 and Cabosil had significantly reduced viscosity, yet they induced less diffusion.

No correlation was observed between zeta potential and macroscopic and histologic diffusion of charcoal particles.

Galenic properties of the suspensions do not appear to explain the experimental diffusion.

DISCUSSION

In order to improve the ease with which charcoal suspensions could be injected into localized residual tumors, several excipients were introduced in the preparation and their formulation characteristics were measured.

We studied whether it could be possible to optimize some physicochemical parameters, such as zeta potential and viscosity, to improve flow and stability and to reduce diffusion. When the suspension without additives was injected into the tumor, charcoal is seen in spots. When excipients are incorporated in the suspension, charcoal particles were most often found outside the cells.

It was difficult to correlate the results with the physicochemical properties of the suspensions. This suggests mechanisms other than those previously implicated in the in vivo migration of charcoal particles, like granulometric distribution. We have found that the finest particles (<2 μm) produce the greatest diffusion and more readily undergo phagocytosis by macrophages (1).

Neoplastic tissue may be divided into three compartments: interstitial, vascular, and cellular (6). The inter-

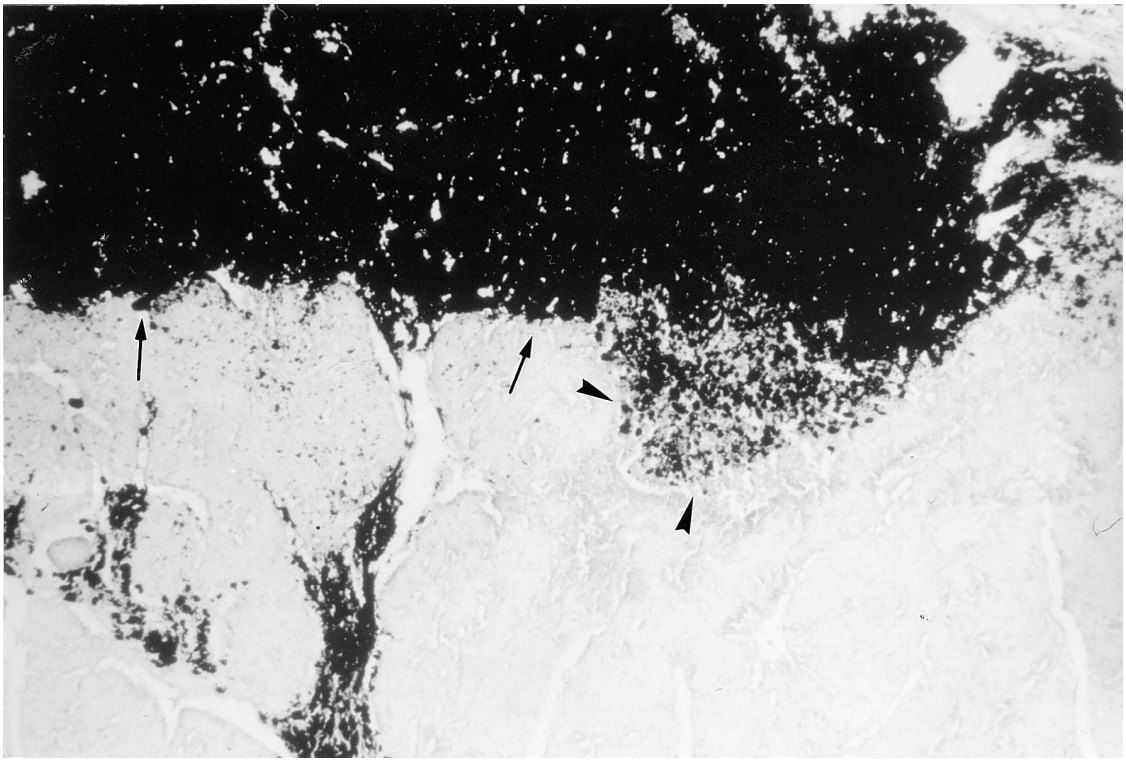

Figure 3. Intratumoral injection of the 10% charcoal suspension. Particles had limited diffusion and are seen in spots at the periphery (→). Charcoal was phagocytosed by some histiocytes that did not migrate (➤) (HES × 100).

stitial compartment is characterized by its rich collagen content and low concentration of macromolecules, such as proteoglycan and hyaluronic acid. This negatively charged compartment forms a hydrophilic gel. The interstitial pressure was high within the tumor and diminished at the periphery. Increase of hydrostatic pressure in the tumor interstitial spaces would be due to the lack of a well-developed lymphoid net and to the cell proliferation in a limited space (7).

Molecular transport in the interstitial space occurs through a concentration gradient (i.e., by diffusion) for the low molecular mass molecules (such as glucose) and according to fluid motion (by convection) for high molecular mass molecules such as dextrans (8). By the large amount of diffusion of molecules such as mannitol, an extracellular marker, charcoal particles could have been easily carried in the bloodstream. This also may be observed for dextran, which easily diffuses in interstitial spaces and is used in research as a vehicle for anticancer drugs. In contrast, water or sodium chloride rapidly diffuse in interstitial spaces and in cellular medium, thus limiting diffusion phenomena.

The tumor vascular compartment (1% to 20% according to the tumor type) includes a preexisting vascular network and vessels formed by tumoral angiogenesis. Tumor vessels are characterized by large interendothelial junctions, by a large number of openings, and by transendothelial canals formed by vesicles and by a discontinued or absent basal membrane (9). As a consequence, this leads to a vascular permeability increase.

A high interstitial pressure in the tumor center generates a diminution of the fluid and macromolecule extravasation. A low interstitial pressure at the periphery leads to an increase of the fluid and macromolecule filtration from the blood vessels at the tumor periphery (10). As a consequence, some suspensions with excipients may have induced an increase in the interstitial pressure when injected, particularly those with higher viscosity (with addition of dextran 40, mannitol, glucose, or egg lecithin).

This could have induced a vascular occlusion and, secondarily, a necrosis, which thus would have favored intravasation of charcoal particles and thus their vascular dissemination. When a macromolecule is extravasated,

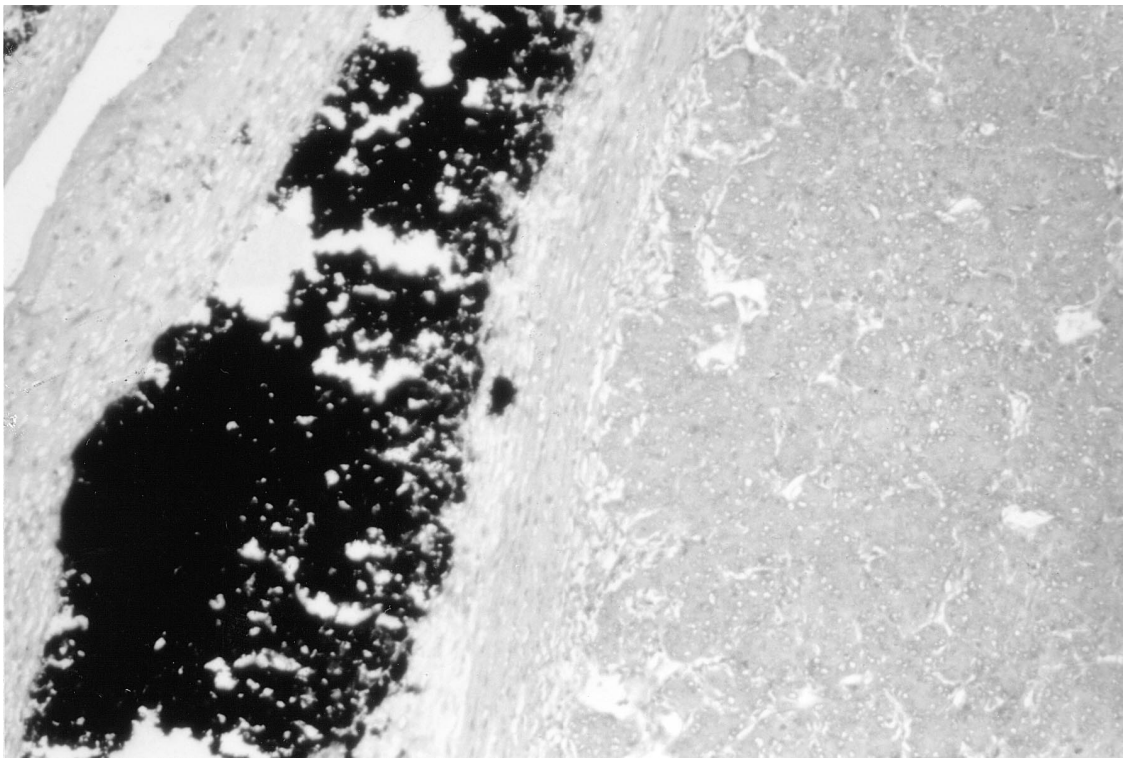

Figure 4. Intratumoral injection of the charcoal suspension containing pluronic F68; limited diffusion is seen, and there is extracellular localization. Sometimes, blue charcoal was phagocytosed by macrophages at the injection site. There was no migration (HES × 100).

its transport is by diffusion and convection through interstitial spaces. For low-viscosity suspensions that may be expected to have greater distribution in the mammary tumor and even at the periphery, where fluid and macromolecule filtration was high, it is possible to think that charcoal particles also could have reached the bloodstream more rapidly.

The tumor cellular compartment contains histiocytes (tissue macrophages) and tumor cells. Charcoal particle migration may be linked to cell migration after phagocytosis, particularly due to tumor macrophage activation that, by liberating chemotactic factors (11), can recruit blood monocytes, which carry charcoal particles they have phagocytosed away from the injection site.

Coating of charcoal particles by some additives, as evidenced by zeta potential variations, could play the role of opsonines and make the particles easier to phagocytose. In order to have phagocytosis, adhesion of those particles on the phagocyte surface is required.

Nonspecific recognition by macrophages could occur through the bias of surface receptors for glucides, which could explain macrophage migration with additives such as glucose, mannitol, dextrans, and polysorbate 80, which contain sorbitane (12). Phagocytosis is also favored by triglycerides. For example, bacteria constitutive lipids suscitate a numerical increase of macrophages. This could explain results obtained with egg lecithin and Cremophor EL.

Arachidonic acid, an egg lecithin constituent, is in other respects a cell activator. The arachidonic acid degradation pathway (lipoxygenase pathway) leads to leucotriene formation, including LTB4, a compound implicated in macrophage chemotaxis and vascular permeability modulation (13).

As far as pyrogenated silica was concerned, migration of charcoal particles away from the injection site was not observed. Selective toxicity in vivo and in vitro of silica in macrophages had been reported (14). Intravenous injection of silica has no effect on monocytes (15).

Similarly, Pluronic F68 did not induce chemokinetic phenomena; that is, it did not increase macrophage migratory activity with macrophage system activation (16).

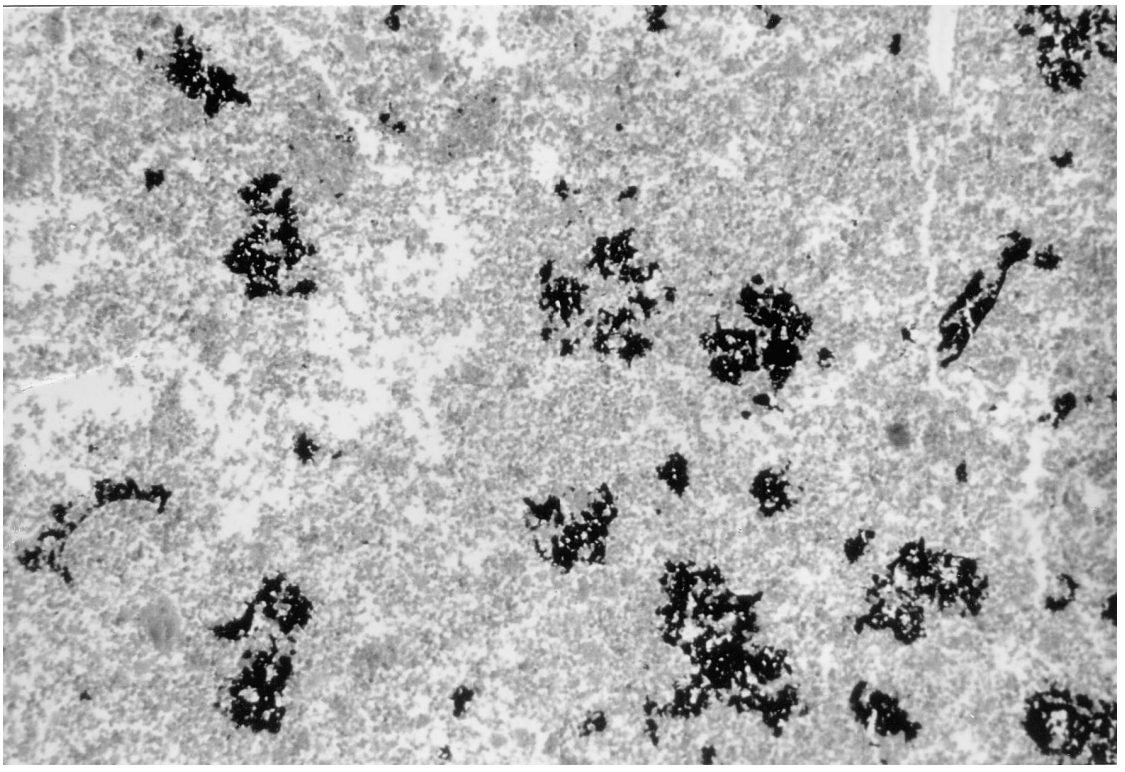

Figure 5. After injection of a 10% charcoal suspension with Cremophor EL, charcoal was seen in a wide zone of necrosis tumor. No phagocytosis was observed (HES × 100).

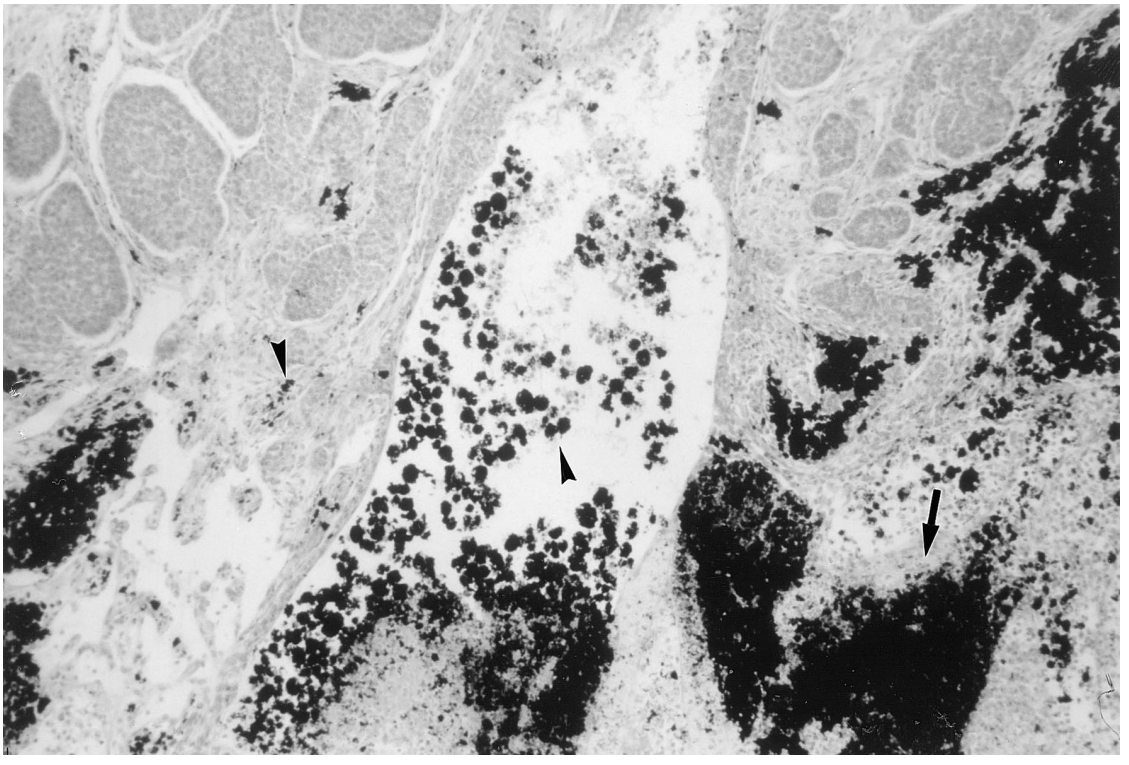

Figure 6. After intratumoral injection of a charcoal suspension containing dextran 40, charcoal particles were diffuse in extracellular spots, surrounded by necrosis (➤), or phagocytosed by histiocytes that have migrated in the tumor stroma (→) (HES × 100).

We have studied the influence of the excipients on in vitro and in vivo macrophage activation, as well as in vivo mobilization, at the studied concentrations. Mobilization was increased by all the additives. In vitro Pluronic F68 had decreased activation; in vivo, all the additives except polysorbate 80 enhanced macrophage activity (17).

In conclusion, Pluronic F68 would be the selected additive since it had limited diffusion in the organs and the tumors. To improve the ability to administer the suspension by syringe by modifying the viscosity of the dispersing phase, we tested different concentrations of Pluronic F68 (0.1%, 0.3%, 0.4%, 0.5%). Pluronic F68 slightly improved the stability of the suspension and did not lead to marked diffusion at the injection site, but it showed slight toxicity, indicating that this additive cannot be used in the formulation (18).

Galenical studies and in vivo studies show than an aqueous suspension of 10% peat charcoal of defined granularity was best for intratumoral injection. Clinical studies show that the suspension allows easy identification of residual breast tumors after chemotherapy with minimal diffusion into surrounding tissue, thus aiding histological examinations (19,20).

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