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Formulation of a charcoal suspension for intratumoral injection: influence of the pluronic F68® concentration

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Abstract

We have developed a charcoal suspension to be injected intratumorally to tatoo human breast cancers prior to chemotherapy. It is an aqueous suspension of 10% peat charcoal of defined granularity. To improve the syringeability Pluronic F68® was tested at concentrations of 0.1, 0.3, 0.4 and 0.5% in mice. 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. At the higher concentrations immediate mortality was noted after the injection, and mice that survived lost weight. Even at the lowest concentration of 0.1% granulomas were formed in the lungs, indicating that this additive can not be used in the formulation. © 1997 Elsevier Science B.V.

Keywords: Charcoal; Suspension; Pluronic F68®; Mammary tumor; Preoperative marking; Intratumoral

A charcoal suspension for intratumoral injection was formulated to enable pathologists to guide the surgeon during excision of residual tumors after chemotherapy.

In previous work studying the nature, granulometry and concentration of the charcoal and suspension vehicle and their characteristics in vitro and in vivo, we concluded that the best formulation was an aqueous 10% micronized peat charcoal suspension (Bonhomme et al., 1996; Mathieu et al., 1994).

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To improve syringeability by modifying the viscosity of the dispersing phase, we tested different concentrations of Pluronic F68®.

Charcoal peat SX4 (Norit, 93153 Le Blanc Mesnil, France) is an activated steam charcoal. The suspension vehicle is water for injection. Pluronic F68® (poloxamer 188), a polyol non ionic surfactant (polyoxyethylene–polyoxypropylene copolymer) was purchased from BASF Ludwigshafen, Germany. The charcoal was crushed in a stainless steel micronizer (Jet O'Mizer Labservice, Macon, France) with a compressed filtered air jet. A concentration of 10% was added to the suspension vehicle with different concentrations of Pluronic F68® (0.1, 0.3, 0.4 and 0.5%). The suspension was sterilized at 120°C for 20 min.

Granulometry: measurements were made with a Counter coulter (models TA II) (Coultronics France SA 95580, Andilly. Each sample was analyzed twice. The data were expressed as means and standard deviations and compared using Student's t-test. Zeta potential: a 250 ml sample was placed in the cell of a Pen Kem 7000 acoustophoretic analyser (Noviprofibre 38320 Eykens). The zeta potential was expressed as acoustophoretic mobility in mm/V per s. (Depraetere, 1988). Sedimentation and resuspendability: 50 ml of the suspension was placed in a 50 ml graduated test tube which was closed with Parafilm. Spontaneous sedimentation was measured at 5, 10, 15, 20, 30, 40, 60 and 120 min and 24 and 48 h, 1 week and 1 and 7 months. Sedimentation was expressed as the ratio between the height of the sediment at time and the height of the suspension at time 0. Resuspendability was assessed after inverting the tube and was expressed in seconds. pH: we used a multiparameter P 407 MCNS 11 pH meter (Bioblock France). Rheology: measurements were made with a CSL 100 Rheometer (Carri-med 99 Rheo 91160 Champlan) with a shear rate given by a geometry cone/plateau (diameter 4 cm, angle 2°).

The various concentrations of Pluronic F68® were studied on four batches of C3H mice aged 6–8 weeks and weighing 20–25 g. They were injected subcutaneously (s.c.) with 0.5 ml of a cell suspension prepared from a syngenetic implantable tumor obtained from solid tissue transplants. The tumor attained a size of 1–2 cm in diameter 3 weeks after the injection, and 100 μl of the charcoal suspension was injected intratumorally at that time. The animals were autopsied 15 days later. Tumor volume (V) was calculated on day 0 and 15 days later according to the formula $V = (\text{length} \times \text{width})/2$. The data were expressed as means and standard errors and compared using Student's t-test; p values of less than 0.05 were considered significant.

The average size of the particles was lowest with the 0.4% concentration, which showed a significant increase in the number particles smaller than 2 μm (31%) compared with the concentration of 0.1% (18%) (Table 1). The DN value increased with the concentration (0.1, 0.3 and 0.5%) of Pluronic F68®, mainly owing to the increase in the number of particles with a size between 2 and 6 μm. The sedimentation rates and resuspendability tests showed a slight improvement in the stability of the charcoal suspensions when pluronic F68 was added. This stability increase is limited for after 7 months of sedimentation, the Hu/Ho values different suspensions being then comparable with those of the suspension without additive. The adsorption of Pluronic F68® to charcoal particles can increase their weight and sedimentation rate. The adsorbed layer would therefore modify the flow of particles at the time of the injection. The time required to resuspend the suspension diminished as the Pluronic F68® concentration increased. The dispersibility of suspensions was good, owing to the wetting. The pH remained stable whatever the concentration of Pluronic F68®. The increase in the Pluronic F68® concentration induced a decrease in viscosity. This effect was particular marked at the concentration of 0.1%. Increasing Pluronic concentrations increased the RAM value, as measured by acoustophoresis. Although weak, this effect would be linked to an increase in the number of particles (deflocculating and dispersing effects of Pluronic F68®).

After intratumoral injection of the charcoal-Pluronic F68® suspensions, immediate death was observed in three mice, two at a concentration of 0.4% one at a concentration of 0.5%. A few days after the injection one mouse died at a

Table 1
The formulation characteristics of the 10% charcoal suspension, with different concentrations of pluronic F68®

	None 10% (1)	Pluronic F68® (0.1%) (2)	Pluronic F68® (0.3%) (3)	Pluronic F68® (0.4%) (4)	Pluronic F68® (0.5%) (5)
Granulometry mean diameter (μm)	7.6 ± 0.2 (A) (B) (C) (D)	6.4 ± 0.3 (F) (G)	6.2 ± 0.2 (H) (I)	5.1 ± 0.1 (J)	5.8 ± 0.1
1–2.4 μm	21.4 ± 3.2 (C) (D)	18.4 ± 1.7 (F)	21.2 ± 1.3 (H)	31.1 ± 2.3 (J)	22.3 ± 0.9
2.4–6.0 μm	55.3 ± 4.5 (A) (B) (D)	68.7 ± 0.7 (E)	64.4 ± 0.3	64.5 ± 2.4	67.8 ± 0.5
6.0–9.6 μm	23.2 ± 1.2 (A) (B) (C) (D)	12.7 ± 2.4 (F)	13.8 ± 1.6 (H) (I)	4.4 ± 0.3 (J)	9.7 ± 0.5
DN (μm)	4.5 ± 0.0 (A) (B) (D)	5.5 ± 0.2 (F) (G)	5.6 ± 0.1 (H) (I)	4.6 ± 0.1 (J)	5.2 ± 0.0
Sedimentation Hu/Ho (%) 20 min/7 months	87/46	91/46	90/46	94/46	88/46
Resuspension: seconds at 48 h and at 7 months	10/13	6/7	6/8	5/6	5/7
pH	5.28 ± 0.02	4.84 ± 0.03	4.73 ± 0.01	4.73 ± 0.01	4.65 ± 0.01
Zeta potential RAM (mm/V per s) × 10 (−10)	244	1.15	1.43	1.61	1.75
Rheology	Apparent viscosity (Pa/s)				
Shear rate (S-1) 10	0.073	0.050	0.0046	0.0034	0.048
150	0.017	0.010	0.0088	0.0086	0.095
500	0.0092	0.0053	0.0050	0.0051	0.0054

Statistical comparisons: * $P < 0.05$ between: 1 vs. 2 = A; 2 vs. 3 = E; 3 vs. 4 = H; 1 vs. 3 = B; 2 vs. 4 = F; 3 vs. 5 = I; 1 vs. 4 = C; 2 vs. 5 = G; 4 vs. 5 = J; 1 vs. 5 = D.

Table 2
Influence of the concentration in pluronic F68® on the weight evolution and on the tumor volume

Experimental group	Weight evolution		Tumor Volume	
	Day 0 Mean ± S.D. (g)	Day 15 Mean ± S.D.	Day 0 Mean ± S.D. (mm^3)	Day 15 Mean ± S.D. (mm^3)
Charcoal 10%	24 ± 0.3	22 ± 1.7	324 ± 278	1082 ± 499
Charcoal 10% + Pluronic F68® 0.1%	23 ± 1.2	21.2 ± 1.8	148 ± 64	694 ± 102*
Charcoal 10% + Pluronic F68® 0.3%	20.4 ± 1.3	17.4 ± 0.6*	138 ± 111	928 ± 129*
Charcoal 10% + Pluronic F68® 0.4%	19.7 ± 0.3	21.2 ± 1.3	330 ± 66	1185 ± 264*
Charcoal 10% + Pluronic F68® 0.5%	20.5 ± 0.4	18.2 ± 0.1*	221 ± 167	1226 ± 206*

Statistical comparison of the intragroup weight and tumoral evolution between day 0 and day 15.
* $P < 0.05$.

concentration of 0.1%, three at a concentration of 0.3%, one at a concentration of 0.4% and two at a concentration of 0.5%. (Table 1). Body weight fell significantly at concentrations of 0.3 and 0.5%, 15 days after the injection. Significant growth in tumor volume was observed 15 days after the injection, regardless of the concentration of Pluronic F68® (Table 2).

On histological sections of the tumors, charcoal particles were found in histiocytes, tumor cells, nodules (most often peritumoral) and necrotic tissue. Diffusion from the site of injection was weaker in tumors injected with the charcoal suspension without additive (Fig. 1). Only rare charcoal particles were found in the lungs and liver in one mouse out of four at a 0.1% concentration, forming granulomas in the lungs (Fig. 2), and in two other mice at other concentrations. Charcoal was found in a vascular embolus (0.4% concentration). After injection of a charcoal suspension containing 0.4% Pluronic F68®, which induced the death of a mouse, charcoal particles were found in the alveolar partions but not in the liver. Kidney vessels were dilated in one mouse at a pluronic concentration of 0.3%. Histology showed granuloma formation in the lungs (0.1%), hypertrophy of kidneys (0.4% concentration) and dilation of hepatic and kidney vessels. No lesions occurred in the organs of mice having received the charcoal suspension without Pluronic F68®. Car-

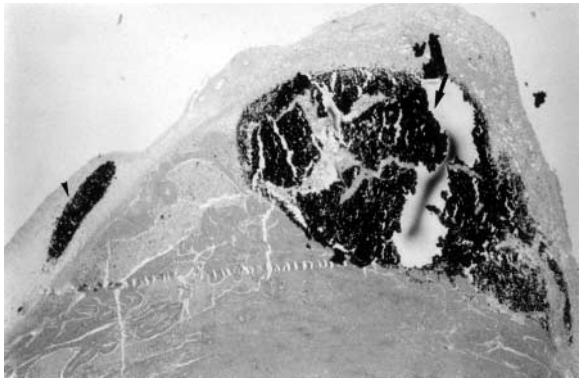

Fig. 1. Tumor after injection of a charcoal suspension containing 0.1% Pluronic F68®. Charcoal forms a beach (→) at the periphery of the tumor nodule and does not spread throughout the tumor. It is engulfed by some histiocytes (⇒) that not diffuse (hematoxylin-eosin-saffron × 250).

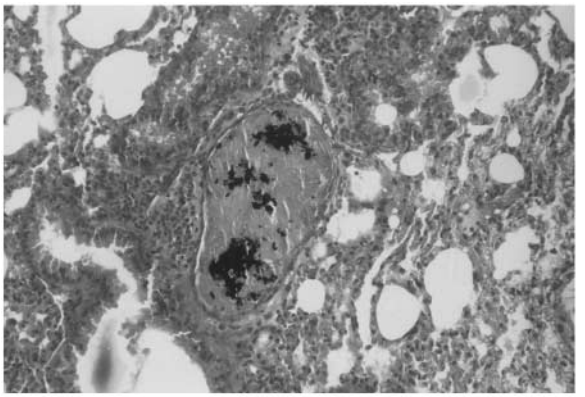

Fig. 2. Ten days after intratumoral injection of a charcoal suspension with 0.1% of Pluronic F68®, charcoal particles are present in some pulmonary vessels. Note that no charcoal particles were found in alveolar macrophages (hematoxylin-eosin-saffron × 200).

diac thrombi were observed, with charcoal particles being found in an auricle. The death of the mouse was due to cardiac arrest following passage of the suspension in the blood circulation.

As the Pluronic F68® concentration in the suspensions increased, charcoal particles diffused more widely in the tumor. In addition, the suspensions became more toxic. These results may be partly explained by the chemicophysical properties of these suspensions. In particular, the average size of the particles fell as the Pluronic F68® concentration increased (0.4 and 0.5%), owing to the increase in the number of particles smaller than 2 μm and leading greater diffusion. The decrease in viscosity when the concentration of Pluronic F68® increased led to greater migration of charcoal particles. The improvement in syringeability had negative effects on diffusion.

Pluronic F68® toxicity could be due to activation of plasma complement C, blockade of the reticuloendothelial system and a modification of polymorphonuclear cell functions (Vercellotti et al., 1982). Pulmonary vessels can also be invaded by granulocytes. In the rat, intravenous administration of Pluronic F68® for several months induces the formation of spumous cells in the lungs, containing phospholipids. The authors of this study showed that it could induce phospholipidosis by an inhibitory action on plasma phospholipase A2 activity and hepatic (Magnusson et al., 1986). This could explain the observed granuloma formation in the lungs at the concentration of 0.1% in this study. Adverse pulmonary reactions have been observed in humans after administration of perflurochemicals emulsified with Pluronic F68®. This additive also dilates pulmonary, hepatic and renal vessels.

Soon after the injection of a suspension containing 0.4% of Pluronic F68®, a thrombus containing charcoal particles was found in an auricle. This could be due to the passage of the charcoal suspension in the blood circulation, but also to a direct effect of Pluronic F68®, which precipitates plasma proteins, activates plasminogen and generates fibrin split products in vitro (Smith et al., 1987).

In conclusion, the addition of Pluronic F68® did not cause marked charcoal diffusion at the injection site or at a distance. Nevertheless, it cannot be included in the formulation because of biologic effects already described in the literature (Lane and Lampkin, 1984; Virmani et al., 1984) and to the toxicity noted in this study.

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