Study of the distribution of magnetite nanoparticles in an experimental model of hepatic metastases.

J.J. Echevarria[1], I. Garcia-Alonso[2], J.A. Larena[1], F. Sanz-Sanchez[1], F. Plazaola[3], M. Insausti[3], N. Etxebarria[3], J. Salado[3], B. Fernandez-Ruanova[1]

1Radiology Department. Galdakao-Usansolo Hospital, Labeaga s/n, 48960 Galdakao, Spain
2Experimental Surgery Laboratory, Medicine Faculty, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain
3Faculty of Science and Technology, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain
fernando.plazaola@ehu.es

The purpose of this work is to assess a magnetic fluid for its affinity for liver metastases at different stages of tumor development in laboratory rats, prior to its potential use in antitumor thermal therapy.

Iron oxide magnetic nanoparticles ranging between 3.8 and 7.1 nm were synthesized using the polyol method. From this synthesis process, magnetic nanoparticles of Fe_3O_4 capped by oleic acid were obtained. The lipid nature of these ligands allows the particles to be suspended in iodized oil Lipiodol, when the mixture is exposed to ultrasonification. A magnetic fluid consisting of suspensions of 2 mg of iron oxide nanoparticles in 0.2 ml of Lipiodol was prepared for transarterial hepatic infusion in each animal.

The magnetic fluid infusion procedure in the selected animals required midline laparotomy to enable the exposure of visceral arterial vessels. The main branches of the celial trunk were clamped, with the exception of the hepatic artery with the aim of driving towards this artery most of the celiac vascular flow. A direct punction of the celiac trunk was carried out using a needle, connected by an elongated catheter to an infusion pump previously filled with the magnetic fluid, and the suspension was slowly and selectively infused into the liver.

The experimental study was carried out using 33 male WAG/RijCrl rats. In order to induce metastases in the liver of laboratory animals, syngenic cells of colon adenocarcinoma, CC-531, were inoculated into the liver of the rats. The splenic reservoir technique was used as a source for the dissemination of tumor cells. A total of 21 rats developed metastases, but six animals died during subsequent surgical procedures. Of the surviving animals, ten were chosen at random to receive the magnetic fluid, via the hepatic artery. The remaining five rats constituted the control group and did not receive the suspension.

Within the first 12 hours after administration of the magnetic fluid, Multi-Detector Computed Tomography (MDCT) and Magnetic Resonance Imaging (MRI) were performed to the animals to check the effectiveness of the infusion procedure. The observation on MDCT of a hyperdense liver due to the presence of Lipiodol in the arterial tree, and the lack of extravasations in the puncture area or the existence of gross intra-arterial contrast media deposits, were considered to be evidence of the correct transarterial administration of the magnetic fluid. MRI studies were carried out on a 1.5 T Siemens Symphony system, using a standard cranial coil and axial Short Time Inversion Recovery (STIR) and gradient-echo (GRE) weighted sequences were performed. The observation on STIR sequences of smooth hyper intense masses into liver was considered as metastatic lesions. The homogeneous decay of signal intensity of liver and metastases on GRE sequences was attributed to the presence of magnetic nanoparticles in the different tissues, and it was considered to be evidence of proper vascular diffusion of the magnetic fluid.

After the imaging studies animals were sacrificed. Livers were extracted, and the number and size of the metastases were determined by visual analysis. Two categories of tumor growth were considered. On the one hand, early stage neoplastic infiltration corresponding to metastases that were smaller than 3 mm, non-overlapping (separated by healthy parenchyma) and with no more than ten visually identifiable lesions. On the other hand, livers that showed extensive neoplastic infiltrations, in an

advanced stage, were characterized by having metastases larger than 3 mm or more than ten visually detectable lesions.

Liver and neoplastic tissue were taken to determine iron concentrations, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analysis was performed with non-parametric tests (Wilcoxon test for related samples). Once significant differences between groups had been demonstrated using the Kruskal-Wallis test, comparisons between possible pairs of groups were performed using the U Mann-Whitney test. The minimum level of significance accepted was $P < 0.05$.

Of the ten animals in which surgical procedures and the magnetic fluid infusion were successfully completed, five showed early stage metastatic development while the other five presented advanced stage metastasis. After subtracting the mean endogenous iron values of the control group, from the iron found in animals that had received the magnetic fluid, the mean concentration attributable to exogenous administration in the early stage group was 172.2 mg/g in tumor tissue and 65.2 mg/g in healthy liver tissues, In these animals tumor tissue accumulated 2.6 times more iron than healthy liver tissue. In contrast, exogenous iron values found for the advanced stage group were 22.7 mg/g and 43.1 mg/g in metastatic and healthy tissue, respectively. In this group, metastases of animals with sever tumor infiltrations, accumulated half as much exogenous iron as healthy liver. Moreover, in the comparative study between concentrations of exogenous iron determined in liver and metastases within groups, significant differences were found in the early stage group, but not in the advanced stage group.

Our model of metastatic adenocarcinoma has revealed important differences in the vascularization of metastatic lesions according the stage of development of the disease. Therefore, we should consider the possibility of there being substantial differences in vascular perfusion in neoplastic lesions of similar type, but in different stages of development. The increase in tumor volume does not necessarily lead to a similar development in its vascularization, rather the increase in tumor mass may lead to the appearance of regions with decreased artery supply.