New antiangiogenic polymer drugs. From synthesis to biological activity.

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Introduction: Inhibition of angiogenesis is one of the main strategies carried out in the treatment of solid tumors. Heparin-dependent growth factors (GF), such as fibroblast growth factor (FGF) or vascular endothelial growth factor (VEGF) are directly involved in this disease being over expressed in the tumoral tissue. The aim of this work is the inhibion of the pro-angiogenic activity of these GF by the action of new heparin-like copolymers synthesized in our laboratory based on a methacrylic derivative of 5-amino-2-naphthalene sulfonic acid (MANSA) and 2-acrylamide-2-methylpropane sulfonic acid (AMPS). The molecular structure-bioactivity relationship of four new families of copolymeric systems is discussed. The physico-chemical characterization of the macromolecules is reported. Also the *in vitro* and *in vivo* antiangiogenic effect of these polymers is demonstrated.

Materials and Methods: Four copolymeric systems were obtained by free-radical copolymerization. AMPS [1] and MANSA [2] were copolymerized with a hydrophilic or a hydrophobic monomer (N-vinylpyrrolidone, VP, or butylacrylate, BA, respectively) in order to prepare macromolecules with different hydrophilic/hydrophobic balance.

Reactivity ratios were determined by *in situ* ^{1}H-NMR in order to estimate the monomer distribution along the polymeric chains. The copolymers conformation was studied using light scattering and zeta potential measurements.

The biological behavior of the new materials was studied *in vitro* using 2D cultures of endothelial cells to determine the inhibitory activity of the polymers on cell proliferation induced by pro-angiogenic factors and 3D cultures on fibrin matrix to determined the inhibition of different steps in the angiogenic process [3].

Finally we studied the *in vivo* activity using 6-week-old female nude mouse. Two silicon tubes filled with Matrigel that contained either PBS (negative control), bFGF (positive control) or bFGF plus the polymers were inserted into a skin pocket in the back of each anesthetized nude mice. Eleven days later, the mice were injected intravenously with FITC–dextran and 10 minutes later, the tubes were removed from the skin pockets and the amount of fluorescence trapped in the implants was measured to evaluate the volume of blood circulating through the newly formed vessels.

Results and Discussion: The reactivity ratios of the AMPS systems (VP-AMPS: r_{VP}=0.12; r_{AMPS}=0.28; BA-AMPS: r_{BA}=3.60; r_{AMPS}=0.28) indicated that VP-AMPS system is azeotropic presenting a moderate

alternating structure whereas BA-AMPS preferably added the most reactive monomer BA to the growing chain.

The reactivity ratios of the different systems (VP-MANSA: r_{VP}=13.40; r_{MANSA}=0.12; BA-MANSA: r_{BA}=14.78; r_{MANSA}=0.26) indicated that both propagating species preferably added the most reactive monomer (VP or BA). Consequently, there was a tendency toward consecutive homopolymerization of the two monomers. At the beginning of the reaction VP or BA-rich copolymers were formed; when these monomers were almost consumed MANSA-rich copolymers were subsequently formed.

This monomer distribution along the copolymeric chains lets the formation of micelles in the case of BA-MANSA and BA-AMPS copolymers with a BA (hydrophobic) core and a MANSA (hydrophilic) corona. The particular self-assembling of these macromolecules, locating the sulfonic groups on the surface of the micelles, made them more accessible to interact with the proangiogenic growth factors.

The anti-angiogenic activity was measured by 2D and 3D *in vitro* assays. Higher activity of the BA-MANSA copolymers was observed, being the micelles more active than linear polymers with similar amount of MANSA in their structure.

Finally the *in vivo* assays also showed a better inhibition in the case of BA-MANSA systems reaching almost a total inhibition.

Conclusions: Anti-angiogenic activity of new copolymers bearing sulfonic groups in their structure (heparin-like synthetic macromolecules) was tested by 2D and 3D *in vitro* and *in vivo* assays, showing good inhibition of different steps of the angiogenesis process. This bioactivity was related to the particular distribution of the monomers along the copolymeric chains and their organization in supramolecular structures.

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References

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