Tailor-Made Biomimetic Random Copolymers for Medical Applications

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SUMMARY: Biospecific copolymers were synthesized by random substitution of a preformed polymer with suitable chemical groups or by random copolymerization of suitable functional monomers. Such polymers contain arrangements of the chemical functions that mimic natural biospecific sites. The probability of occurrence of such arrangements will depend on the average composition of the copolymer. Two examples of such bioactive copolymers are presented. Some *O*-[(*N*-benzylcarbamoyl)methyl]dextrans (DMCB) exhibit an inhibitory effect on the growth of human breast cancer cell lines. Its derivatives, associated or conjugated to sodium phenylacetate (NaPA), were found to have a strong antitumoral activity on malignant human melanoma 1205LU. Preliminary in vivo tests on nude mice are performed. Adhesion of *Staphylococcus aureus* to biospecific random polystyrene derivatives or acrylic terpolymers carrying sulfate and carboxylate groups is hindered in a composition-dependent way. In addition, a correlation between the bacterial adhesion and proliferation has been evidenced. As a result, biospecific random copolymers endowed with both bacteriophobic and bacteriostatic activities were synthesized.

Introduction

Molecular recognition of chemical messengers by living systems is generally based on the formation of a specific complex between the messengers and its receptor. The complex is the result of interactions between complementary chemical groups in the messenger and the receptor. It is now well established that synthetic or modified natural polymers with a statistic distribution of various functional groups along the chain are biomimetic copolymers capable of specific interactions with biological species such procaryotic or eucaryotic cells. The purpose of this paper is to consider examples that exhibit some inhibitory effect on two types of cells. The first is the malignant human melanoma 1205LU, and the second one is *Staphylococcus aureus*.

Antiproliferative and antitumoral activity

Some *O*-[(*N*-benzylcarbamoyl)methyl]dextrans (DMCB) exhibit an inhibitory effect on the growth of human breast cancer cell lines[1)]. The inhibitory effect on the cell growth depends

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on the benzylcarbamoyl grouping content[2]; the hydrophobic nature of the grouping may be significant. The development of solid tumors could be attributed to the actions of growth factors secreted by breast cancer cells in autocrine and paracrine modes. For example, our studies showed that some DMCBs inhibit the growth of breast MCF-7 and MCF-7ras (cell line transfected with the ras oncogen). We demonstrated a blockage of the paracrine effect and receptor binding of transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF)[3]. We also showed that a DMCB sample inhibits specifically the mitogenic effect of both fibroblast growth factors FGF-2 and FGF-4[4]. Moreover, some of these derivatives were found to have a slight antitumoral activity on human melanoma cell line 1205LU. In the same way, sodium phenylacetate (NaPA), a common metabolite of phenylalanine, normally found in human plasma in micromolar concentrations, exhibits an antiproliferative effect on both MCF-7ras cell line[5] and malignant melanoma A375[6]. These findings suggest that NaPA could have an antiproliferative effect given alone or in combination with a dextran derivative termed LS17-DMCB. It was also interesting to examine the effect of the compound obtained after esterification of LS17-DMCB with phenylacetic acid and termed LS17-NaPAC (Fig. 1).

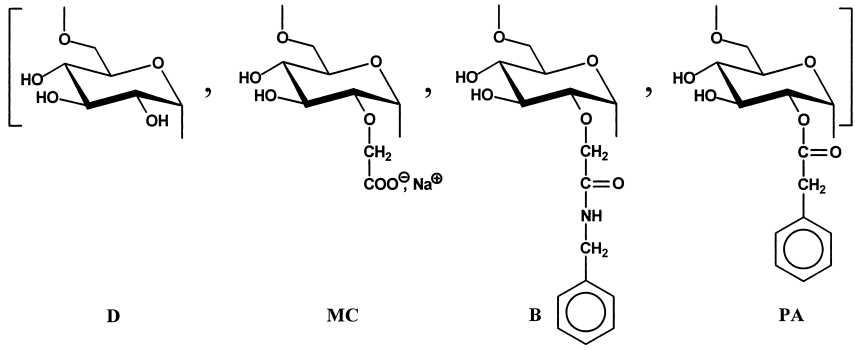

Fig. 1: Structure of LS17-NaPAC units: D native dextran, MC sodium (carboxymethyl)dextran, B [(*N*-benzylcarbamoyl)methyl]dextran, PA (phenylacetyl)dextran.

First, we investigated samples of DMCB having degree of substitution (DS) with (*N*-benzylcarbamoyl)methyl groups from 0.2 to 0.8. These samples induce a slight dose-dependent inhibition of 1205LU cell viability after 72 h exposure. The IC-35 dose (of the concentration causing 35 % inhibition) was around 500 μg/mL (≈ 60 μg/mL NaPA) for the most active derivatives. This is the reason why we have chosen to covalently bind phenylacetic acid to the LS17DMCB with a DS of about 0.3. Several approaches have been

used to obtain the conjugates; they are currently under investigation to clarify their chemical structure. Second, we evaluated the effect on the growth of 1205LU cells in culture of NaPA alone, the combination of NaPA with LS17DMCB and LS17-NaPAC. Figure 2 shows the dose-response viability of 1205LU cells in the presence of free NaPA or a combination of NaPA with dextran derivative LS17DMCB. The combination had a lower IC 50 (around 1.8 mM) than that of the free NaPA (6 mM) and was more efficient in inhibiting the growth of 1205LU cells than were free NaPA or the dextran derivative. In contrast, the conjugate LS17-NaPAC has almost the same IC 50 as free NaPA in these *in vitro* conditions (results not shown).

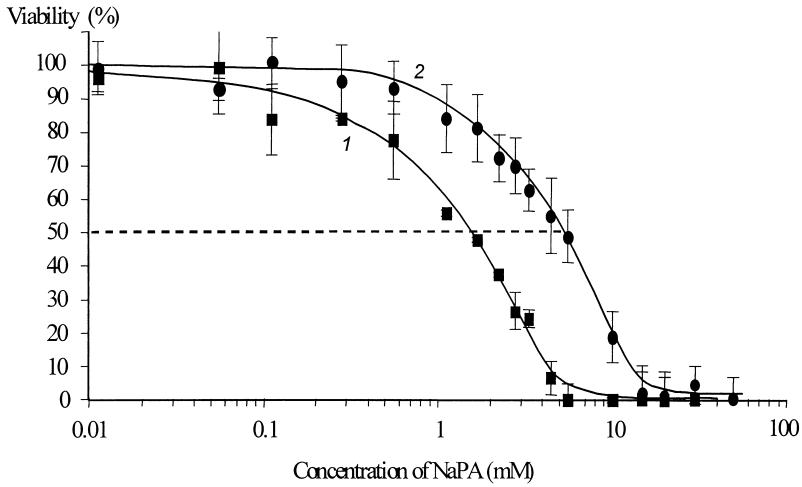

Fig. 2: The viability of 1205LU human melanoma cells in the presence of a combination LS17DMCB and NaPA (*1*) or NaPA alone (*2*). (The error bars, standard deviations, for some points are smaller than their size.)

The incorporation of NaPA into a polysaccharide can protect the antitumoral agent from degradation but also change the internalization of free NaPA in cells. It was proposed that tumors might stimulate production and release of growth factors in and from capillary endothelial cells and also stimulate angiogenesis. The ability of NaPA associated with DMCB to interfere with the secreted growth factors promoting growth of malignant melanoma will be investigated.

To determine the efficacy of NaPA and conjugate LS17-NaPAC *in vivo*, studies were performed including an animal model involving nude mice bearing 1205LU cells transplanted subcutaneously. After 1205LU cell inoculation, 100 % of single subcutaneously palpable tumors appear after four days from inoculation. Then, NaPA (6.9 mg/kg) or LS17-NaPAC (60

mg/kg, equivalent to 6.9 mg/kg of free NaPA) was applied by subcutaneous injection in 0.1 mL of phosphate buffer saline (PBS) twice a week, for five weeks. The control group received 0.1 mL of PBS. The volume of ellipsoid tumor was calculated from the formula $V = (4/3)\pi (R_1)^2 R_2$ where R_1 and R_2 are its semiaxes ($R_1 < R_2$). Statistical comparisons were performed using the Mann-Whitney test; $p = 0.05$ was considered statistically significant. Figure 3 shows the mean of tumor volume in the control group and that in both groups of mice treated either with NaPA or LS17-NaPAC versus the time of treatment. It appeared that in the conjugate LS17-NaPAC treatment, the volume of the tumors is reduced approximately 2.5 times after five weeks ($p < 0.001$).

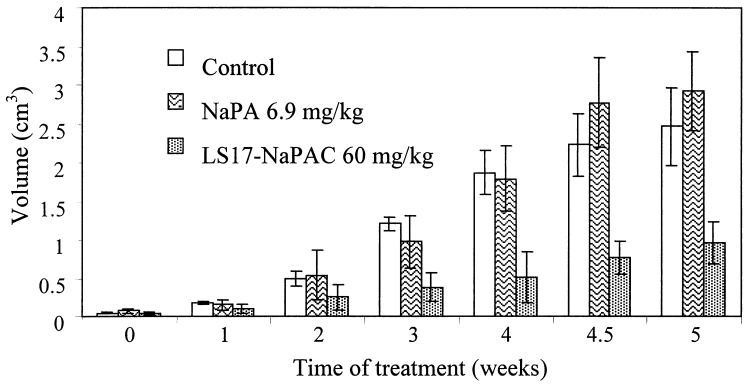

Fig. 3: Mean of tumor volume versus the time of treatment. After four days of treatment, the tumors are significantly smaller than for the control group.

Surprisingly, the incidence of tumor development was not significant after the free NaPA treatment. The dose of free NaPA is probably too low to exhibit any effect. The animal data are consistent with the fact that the dextran derivative protects NaPA without producing significant toxicity. It is important to conclude that the the polysaccharide part of the polymer conjugate enhanced the ability of NaPA to inhibit the growth of melanoma tumors. There is considerable interest in the use of non-toxic conjugates in cancer chemotherapy. Further assays are now under investigation to clarify the mechanism of the ability of NaPA conjugated or associated to dextran derivatives to inhibit the growth of human melanoma cancer.

Modulation of procaryotic and eucaryotic cell adhesion and proliferation

Potential uses of medical devices and implants such as catheters, sutures, vascular prostheses, joint prostheses or contact lenses have significantly increased over recent years. One of the major complications related to the uses of medical devices is bacterial infection. Infection

involving artificial organs, synthetic vessels, joint replacements, or internal fixation devices, usually require re-operation, and may result in amputation, osteomyelitis, or death. Both the presence of implanted materials and the growing proportion of multiple antibiotic-resistant bacterial strains complicate the therapy of such infections, which are persistent and difficult to cure without implant removal and replacement. Clinical studies indicate that a few species seem to predominate in biomaterial infections. *Staphylococcus aureus* and *S. epidermidis*, most frequently isolated from infected biomaterial surfaces, are the primary cause of infection of implanted polymeric biomaterials such as artificial hearts, vascular grafts, catheters and shunts. *S. aureus* is also the major pathogen isolated from metal-bone and joint infections.

A reason for frequent isolation of *S. aureus* from device-associated infections is the ability of this species to adhere to biomaterials by recognizing specific host proteins deposited on the material. Indeed, bacterial adhesion through specific adhesion to a variety of plasma proteins is the critical step of colonization and infection on biomaterials. Several *in vitro* studies have shown that *S. aureus* attachment to polymeric surfaces is strongly promoted by fibronectin (Fn), a plasma protein that rapidly coats synthetic materials after implantation[7]. As microbial adhesion is the initial and fundamental mechanism in the development of infection, it is therefore interesting to design materials that could directly inhibit staphylococcal adhesion mediated by Fn. For that purpose, advantage was taken of the fact that heparin-like polystyrene (PS) derivatives can modulate Fn conformation depending on their chemical composition, and therefore modulate the proliferation of fibroblastic and endothelial cells. Moreover, inhibition of staphylococcal adhesion by heparin and soluble random heparin-like dextran derivatives has been observed on Fn-coated poly(methyl methacrylate) (PMMA)[8].

On the basis of these findings, it was checked whether insoluble random heparin-like copolymers could be endowed with bacteriophobic and bacteriostatic properties, i.e. whether they would be able to inhibit bacterial adhesion and thus to inhibit bacterial proliferation. Therefore, biospecific random PS derivatives bearing carboxylate and sulfonate groups of various chemical compositions were synthesized and tested for their ability to modulate the adhesion of *S. aureus.* Some of them showed bacteriophobic properties[9-11]. However, as the practical use of PS derivatives in the biomedical field is questionable because of their poor mechanical properties, two series of random copolymers have been synthesized by copolymerization, namely vinyl chloride (VC)-based random copolymers and acrylic-based copolymers[12]. As PVC is the most widely used polymeric biomaterial for the manufacture of catheters, CEC tubing, blood bags, etc, the synthesis of VC-based random copolymers has been designed as an essential goal. Acrylic-based random copolymers have been designed

with regard to their use as biomedical devices. It was therefore interesting to check whether bacteriophobic effects on PS and VC-based copolymers could be extended to acrylic random copolymers[9-11]. The synthesis of the three classes of biospecific random copolymers was accomplished.

Indeed, PS bearing sulfonate (PS-SO_3^-) or sulfonate and -$SO_2NHCH(COO^-)CH_2COO^-$ groups (PS-SO_2Asp) was synthesized by random substitution, whereas VC- and acrylic-based copolymers were prepared by copolymerization with monomers carrying functional groups. It was shown that the synthesis was reliable and reproducible. These materials were characterized by elemental analysis and IR spectra to determine the content of sulfonate groups. Concerning acrylic copolymers, terpolymerization was performed with methyl methacrylate, methacrylic or acrylic acid and styrenesulfonate as comonomers bringing carboxylate and sulfonate groups. These terpolymers were characterized by NMR allowing the determination of proportions of chemical functional groups.

The specific area available for protein adsorption was assessed by radiolabelled bovine serum albumin adsorption, as it was shown that albumin adsorption on polymers is non-specific and of low affinity. Competitive adsorption studies of albumin and Fn showed that the affinity of the latter for all the random copolymers was high, whatever their composition was. For PS derivatives, it was shown that high quantities of Fn have displaced adsorbed albumin. As the molar ratios of Fn to albumin at saturation are high, we suggest that Fn adsorbs on PS derivatives in a monomolecular layer, and that the differences observed within the PS derivatives could be explained by variations in Fn conformation when adsorbed on different PS derivatives. The results are in agreement with previous findings on modulation of Fn conformation by PS derivatives[13].

With VC-based copolymers, the quantity of absorbed Fn was markedly lower than on PS derivatives. The copolymers containing sulfonate groups exhibit higher abilities to bind Fn than polymers containing either no functional group or only carboxylate groups. The modulation of bacterial adhesion to various synthesized biospecific random copolymers was studied by using a protocol of staphylococcal adhesion on polymers adapted from Vaudaux *et al.*[14]. Adhesion of two strains of *S. aureus* (Cowan I and 8325-4) was modulated by PS derivatives depending on their content in aspartic acid. It was found that bacterial adhesion was minimal on PS that contains only sulfonate groups and that the adhesion increases proportionally with the content of carboxylate groups. This modulation of bacterial adhesion was equivalent when standardized to the quantity of adsorbed Fn on the various PS

derivatives. Thus, the inhibition of staphylococcal adhesion is independent of the quantity of adsorbed Fn.

These findings establish that PS-based random copolymers are able to inhibit bacterial adhesion to Fn-coated samples as a result of differences in conformation of the Fn adsorbed on these copolymers of different compositions. Interestingly, the derivatives to which the bacterial adhesion is markedly reduced, are also able to inhibit the proliferation of adsorbed bacteria, i.e. to exhibit a bacteriostatic effect. Moreover, the same materials, PS derivatives or acrylic random terpolymers, are able to modulate proliferation of eucaryotic cells such as fibroblastic Mac Coy and endothelial EAhy 926 cell lines as well as bacterial adhesion.

The acrylic-based terpolymers were synthesized to get biomaterials endowed with mechanical properties suitable for practical use in biomedical devices. Figure 4 illustrates the above properties of the acrylic random terpolymers. The change in the conformation of the adsorbed Fn does not have the same effect on fibroblastic cells and bacteria.

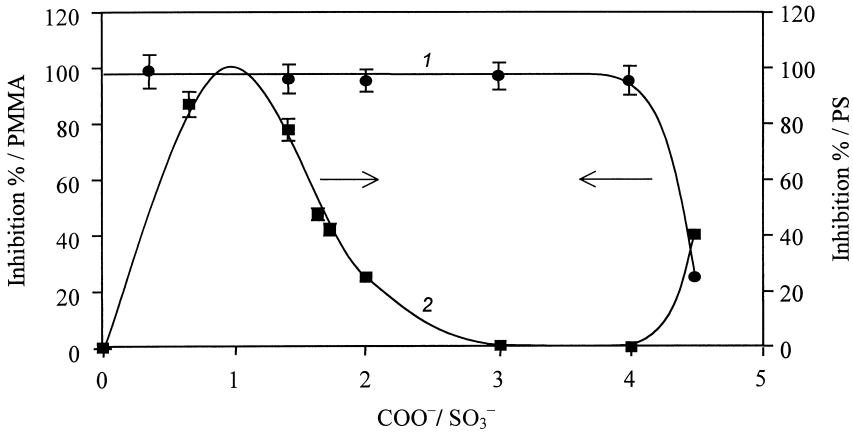

Fig. 4: Inhibition of bacterial adhesion (*1*) and fibroblastic proliferation (*2*) on acrylic terpolymers relative to PMMA (*1*) and PS (*2*) in dependence on the COO^-/SO_3^- molar ratio.

As shown in Fig. 4, in relation with the molar ratio carboxylate/sulfonate, three major domains can be distinguished, corresponding to different medical applications:

- $0.6 < COO^-/SO_3^- < 1.4$ – bacteriophobic and cellular antiproliferative properties,
- $3 < COO^-/SO_3^- < 4$ – bacteriophobic and normal cell proliferation properties,
- $COO^-/SO_3^- > 4$ – normal bacterial adhesion and cell proliferation compared to standard.

Concerning VC-based random copolymers, a maximum inhibition of bacteria (*S. aureus* Cowan I) was observed between 0.01 and 0.14 mequiv SO_3^-/g, whatever the carboxylate

content was. The inhibition of bacterial adhesion was completely independent of the adsorbed amount of Fn. It was shown that bacterial proliferation was markedly inhibited by VC-based random copolymers containing sulfonate groups. Moreover, anticoagulant properties of these copolymers have been tested, allowing identification of the materials that are endowed with anticoagulant, bacteriophobic and bacteriostatic activities.

Conclusion

It has been shown that several types of random copolymers modulate proliferation of some eucaryotic and procaryotic cells. On the basis of these results, random copolymers as antitumoral agents or materials exhibiting bacteriophobic and bacteriostatic properties were synthesized. Our results open numerous possibilities for biomedical applications in the field of oncology or vascular devices. We are currently attempting to establish the mechanism of antitumoral action of dextran derivatives. Concerning VC-based random copolymers, we are interested in evaluation of the implication of bacterial adhesion.

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