Polymer Bulletin 39, 53–58 (1997)

Polymer Bulletin

Hydrolysis of poly(ester-ether-ester) block copolymers in the presence of endothelial cells: in vitro modulation of endothelin release

P. Cerrai[1], C. Cristallini[2], M. G. Del Chicca[3], G. D. Guerra[1,*], S. Maltinti[2], R. Sbarbati-Del Guerra[1,3], M. Tricoli[1]

[1] C.N.R., Centro di Studi sui Materiali Macromolecolari Polifasici e Biocompatibili, Via Diotisalvi 2, I-56126 Pisa, Italy
[2] Dipartimento di Ingegneria Chimica, Chimica Industriale e Scienza dei Materiali, Università di Pisa, Via Diotisalvi 2, I-56126 Pisa, Italy
[3] C.N.R., Istituto di Fisiologia Clinica, Via P. Savi 8, I-56126 Pisa, Italy

Received: 13 January 1997/Revised version: 2 May 1997/Accepted: 3 May 1997

Summary

In previous papers, we studied the hydrolytic degradation of six poly(ester-ether-ester) block copolymers, i.e. three poly(ε-caprolactone)-*block*-poly(oxyethylene)-*block*-poly(ε-caprolactone) copolymers and three poly(L-lactide)-*block*-poly(oxyethylene)-*block*-poly(L-lactide) copolymers. Their degradation products, 6-hydroxyhexanoic acid and L-lactic acid, have now been found to modulate endothelin release by human umbilical vein endothelial cells, with no significant alteration of the vasoconstrictor-vasodilator balance previously determined. The influence of the same degradation products on the cell proliferation has also been determined and discussed.

Introduction

Endothelin (ET1) is the most potent vasoconstrictor synthesized and released by endothelial cells (1). It also has growth promoting properties for vascular smooth muscle cells and plays a fundamental role in the homeostasis of the vessel wall in health and in disease. Aim of this research was to investigate the influence on ET1 release of the presence of biodegradable materials, *in vitro*.

Poly(ester-ether-ester) block copolymers are biodegradable materials, obtained by thermally copolymerizing, without any catalyst, cyclic ester monomers with a preformed poly(ethylene glycol) (PEG). In this paper, we have tested three poly(ε-caprolactone)-*block*-poly(oxyethylene)-*block*-poly(ε-caprolactone) copolymers (PCL-POE-PCL) and three poly(L-lactide)-*block*-poly(oxyethylene)-*block*-poly(L-lactide) copolymers (PLA-POE-PLA), obtained by reacting PEG with ε-caprolactone and L-lactide, respectively.

The synthesis, the physicochemical characterization and the biocompatibility of such copolymers have been previously reported (2, 3). Concerning the copolymer hydrolytic degradation products, 6-hydroxyhexanoic acid (HHA) and L-lactic acid (LLA), their releasing mechanism has been described elsewhere (4, 5). In a recent paper (4) the biodegradation characteristics of the two series of copolymers PCL-POE-PCL and PLA-POE-PLA have been assessed *in vitro* in the presence of both murine fibroblasts and

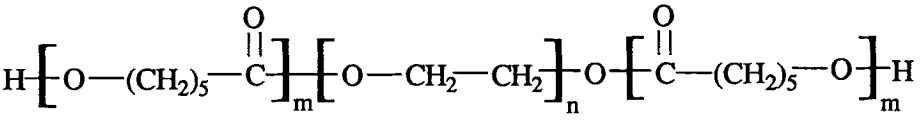
* Corresponding author

human endothelial cell populations. Furthermore, two aspects of the endothelial cell metabolism, i.e. prostacyclin (PGI_2) and angiotensin II (AII) releases, in the presence of both series of biomaterials and of their hydrolytic breakdown products, have been studied (5).

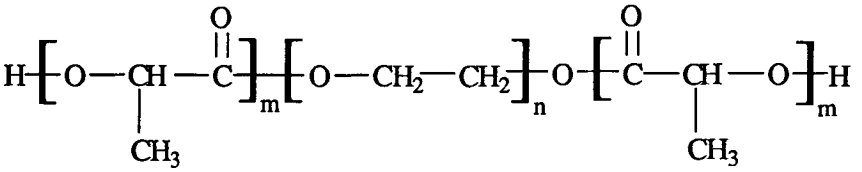
Experimental

Copolymer synthesis

The PCL-POE-PCL and PLA-POE-PLA three-block copolymers were synthesized by reacting PEG (molecular mass = 35000) with ε-caprolactone at 185°C and with L-lactide at 140°C, in bulk, without catalyst, under vacuum, using reagents purified as already reported (2-4). The structural formulas of the copolymers are shown in Scheme 1; their composition and number average molecular weights, calculated from ^1H NMR spectra, are reported in Table 1.



PCL-POE-PCL



PLA-POE-PLA

Scheme 1. Structural formulas of PCL-POE-PCL and PLA-POE-PLA.

Endothelin assay

In a preceding paper, we reported the *in vitro* biodegradation assay of the copolymers, in the presence of human umbilical vein endothelial cell (HUVEC) populations (4). The degradation products of PCL-POE-PCL and PLA-POE-PLA copolymers, i.e. respectively HHA and LLA, were quantified in the HUVEC supernatant by HPLC (4). ET1 was assayed during 3, 5, and 10 days after HUVEC confluence by a commercial Radio Immuno Assay (RIA) kit (Biomedica Gruppe, Biomedica GmbH, Austria). The initial number of the cells, seeded in six-well culture plates, was about 4.85×10^4 per well.

Table 1. PCL-POE-PCL and PLA-POE-PLA three-block copolymers. OE: molar percentage of repetitive oxyethylene units; CL: molar percentage of repetitive oxycaproyl units; LA: molar percentage of repetitive lactyl units; n: number of OE units in polyether blocks; m: number of CL or LA units in each polyester block (see Scheme 1).

copolymer	OE	CL or L A	n	m	hydrophilicity	10^{-4} M_n
CL24	86	14	790	65	high	4.99
CL28	52	48	790	360	medium	11.75
CL27	34	66	790	740	low	20.37
LA4	81	19	790	90	high	4.54
LA5	42	58	790	640	medium	10.78
LA3	27	73	790	1350	low	18.93

Results and discussion

Each series of copolymers (see Table 1 and Scheme 1) comprises one with high oxyethylene (OE) content and high hydrophilicity; one with medium OE content and medium hydrophilicity; one with low OE content and low hydrophilicity. Our previous studies (4) showed that the hydrolytic degradation rate increases with increasing the hydrophilicity of the material.

ET1 release by confluent HUVEC in the presence of PLA-POE-PLA copolymers and of their hydrolytic breakdown products showed at day 10 after cell confluence no significant differences (Fisher's PLSD test, significant level 5%) compared with the negative control wells where no copolymers were present (Fig. 1). In the presence of the least hydrophilic copolymers (LA3, LA5) the trend was towards lower ET1 levels.

The LA5 copolymer, which is the one with intermediate hydrophilicity, inhibits significantly ET1 release by HUVEC at days 3 and 5, compared with the negative control. At day 10 the same trend persists, even if not significant.

In a previous work (5) we reported that LA5 has a positive effect toward the stimulation of the production of PGI_2, a powerful vasodilator, whose action might be antagonized by ET1. The similarity between PGI_2 and the tritium-labelled 6-keto-PGF1α, which reacts with ET1 in the RIA test, can lower the sensitivity of the test itself.

The results in the presence of PCL-POE-PCL copolymers showed at day 3, 5, 10 after HUVEC confluence no statistical differences between all the copolymers under study and the negative control wells (Fig. 2).

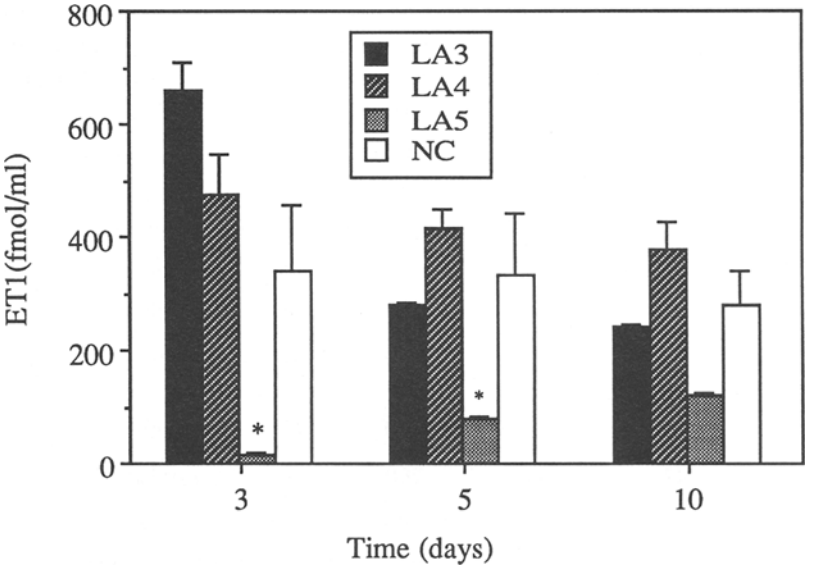


Fig. 1. ET1 release by HUVEC in the presence of PLA-POE-PLA copolymers. The data are the mean ± SE of four determinations. Fisher's PLSD test; significance level 5%, referred to NC: $*p < 0.05$.

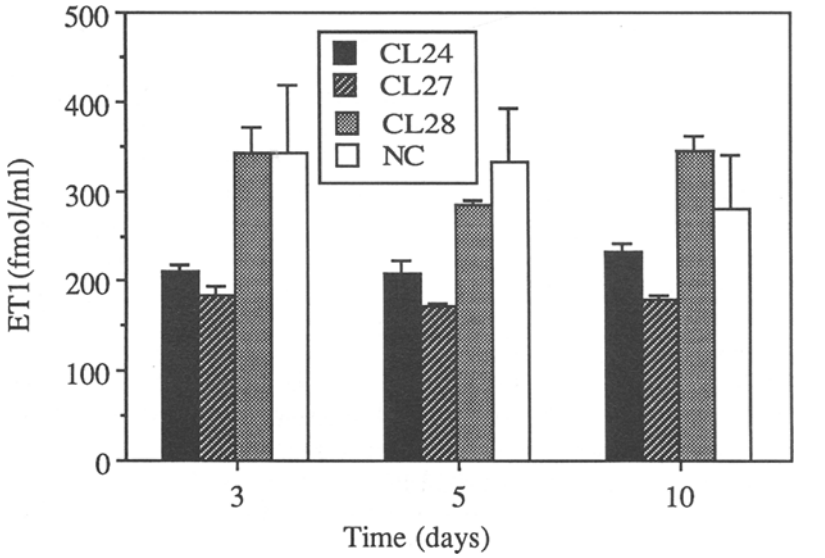


Fig. 2. ET1 release by HUVEC in the presence of PCL-POE-PCL copolymers. The data are the mean ± SE of four determinations.

Table 2 shows the cell number found in each well, as well as the values of ET1 release per cell number, at day 10 after cell confluence. The maximum cell proliferation occurs in the presence of the most hydrophilic materials, CL24 and LA4, as well as in the presence of the less hydrophilic ones, CL27 and LA3. The minimum cell proliferation occurs in the presence of the medium hydrophilic materials, CL28 and LA5.

Table 2. Cell number found in each well (CN) and ET1 release per cell number (ET1), at day 10 after cell confluence. NC: negative control (no copolymer in the wells). Fisher's PLSD test; significance level 5%, referred to NC: $*p < 0.05$; $**p < 0.01$.

copolymer	10^{-4} CN	ET1 (fmol/l)
CL24	12.60	*1.85 ± 0.06
CL28	6.25	5.51 ± 0.28
CL27	12.25	**1.13 ± 0.04
LA4	12.00	3.96 ± 1.27
LA5	5.50	*1.65 ± 0.06
LA3	12.30	*1.96 ± 0.04
NC	5.00	5.58 ± 1.22

Such a behaviour may be explained by the different compositions (see Table 1) and degradation rates (4) of the copolymers. In the case of CL24 and LA4, the copolymers undergo a rapid hydrolysis of their polyester moieties, releasing quite few HHA and LLA molecules, because of the little number of 6-oxycaproyl (CL) and lactyl (LA) units in the copolymer chains. In the case of CL27 and LA3, containing the greatest number of CL and LA units in the chains, the copolymers undergo a slow release of many HHA and LLA molecules. In both cases, the cells can metabolize easily the hydrolysis products, and so proliferate. In the case of CL28 and LA5, there is a quite fast release of relatively numerous HHA and LLA molecules. Probably, in such conditions, the cells may have a lesser opportunity of metabolizing the copolymer hydrolysis products, so that the cell proliferation is not very different from that observed in the NC. As regarding the values of ET1 release per cell number, although four copolymers show significant differences in comparison with the NC, we think that they do not modify substantially the vasoconstrictor-vasodilator balance found in our previous work by the investigation of AII and PGI_2 release (5), owing the very small quantities of the ET1 released by the HUVEC cells in the presence of all copolymers. In addition, the ET1 released by HUVEC in the presence of the copolymers is never more than that released in the NC; this fact indicates that the presence of vascular grafts containing PCL-POE-PCL or PLA-POE-PLA

copolymers should not influence negatively the blood flow because of the releasing of such a powerful vasoconstrictor by the endothelial cells.

Conclusions

These specific biocompatibility tests were an attempt to closely simulate an *in vivo* situation. We can conclude that both series of copolymers demonstrated to not enhance ET1 release by human endothelial cells. We have already demonstrated that neither AII nor PGI_2 release by HUVEC is significantly changed by the same copolymers (5). Our *in vitro* studies reasonably suggest that the balance vasoconstrictor-vasodilator, which is fundamental in healthy vessels, would not be altered by the presence of vascular grafts, containing poly(ester-ether-ester) block copolymers as biodegradable materials.

As concerning the cell proliferation on the medium hydrophilic copolymers, the interpretation given above does not agree completely with the copolymer decomposition in the presence of HUVEC cells (5), and must be checked by more appropriate tests.

References

1 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T (1988) Nature 332: 411
2 Cerrai P, Guerra GD, Lelli L, Tricoli M, Sbarbati Del Guerra R, Cascone MG, Giusti P (1994) J Mater Sci Mater Med 5: 33
3 Cerrai P, Tricoli M, Lelli L, Guerra GD, Sbarbati Del Guerra R, Cascone MG, Giusti P (1994) J Mater Sci Mater Med 5: 308
4 Sbarbati Del Guerra R, Cristallini C, Rizzi N, Barsacchi R, Guerra GD, Tricoli M, Cerrai P (1994) J Mater Sci Mater Med 5: 891
5 Sbarbati Del Guerra R, Gazzetti N, Lazzerini G, Cerrai P, Guerra GD, Tricoli M, Cristallini C (1995) J Mater Sci Mater Med 6: 824