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Local photodynamic therapy with Zn(II)-phthalocyanine in an experimental model of intimal hyperplasia

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Abstract

Photodynamic therapy (PDT) appears to be a novel promising modality to prevent intimal hyperplasia (IH) and restenosis after angioplasty. Local PDT, that consists of local delivery of photosensitizing agents followed by intraluminal local irradiation, represents a recent advancement. This methodology requires optimization in order to achieve the best prompt outcome especially in terms of pharmacokinetics of the photosensitizing agent. We studied the pharmacokinetic properties by using the photosensitizing agent Zn(II)-phthalocyanine (ZnPc), locally released by a channeled balloon. The efficacy of local PDT in reducing IH was evaluated in an experimental rabbit model of arterial injury. The maximum accumulation of ZnPc was found at 30 min: the injured portion of the artery gave a ZnPc recovery of 1.18 μmol/mg, as compared with undetectable amounts of ZnPc in the non injured arteries; within 90 min after the local delivery, clearance of the agent was almost complete. Local PDT produced an effective reduction of IH in our vascular injury model: at 7, 14, 21 and 28 days IH and intima/media ratio (IMR) was significantly reduced as compared with balloon injured arteries. The local delivery of ZnPc showed favourable pharmacokinetic properties, that allow the performance of PDT immediately after the vascular injury. Local PDT performed in these conditions represents a promising approach to prevent IH after balloon injury. Further studies are needed to better clarify the biological response of the injured arterial wall to local PDT.

Keywords: Local delivery Zn(II)-phthalocyanine; Reduction of intimal hyperplasia

1. Introduction

Percutaneous transluminal angioplasty (PTA) represents the most widely used non-surgical approach for the recanalization of atherosclerotic vascular obstruction. However, the long-term success of this procedure is limited by the occurrence of restenosis in about 30–50% of the cases. One characteristic feature of restenosis is represented by the proliferation of vascular smooth muscle cells (SMC) and their migration from the media to the intima, thereby originating intimal hyperplasia (IH). Although the incidence, timing, clinical and anatomic correlations of restenosis have been studied in depth, there is no effective treatment to prevent restenosis and therefore to overcome this huge clinical and economical problem [1–5].

Photodynamic therapy (PDT) involves the local activation of a systemically administered photosensitizing drug by light, whose wavelength matches the absorption spectrum of the sensitizer. In the presence of tissue oxygen, PDT causes the generation of reactive oxygen species, such as hydroxyl radicals, superoxide anion and singlet oxygen, which exert their cytotoxic effects on cellular organelles and membranes resulting in cell death [6,7]. Encouraging results on the efficacy of PDT in reducing experimental IH have been recently reported [8–12].

The photosensitizers used to prevent restenosis in animal models often have been delivered intravenously [8–12]. Local delivery of the photosensitizing dye seems to be more efficacious, allowing the administration of higher and more precisely defined doses with less risk of systemic toxicity. Thus, a local device balloon catheter, which is

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used during or immediately following the procedure, might represent an important improvement in the PDT of restenosis. In order to optimize the efficiency of this approach, it is essential to define the pharmacokinetic properties of the photosensitizing agent in a suitable animal model. In this study, the local delivery of Zn(II)-phthalocyanine (ZnPc) was investigated, and the efficacy of the local PDT was evaluated in an experimental rabbit model in which by arterial injury was performed.

2. Materials and methods

2.1. Induction of arterial injury

Fifty-two New Zealand white male rabbits (body weight 3.5–4 kg) have been used. Throughout this study the rabbits were kept on a normal diet in standard cages with free access to tap water. The animal protocols used in this study were approved by the institutional animal care of Department of Biology, University of Padua.

All the animals were pre-anesthetized by intramuscular injection of ketamine (25 mg/kg) and a mixture of atropine (40 μg/kg) and chlorpromazine (15 mg/kg). The animals were anesthetized with ketamine (15 mg/kg, i.v.) and diazepam (5 mg/kg, i.v.).

The superficial femoral artery was surgically exposed by blunt dissection and arteriotomy was performed for vascular access. A 2 F (4 mm diameter) Fogarty arterial embolectomy catheter (Baxter Health Care, Edwards Div., Irvine, CA) was introduced through the arterial incision.

Before inducing the intra-arterial lesion of the iliac artery, all the animals received intravenous heparin in a bolus of 100 U/kg. Arterial injury was obtained by inflating the balloon through the introduction of distilled water (70 μl) into the catheter, and by pulling back the balloon against resistance by continuous inflation and deflation. This procedure was repeated four times, rotating the balloon of 90° every time in order to obtain a uniformly distributed lesion along the circumference of the artery. This procedure allowed us to achieve a standardized vascular injury for an extension of 4 cm from the iliac bifurcation. Markers on the catheters and careful documentation of distances into the arteries by a caliper allowed a precise positioning of the subsequently used channeled balloon catheter for local delivery and the light emitting fiber, without fluoroscopical monitor.

This type of lesion was induced in: (i) 16 animals, which represent the control group, sacrificed at 7 days (four), 14 days (four), 21 days (four), and 28 days (four); (ii) 12 animals considered for pharmacokinetic studies (groups of four animals sacrificed at 30, 60, and 90 min); (iii) six animals treated with locally delivered ZnPc (three) or with local exposure to light (three) only; (iv) 18 animals treated with PDT after arterial injury and sacrificed at 7 days (four), at 14 days (four), 21 days (five), and 28 days (five).

2.2. Local delivery of ZnPc

Small unilamellar vesicles of POPC (CGP 31 586) and OOPS (CGP 31 524 A) in a weight ratio of 9:1 containing intimately mixed ZnPc were supplied in a freeze-dried state by Ciba-Geigy and rehydrated by adding a suitable volume of water immediately before use. The photosensitizer:lipid molar ratio in the liposomes was 1:100, while the ZnPc concentration was usually 20 μg/ml.

Previous studies from our laboratory showed that ZnPc can be incorporated in a stable form into the phospholipid bilayer of these liposomes and, once injected into the bloodstream, is released to lipoproteins. This is important for enhancing the selectivity of drug delivery to the injured arterial wall [13].

A local delivery device balloon catheter was chosen to infuse the ZnPc through adequate pores under a sufficient pressure. The balloon should yield a different pressure for balloon inflation (3 atm) and drug infusion (2 atm), should not permit systemic dispersion and could be used as an angioplasty catheter. The channeled balloon (Boston Sci., Natick, MA, USA), first described by Hong [14], fulfils these requirements. It is an over-the-wire, triple lumen catheter with a channeled balloon near the distal tip. One lumen is used for the inflation of the balloon, one lumen permits the infusion of the solutions, including ZnPc, and the third lumen permits the use of guide wire to facilitate advancement of the catheter. The balloon design consists of a balloon with 18 channels running lengthwise on the outside of the balloon. The inner balloon provides an inflatable segment of known diameter and length at the recommended inflation pressure. The outer balloon has holes located circumferentially along the mid section of the balloon for the controlled selective infusion of the solutions. This catheter is designed to obtain local drug delivery with minimal dispersion and no adjunctive arterial injury.

A 3 F channel balloon infusion catheter was positioned in the injured arterial segment; then the balloon was inflated at 3 atm and the layer of perforated tubes was independently perfused by using a separate pressure of 2 atm through the supplementary port named ‘infusion’. ZnPc was delivered from the aqueous suspension of the liposomes having a phthalocyanine concentration of 10 μg/ml; typically 2 ml containing the ZnPc suspension were delivered within a minute, hence the total amount of ZnPc delivered was 20 μg. The time required to deliver the sensitizer was less than 1 min. Thus, blood occlusion for this short span of time did not induce ischemic problems. The rabbits were sacrificed at predetermined time intervals (groups of three animals at 30, 60 and 90 min after the ZnPc delivery).

The photosensitizer concentration was measured by

spectrophotofluorimetric analysis after chemical extraction from serum or tissue homogenates [15]. The ZnPc levels were assessed in the injured arterial segment, and in blood, control non-injured arterial segments, liver, spleen, lung, kidney, and skin to evaluate any systemic diffusion of the photosensitizer.

We also evaluated whether the local delivery procedure with the channeled balloon could induce an additional damage to the arterial wall, thus promoting a more relevant IH. To this purpose, we evaluated the degree of IH at 30 days in two groups of rabbits: (i) four animals which were subjected to the standardized arterial injury with the Fogarty catheter (controls), as previously described; (ii) three animals which, after the arterial injury, received ZnPc (20 μg) locally delivered with the channeled balloon.

2.3. Local PDT

In a typical experiment, 20 μg of ZnPc were delivered, under low ambient light, with the channeled balloon from 2 ml of the liposomal suspension. We treated the central portion of the injured segments for 4 cm. To this purpose, the delivery procedure was repeated twice (1 ml each), to cover the entire injured arterial segment (4 cm) considered. Immediately after the local delivery of ZnPc, the injured iliac artery was irradiated intraluminally, by positioning a radial emitting fiber where the photosensitizer was delivered using the distance marker. Light from a quartz halogen lamp (Teclas, Lugano, Switzerland) was locally applied using a radial emitting fiber (Medlight, Ecublens, Switzerland). The lamp was equipped with bandpass optical filters to isolate the 600–700 nm wavelength range. The lamp was operated at a fluence rate of 180 mW/cm^2 (measured at the end of the fiber) for a total delivered fluence of 300 J/cm^2. The emitting fiber (1 m length), used for intraluminal PDT, produces a radial light pattern which is homogeneous all along the diffuser tip (2 cm length).

We also evaluated whether ZnPc locally delivered alone, or endoluminal irradiation alone might influence the development of IH. For this purpose, three animals, which received 20 μg of ZnPc, and three animals, which were exposed to endoluminal irradiation, were sacrificed at 30 days after treatment.

After the procedure, the arteriotomy was tied off with a 2.0 silk suture and the subcutis was closed with a 3.0 silk suture. Antibiotics were given immediately after surgery. The animals that received PDT were sacrificed at 7 days (four), 14 days (four), 21 days (five) and 30 days (five) after the procedure by giving an overdose of pentobarbital. Harvesting of normal and injured iliac arteries was done after flushing with saline, and in situ fixation was performed under a pressure of 100±10 mmHg for 30 min with a 4% solution of paraformaldehyde. Serial sections of each arterial segment were assessed by light microscopy.

2.4. Histology

The arterial sections, after the fixation, were progressively dehydrated in alcohol, and embedded in paraffin. Thin sections from uninjured and injured arterial tissue (~4 μm) were stained with haematoxylin and eosin and Van Gieson. The specimens were analysed morphometrically using a computerized image analysis system (IMAGE-PRO plus Windows 4.1, Microsoft). The internal elastic lamina (IEL), the external elastic lamina (EEL) and the lumen (L) were delineated manually and the areas were automatically measured. The IH was defined as the ratio of (IEL−L)/IEL. The IMR was defined as as the ratio of (IEL−L)/(EEL−IEL).

2.5. Statistical analysis

All the results are expressed as means±standard deviation. Statistical analysis was performed with a two-tailed Student *t*-test for comparison of morphometric differences between control and PDT-treated arteries and *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Pharmacokinetic properties

The recovery of locally delivered liposomal ZnPc from the injured arterial segment, as well as from control untreated arterial districts at predetermined time intervals, is shown in Table 1. As one would expect for a local administration, the largest recovery of the phthalocyanine from the injured arterial area, i.e., 1.18±0.17 μmol/mg of tissue, was obtained at the shortest post-deposition time analysed by us, i.e., 30 min; the amount extracted was about 30% of the totally deposited amount of ZnPc after 60 min. The ZnPc underwent a fairly rapid clearance from the injured artery, since after 90 min less than 10% of the

Table 1
Recovery (average±standard deviation) from selected tissues (expressed as μmol/mg) and from plasma (expressed as μmol/ml) of ZnPc at various time intervals after local delivery of 20 μg phthalocyanine in 2 ml of liposomal suspension[a]

Tissue	Time after local delivery of ZnPC		
	30 min	60 min	90 min
Iliac artery	1.18±0.17	0.81±0.13	0.07±0.02
Control artery	ND	ND	ND
Liver	0.20±0.02	0.15±0.02	0.15±0.05
Spleen	0.35±0.01	0.40±0.01	0.27±0.02
Kidney	0.08±0.00	0.08±0.01	0.11±0.01
Skin	0.04±0.01	0.03±0.01	0.04±0.01
Plasma	0.03±0.00	0.03±0.00	0.02±0.01

[a] Total number of animals, 12; ND, not detectable.

phthalocyanine recovered at 30 min was still present in the lesion. Only traces of ZnPc, if any, were found in the control arterial segments at all times, which suggests that a very limited diffusion of the phthalocyanine from the deposition site took place. This conclusion was further supported by the low concentrations of ZnPc which were present in the rabbit serum at all the analysed times (Table 1). As a consequence, there was a reduced accumulation of the photosensitizer in the cutaneous tissue (Table 1); this would guarantee against the onset of persistent skin photosensitivity, which represents a frequent undesired side effect of PDT [16].

3.2. Light microscopy and morphometric analyses after PDT treatment

Four rabbits died during the course of the study (two in the control group, and two in the PDT-treated group), owing to the complication from the procedure. Autopsy examination failed to reveal any appreciable tissue damage. All rabbits treated with PDT appeared to be healthy, without any detectable complication, such as skin photosensitivity (data not shown).

Histology of arterial specimens (four sections each) at 7, 14, 21 and 28 days after the arterial vascular injury and subsequent PDT showed a significant reduction of IH and IMR after local PDT treatments as compared with controls (Table 2). IH, defined as the layer above the IEL, showed a pattern characterized by smooth muscle cells (SMCs) proliferation with different orientation, frequently organized in two layers, and restitution of medial cellularity. In control injured arteries, IH was observed 1 week after the injury with progressive increase in IH occurring at 3 weeks. The medial layer showed SMC and no signs of IEL disruption at 7 days. In the PDT-treated common iliac arteries, a complete acute depletion of SMC in the media was still present at 7 days, without signs of IH. However, despite the complete absence of SMC in the media, IH developed at 15, 21 and 28 days, even if significantly reduced as compared with controls (Fig. 1).

Arteries injured and locally irradiated only (three) as well as those treated with locally delivered ZnPc only (three), demonstrated an equivalent pattern of IH as compared with the above-mentioned control group. IH was present, reaching at 30 days 39.23±22.81% lumen reduction in animals which underwent local irradiation, and 36.00±7.71% in those which received the local delivery of ZnPc with channel balloon. Thus, light irradiation alone and ZnPc alone did not affect the healing response of the injured arteries.

Moreover, the pressure exerted by channel balloon does not appear to induce any adjunctive injury. The pattern of IH and the degree of lumen reduction were similar to those obtained in the control group using only one catheter.

4. Discussion

The successful outcome of PDT with both porphyrin- and phthalocyanine-type photosensitizers for the treatment of atherosclerotic plaques or the prevention of arterial restenosis has been reported [17–21]. The rationale for the utilization of this approach in the treatment of the above-mentioned pathologies was provided by the demonstration that some porphyrin-related photosensitizing drugs undergo a preferential uptake into actively proliferating SMC. If such differential sensitization is mandatory, the use of PDT to prevent restenosis in the clinical setting would be limited to treating patients at some interval after the angioplasty. As a consequence, the time delay of at least a few days between angioplasty and PDT (when cells proliferation was ongoing), and the repetition of invasive procedures, may well lead to undesired complications and increased costs. Previous data, however, showed that it is not necessary to delay PDT after balloon injury in order to prevent IH, because PDT performed at the time and site of angioplasty induces SMC death with absence of IH formation, also in normal arteries [11]. Recent clinical data from a pilot study suggest that endovascular PDT (with illumination given during the same procedure as balloon angioplasty, following systemic administration of the sensitizer 18–24 h before) is safe and effective in reducing restenosis [22].

The aim of our study was to evaluate the efficacy of a

Table 2
Comparison of IH (%=(IEL−Lumen)/IEL) and IMR (%=(IEL−L)/EEL−IEL) between treated (PDT) and control (CON) animals at various time intervals after the balloon injury[a]

		1 week	2 weeks	3 weeks	4 weeks
PDT	IH	0.00±0.00*	16.89±3.43**	33.28±12.29***	40.35±7.80
	IMR	0.00 80.00†	0.33±0.26	0.44±0.05††	0.62±0.17†††
CON	IH	13.24±3.09	26.05±6.34	67.55±8.41	49.42±21.50
	IMR	0.22±0.03	0.42±0.39	1.18±0.27	1.21±0.46

[a] The values are expressed as means±standard deviation. Total number of animals, 34.

*$P<0.001$ PDT versus control; **$P<0.09$ PDT versus control; ***$P<0.05$ PDT versus control; †$P<0.004$ PDT versus control; ††$P<0.01$ PDT versus control; †††$P<0.05$ PDT versus control.

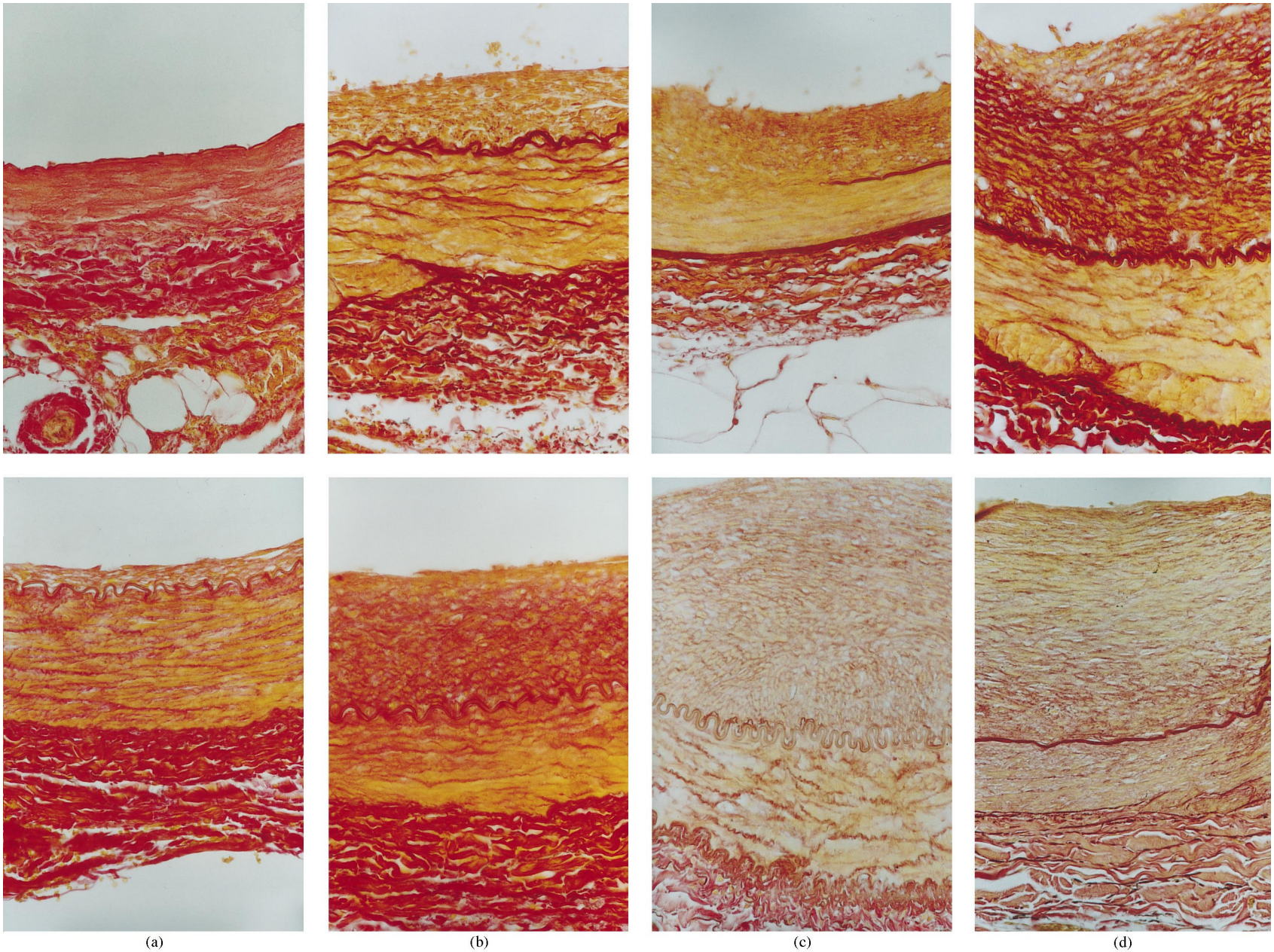

Fig. 1. Composite of light micrographs (Elastic van Gieson stain, ×240) of iliac arteries from animals sacrificed at various weeks following local photodynamic therapy (PDT, upper panels) as compared with controls (CON, lower panels). (a) Sections of an iliac artery from an animal sacrificed at 1 week after PDT as compared with CON (arterial injury only); (b) sections of an iliac artery from an animal sacrificed at 2 weeks after PDT as compared with CON (arterial injury only); (c) sections of an iliac artery from an animal sacrificed at 3 weeks after PDT as compared with CON (arterial injury only); (d) sections of an iliac artery from an animal sacrificed at 4 weeks after PDT as compared with CON (arterial injury only).

new procedure, local PDT, in reducing IH in a rabbit animal model of arterial injury. Two aspects of this local PDT treatment deserve consideration: the local endoluminal delivery of ZnPc and irradiation, and the timing of the procedure, performed immediately after the arterial injury, when SMC proliferation is not present.

As regards the pharmacokinetic aspects of the local delivery of ZnPc with a channeled balloon, significant amounts of ZnPc, an effective second-generation photosensitizer, are retained by the injured arteries after local delivery. The localization of the phthalocyanine in the arterial lesions appears to be characterized by a high degree of selectivity for two reasons. First, the recovery of ZnPc from non-injured areas of control arteries was found to be at the lowest detection limit of our analytical method, namely 9 nmol/mg of tissue, at least in the 30–90 min post-deposition time interval. Secondly, no significant diffusion of ZnPc outside the deposition sites was observed, in agreement with the small concentrations of this photosensitizer which were found in the serum. Data obtained by previous investigators showed that the locally released photosensitizing agent is preferentially localized in the intimal layers with a gradually decreasing concentration at the level of the media and adventitia [23]. This situation should favour a selective phototherapeutic action of the locally released ZnPc in the injured area, since the reactive intermediate species which are usually responsible for PDT damage exhibit a very short lifetime, so that their cytotoxic effects are restricted within a sphere of a few nanometers diameter surrounding the photosensitizer binding site [24]. Moreover, a further advantage of local photosensitizer delivery is given by a significantly lower serum concentration of drug that lessens the likelihood of more serious systemic side effects, such as skin photosensitivity and respiratory distress, that has been observed in rabbits receiving high doses of the photosensitizer [25]. The elimination of ZnPc from the rabbit organism appeared to occur largely via the bile–gut pathway, as shown by the markedly larger amounts of phthalocyanine which were recovered from the liver as compared with kidneys. This behaviour is usual for hydrophobic compounds, such as ZnPc which has an *n*-octanol/water partition coefficient greater than 100 [26]. The large recoveries of ZnPc from liver and spleen are also in agreement with the well-known high affinity of liposome-delivered phthalocyanines for the components of the reticuloendothelial system [27]. Previous studies [28] showed that systemically injected ZnPc is completely cleared from the organism in about 1 week. It is likely that this time period also applies under our condition, owing to the relatively small amounts of phthalocyanine entering the circulation.

The endolesional concentration of ZnPc reaches quite large values within 30 min after local delivery, and the amount of ZnPc which was found to be present in the arterial lesion after 30 min, namely 1.2 μmol/mg of tissue, has been shown to induce an extensive tumour response upon in vivo irradiation [13]. Thus, we performed the local delivery of the photosensitizing agent shortly after the arterial injury and, immediately after the local delivery, we proceeded to irradiation with a suitable light wavelength. We obtained SMC depletion and inhibition of experimental IH also with this favourable timing, confirming a previously hypothesis of Nyamekye, who demonstrated that PDT performed at the time of the balloon injury produces a depletion of SMC of the media [11] also in normal arteries. This modality offers a new approach to the management of restenosis after angioplasty, confirming that it is not necessary to delay PDT in order to prevent IH, since also local PDT induces SMC death in the absence of active SMC proliferation. Furthermore, endoluminal irradiation appears as a more practical approach as compared with external irradiation. The extraluminal irradiation has been proposed considering a possibly more important role of adventitia in the restenotic process than previously thought [29,30]. We investigated these pharmacokinetic aspects of ZnPc in normal injured arteries and we obtained data which are consistent with the previous observations: pthtalocyanines showed a peak accumulation at 30 min after sensitization, confirming that normal arteries could be effectively sensitized with both systemic and local delivery of phthalocyanine.

IH after arterial injury was significantly reduced by local PDT, confirming previous observations in different animal models and different settings [23,31]. The local PDT of balloon-injured arteries produced a complete local depletion of SMCs associated with a lack of IH until 7 days after the balloon catheter denudation. Despite an irrelevant repopulation of the media by SMC, a certain grade of IH, even if significantly reduced as compared with the control group, was observed at 14, 21 and 28 days after the injury. We treated the central portion of the injured arterial segment, and intimal hyperplastic response may progress from adjacent proliferating and migrating intimal SMC. This hypothesis is in agreement with the observations of Statius van Esp et al., who speculated that delayed IH developed from the injured vessel margin that did not receive the PDT treatment [32]. Therefore, it is reasonable to treat the whole injured segment together with a tract of uninjured artery to avoid this delayed IH.

Our follow-up was limited to 1 month, and, with this time of observation, we confirm that also local PDT does not affect the integrity of the arterial wall. In fact, we observed no diameter dilatation or aneurysm formation in our animals. Long-term observations (26 weeks) of normal arteries treated with PDT showed the safety of the treatment which did not induce degeneration, weakening or aneurysm formation in the artery. Rather than arterial weakening, the experiments of Grant and Brown on the magnitude of intraluminal pressure required to burst normal PDT treated arteries and normal non-treated arteries, showed that treated vessels require a higher in-

traluminal pressure before bursting than non-treated arteries do [11]. PDT appears to strengthen the connective tissue probably by increasing collagen synthesis in the early phase of injury [33,34].

The balloon catheter denudation model to induce arterial injury (well documented in the literature) is limited by induction of IH, that represents only one aspect of the restenosis. It is important to outline, however, that IH is the main component of instent restenosis. Considering the widespread utilization of stent to avoid mechanical factors of restenosis, like elastic recoil and remodelling, it seems that a reduction of IH remains the most important goal of preventing restenosis. Moreover, the injury of non-diseased artery in an animal model provides information that cannot be extrapolated for restenotic lesions of human atherosclerotic plaques, which are characterized by fibrosis and calcification. However, data from autopsy in humans revealed that activation of SMCs in synthetic phenotype, as well as increase of extracellular matrix synthesis, are present also in humans [23].

The impact of the adjunctive use of channelled balloon and the effectiveness of light alone and irradiation alone were also examined. The local delivery of liposome-incorporated ZnPc has been achieved by using a specifically engineered channelled balloon, designed for balloon dilatation and infusion of fluids and also for post-delivery expansion of expandable stents. It allows the controlled infusion of the photosensitizer through pores of adequate size under a pressure of 3 atm. We used it only for the delivery, but not to induce arterial lesion, with the aim to make the lesions of the controls perfectly comparable with those treated by PDT. The use of two balloons could increase the arterial injury, as described for an other macroporous balloon, which was successfully used for the delivery of other photosensitizers (Photofrin) [35,36]. In our experiments, the pressure exerted by the channelled balloon, used after the embolectomy catheter, does not appear to induce any adjunctive injury, since we observed no significant difference in SMC proliferation and vessel wall remodelling. The pattern of IH and the degree of restenosis were similar to that obtained using only one catheter.

Previous reports documented cytotoxic effects of hematoporphyrin derivatives even without photoactivation; such effects included cytoplasmatic protrusion, vacuoles, and even complete cell lysis [37]. On the basis of these observations, we performed a set of experiments which clearly indicate that locally delivered ZnPc alone, in the absence of light, or local irradiation alone, without ZnPc, do not alter the healing process after arterial injury and do not affect IH.

In summary, our findings indicate that the pharmacokinetic properties of ZnPc under our experimental conditions are appropriate for local delivery of ZnPc. Local PDT performed immediately after the procedure of arterial injury showed efficacy in reducing intimal hyperplasia, at least in the short-term period. Different strategies, i.e., PDT of an injured section as well, should ameliorate the long-term efficacy of PDT to control the development of restenosis after angioplasty.

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