Review

Endothelin and restenosis

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1. Introduction

Percutaneous transluminal angioplasty (PTCA) was first introduced into the therapy of patients with coronary artery stenosis in the late seventies [1]. In the two decades since, this method has become standard therapy for patients suffering from all forms of coronary artery disease. The success rate of the procedure itself has increased from 61 percent in the late seventies to well over 90 percent from the mid-eighties onwards [2]. However, long-term success of PTCA remains limited by late restenosis caused by vessel wall proliferation that occurs in 20 to 40 percent of all patients to such an extent that a second PTCA is necessary [2]. As in the mid-90s about 500,000 primary PTCA procedures were carried out in the USA [3] and about 200,000 in Europe [4], this accounts for more than 230,000 patients per year eligible for a second invasive procedure due to recurrent angina. A hypothetical reduction of the incidence of restenosis by 10–15 percent per year would reduce annual treatment costs by almost 1.5 billion dollars in the USA and Europe alone. Up to now a large number of clinical trials performed to investigate whether systemic administration of drugs which were effective in animal models of restenosis were efficacious in men have failed. The reason for this discrepancy between pre-clinical and clinical studies could be that doses effective in experimental settings have not been applicable to patients due to other cardiovascular effects of these drugs [5]. If technical advances, such as the use of stents, will by themselves be able to achieve the goal of a significant reduction of restenosis rates long term still needs to be studied. Two clinical trials with 6 months follow up have been published, both showing a reduction in restenosis [6,7] due primarily to a larger increase in lumen immediately after stenting as compared to PTCA. However, both studies showed a larger amount of late lumen loss with stents. There is increasing evidence that stents, while preventing vascular constriction, induce at least the same amount of vascular smooth muscle cell (SMC) proliferation as ‘classical’ PTCA because of additional circumferential deep vessel wall injury [8]. As in the case of stents additional treatment to limit late lumen loss can finally concentrate on inhibiting SMC proliferation and extracellular matrix formation pharmacological agents not limited by systemic side-effects have huge therapeutic potential.

2. Mechanisms of restenosis

The precise molecular processes responsible for pathological restenosis after PTCA with or without stent implantation in humans are still not well understood to date. Several animal models to investigate mechanisms and pharmacological prevention of restenosis have been developed and extensively used, however, none of the models has been able to accurately reflect the complexity of the processes underlying the human disease [9]. Nevertheless experiments in rats, rabbits and increasingly so in pigs are the only way to test new pharmacological principles for the prevention of restenosis before applying them clinically and, in addition to cell culture experiments in vitro, they provide at least limited insights into the pathophysiology of restenosis. The different mechanisms involved in restenosis as well as their relative importance have recently been reviewed in this journal by Bauters and colleagues [10]. In our review we will therefore only

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briefly recapitulate the response to experimental injury and then focus on the emerging role of endothelin-1 (ET) and ET receptor antagonists in restenosis.

Immediately after injury of a coronary artery by PTCA platelets accumulate at the site of injury and on activation by thrombin release a multitude of substances, including serotonin, platelet derived growth factor, and further promote thrombin release [11]. Medial SMCs shift from a contractile to a synthetic phenotype, migrate, proliferate, and produce extracellular matrix. Taken together these responses lead to neointimal hyperplasia [12] and constitute, at least in experimental models, a major component of restenosis.

3. Endothelin in restenosis

Endothelin, a 21 amino acid peptide, has been described as the most potent endogenous vasoconstrictor known [13]. As excellently reviewed by Rubanyi and Polokoff [14], ET binds in an autocrine/paracrine fashion to two different specific high affinity receptors, named ET_A and ET_B. While within the vasculature ET_A receptors are only located on SMCs leading to vasoconstriction and SMC proliferation, a variable portion of ET_B receptors were also described on SMCs promoting that at location the same effect as ET_A receptors via the same intracellular signalling pathways [14]. In contrast, the majority of ET_B receptors is located on endothelial cells, there leading to increased NO and prostacyclin levels and thus promoting vasodilatation [14].

Over the last few years a considerable effort has been made by several groups to better define the role of ET and its two receptor subtypes, ET_A and ET_B, in restenosis. Increased tissue ET immunoreactivity has been shown in human atherosclerotic plaques [15,16]. This is in accordance with the finding that immediately after PTCA plasma ET levels were elevated in the coronary sinus of patients [17] and even more so in the dilated coronary artery distal to the site of PTCA [18]. However, these elevated levels need not necessarily originate from the atherosclerotic plaque alone. It has been shown in cell culture experiments that SMCs themselves are able to produce and secrete endothelin [19]. In rat carotid arteries mRNA levels for endothelin converting enzyme (ECE, [20]) as well as ECE activity itself were increased after balloon injury [21]. Application of the rather unspecific neutral endopeptidase inhibitor phosphoramidon, which also blocks ECE activity and therefore should reduce ET levels, was able to reduce neointima proliferation in rats after balloon injury of the carotid artery [21].

While both ET_A and ET_B receptors have been shown to be present in human coronary arteries the overwhelming majority of receptors located on SMC are of the ET_A subtype [22]. Despite up-regulation in ET receptor density in human atherosclerotic coronary arteries the relation between the two receptors remained essentially unchanged [23]. Up to now no study has been published investigating ET receptor density and subtype distribution after PTCA in men. However, several papers on experimental studies have been published, characterising the most widely used species in restenosis. In rats after PTCA of carotid arteries a significant increase in mRNA levels of more than 20 times over controls was seen for ET_A receptors at both day 3 and day 7 after injury [24]. Stimulation of mRNA levels for ET_B receptors was also significantly elevated, albeit less pronouncedly so (10±15 fold over controls). In contrast, in rabbit carotid artery investigated autoradiographically four weeks after endothelial denudation it was found that ET_A receptors were predominately located in the media, while ET_B receptors were localised mainly in the neointima [25]. This finding appears to be species specific and valid for rabbits only. Therefore the predictive value of this species for the evaluation of ET receptor antagonists in restenosis with regard to compounds of different subtype selectivity may be limited. A recent study in our laboratory has investigated ET receptor density and distribution 4 weeks after PTCA in pig coronary arteries. In the tunica media specific binding to ET_A as well as ET_B receptors was approximately 3 times higher in injured versus control segments of the left anterior descending artery (LAD); however, ET_A receptor expression was predominant (ratio ET_A:ET_B approximately 2:1 [26,27]. The same held true for specific binding to neointimal receptors thus suggesting important similarities between injured pig coronary arteries and atherosclerotic human tissues concerning the receptor ratio.

We have tried to schematically visualise the role of ET in all processes involved in restenosis after PTCA in Fig. 1. In the following sections experimental evidence supporting the proposed mechanisms will be discussed; however, the role of ET in monocyte adhesion and SMC phenotype changes still warrants further investigations. The effect of elevated ET levels on vascular SMC hypertrophy and hyperplasia has been studied both in vitro and in vivo by injecting exogenous ET. In SMCs cultured from human vascular smooth muscle ET from 10^{-11} to 10^{-7} M induced concentration dependent mitogenesis and proliferation; this was mediated via ET_A receptors and correlated closely with receptor density [28]. In cultured rat aortic SMCs ET from 10^{-11} to 10^{-7} M also induced concentration dependent mitogenic effects [29]. This ET-induced mitogenesis could be enhanced significantly by adding angiotensin II in the same concentration range [29]. It has been suggested that ET exerts its direct mitogenic activity mainly through protein kinase C activation [30]. In isolated porcine coronary SMCs ET concentration dependently stimulated the synthesis of collagen type I with a maximum at 10^{-8} M ET [31]. This effect was shown to be mediated via ET_A receptors [31], as addition of the ET_A receptor antagonist BQ 123 (10 μM) almost totally blocked ET induced extracellular matrix production. In vivo, in the rat model of
importantly still, chronic infusion of Ang II over two weeks resulted not only in elevated blood pressure, but also in a significant increase of media thickness of basilar and small mesenteric arteries in rats [36] as well as elevated arterial tissue ET levels. As application of the selective ET\(\alpha\) receptor antagonist LU 135252 was able to totally inhibit the vascular hypertrophy but only partially the concomitant blood pressure increase, part of the structural effect of the two co-mitogens seem to be pressure independent [36]. Thrombin, one of the substances activated at the site of injury and promoting platelet aggregation as well as acting as a growth promoter, has also been shown to stimulate endothelin secretion [37]. Consequently the thrombin inhibitor hirudin was able to inhibit thrombin induced ET release [38]. It has been suggested that in a canine model of coronary artery endothelial injury and constriction the extend of platelet accumulation was positively correlated to the severity of neointimal proliferation [39]. Using the same experimental model we recently have been able to demonstrate that ETA receptor blockade by LU 135252, but not ET receptor antagonist by BQ 788, was able to reduce cyclic coronary flow reductions by 50% compared to controls [40]. Although outside the scope of that acute study this might indicate an additional beneficial impact on restenosis by reduction of local thrombin formation early after PTCA.

4. Endothelin antagonism in experimental restenosis

Up to now, several different ET receptor antagonists have been tested in different models of restenosis in rats and pigs, as summarised in Table 1. The model most commonly used to test the effects both of selective ET\(\alpha\) receptor antagonists as well as balanced ET\(\alpha/B\) receptor antagonists was endothelial denudation by a balloon catheter in rat carotid arteries. All studies done in this model [41–46] have one specific methodological detail in common: The rats were treated from 3 days prior to 14–21 days after balloon injury. One of the first substances to be tested was the peptidic, selective ET\(\alpha\) receptor antagonist BQ 123 [41]. The drug was applied once daily intraperitoneally with either 0.8 or 8 mg/kg and showed no effect on neointima proliferation [41]. This is perhaps not surprising, as BQ 123 has a very short half life, intraperitoneally applied substance is almost immediately absorbed, and thus one might assume that no or only very little substance was present at the site of injury for the by far greater part of the experimental time. Two further peptidic compounds were applied in the rat model, the mixed ET\(\alpha/B\) receptor antagonist TAK 044 [44] and the selective ET\(\alpha\) receptor antagonist FR 139317 [45], both of them given subcutaneously. In contrast to the results discussed above, both the selective ET\(\alpha\) receptor antagonist FR 139317 and the mixed ET\(\alpha/B\) receptor antagonist TAK 044 were able to reduce neointima proliferation by 76% [45] and 80% [44]
respectively. The balanced ET$_{A/B}$ receptor antagonist SB 209670, also applied intraperitoneally, was shown to reduce the neointima/media ratio by 52% [42]. Up to now two studies in rats have been published in which a selective ET$_A$ receptor antagonist was applied orally. The compound BMS 182874, tested with a daily oral dose of 100 mg/kg, was able to reduce neointima/media ratio by 35% [43]. In our laboratory we evaluated the dose response of the ET$_A$ receptor antagonist LU 135252 from 20 to 100 mg/kg/day orally and found a reduction of neointima/media ratio of 14 to 25% [46]. In a somewhat different approach the immediate restenotic response after endothelial injury to rat thoracic aorta was investigated by measuring incorporation of radiolabeled thymidine [47]. In this study, which was terminated 3 days after injury, the selective ET$_A$ receptor antagonist BMS 182874 reduced thymidine incorporation by 55%. With the balanced ET$_{A/B}$ receptor antagonist bosentan a 35% reduction was achieved [47]. However, as both substances were only used in one and the same dose, no differences in the efficacy of those two antagonists should be deduced.

In the Yucatan micropig [48] PTCA in an iliac artery as well as in a carotid artery was carried out 3 days after oral pre-treatment with the ET$_A$ receptor antagonist A 127722.5 (7.5 mg/kg, twice daily). Four weeks after PTCA the two arteries were excised and morphological changes were assessed. As compared to placebo, both medial area and neointima/media ratio were significantly reduced by treatment with this selective ET$_A$ receptor antagonist. In addition, collagen disposition was significantly reduced [48], thus confirming results predicted from in vitro studies of ET$_A$ receptor antagonism and collagen synthesis [31]. However, antiproliferative effects in the carotid artery were markedly less pronounced than in the iliac artery [48], the reason for this difference being not clear at present.

Only one study not involving pre-treatment over several days, which would be impractical in a routine clinical setting was carried out so far. In our laboratory a study in land race pigs applying PTCA in the LAD according to standard clinical protocols was done [27]. 5 minutes prior to balloon inflation 3 mg/kg of the ET$_A$ receptor antagonist LU 13522 was administered intravenously and after recovery from anaesthesia the animals received LU 13522 at a dose of 30 mg/kg/day orally for 4 weeks. At the end of the experiments the LAD was excised and frozen to allow not only histological examination, but also ET receptor autoradiography and receptor distribution studies. Treatment with the ET$_A$ receptor antagonist normalised both ET$_A$ as well as ET$_B$ receptor numbers and led to a 45% reduction in neointima/media ratio [27]. In a second series of experiments we investigated the effect of 30 mg/kg/day orally of the ET$_A$ receptor antagonist LU 135252 in the pig model with additional implantation of a stent post PTCA of the LAD, again without pretreatment of the animals. A similar reduction in the proliferative response of 35% was seen 4 weeks after this procedure (Fig. 2, unpublished results from our laboratory). The selective ET$_A$ receptor antagonist A 127722 was also tested in pigs with coronary artery stents [49]. After 28 days of oral treatment (b.i.d.) a maximal reduction in neointima formation of about 30%, which compares to the one seen by us (Fig. 2), has been reported [49]. Further experiments to determine dose-response relationships of different ET receptor antagonists in coronary restenosis after PTCA or stenting are currently under investigation.

From the results described above it can be concluded that both in rat and in pig experiments blockade of the ET$_A$ receptor is responsible for reduction of restenosis. This conclusion is also valid in the light of the effects discussed for the causative role of endothelin itself in restenosis. The ET$_B$ antagonising component in balanced receptor antagonists does not seem to impair the positive effects of

<table>
<thead>
<tr>
<th>Substance</th>
<th>ET receptor specificity</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Treatment (days)</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQ 123</td>
<td>A</td>
<td>i.p.</td>
<td>8 q.d.</td>
<td>14</td>
<td>none</td>
<td>[41]</td>
</tr>
<tr>
<td>SB 209670</td>
<td>A/B</td>
<td>i.p.</td>
<td>2.5 q.d.</td>
<td>14</td>
<td>neointima/media: – 52%</td>
<td>[42]</td>
</tr>
<tr>
<td>BMS 182874</td>
<td>A</td>
<td>p.o.</td>
<td>100 q.d.</td>
<td>21</td>
<td>neointima/media: – 35%</td>
<td>[43]</td>
</tr>
<tr>
<td>TAK 044</td>
<td>A/B</td>
<td>s.c.</td>
<td>5 q.d.</td>
<td>14</td>
<td>neointima: – 80%</td>
<td>[44]</td>
</tr>
<tr>
<td>FR 139317</td>
<td>A</td>
<td>s.c.</td>
<td>32 b.i.d.</td>
<td>21</td>
<td>neointima: – 76%</td>
<td>[45]</td>
</tr>
<tr>
<td>LU 135252</td>
<td>A</td>
<td>p.o.</td>
<td>20–100 q.d.</td>
<td>14</td>
<td>neointima/media: – 14 to – 25%</td>
<td>[46]</td>
</tr>
</tbody>
</table>

**Rat, balloon-induced endothelial denudation in carotid artery**

<table>
<thead>
<tr>
<th>Substance</th>
<th>ET receptor specificity</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Treatment (days)</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS 182874</td>
<td>A</td>
<td>p.o.</td>
<td>30 q.d.</td>
<td>3</td>
<td>thymidine incorporation: – 55%</td>
<td>[47]</td>
</tr>
<tr>
<td>Bosentan</td>
<td>A/B</td>
<td>p.o.</td>
<td>30 q.d.</td>
<td>3</td>
<td>thymidine incorporation: – 35%</td>
<td>[47]</td>
</tr>
</tbody>
</table>

**Pig, PTCA in iliac artery**

<table>
<thead>
<tr>
<th>Substance</th>
<th>ET receptor specificity</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Treatment (days)</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 127722.5</td>
<td>A</td>
<td>p.o.</td>
<td>7.5 b.i.d.</td>
<td>28</td>
<td>neointima/media: – 40%</td>
<td>[48]</td>
</tr>
</tbody>
</table>

**Pig, PTCA in coronary artery**

<table>
<thead>
<tr>
<th>Substance</th>
<th>ET receptor specificity</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Treatment (days)</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LU 135252</td>
<td>A</td>
<td>p.o.</td>
<td>30 q.d.</td>
<td>28</td>
<td>neointima/media: – 45%</td>
<td>[27]</td>
</tr>
</tbody>
</table>

Abbreviations: q.d.–once daily, b.i.d.–twice daily, p.o.–orally, s.c. subcutaneously.
concomitant ET\textsubscript{A} receptor antagonism. However, the question can only be resolved by using a selective ET\textsubscript{B} receptor antagonist with acceptable oral availability and half life. Such drugs only now become available and hopefully a comparison between ET\textsubscript{A} and ET\textsubscript{B} receptor blockade will be published soon to further our knowledge about ET receptor subtypes and experimental restenosis.

5. Conclusions and future directions

Evidence obtained so far from experimental studies indicates an important role of endothelin as a (co-)mitogen for smooth muscle cell proliferation as well as a factor stimulating extracellular matrix production at the site of vascular injury. Analogous conclusions have been drawn for AngII, and several experimental studies have indicated a beneficial role for both ACE inhibition and AngII receptor blockade (reviewed recently by Pratt and Dzau [5]). However, in all clinical trials conducted so far, ACE inhibitors and AngII receptor antagonists had to be used in doses 10–100 times lower than required for efficacy in all experimental restenosis studies [10] to avoid unwanted pronounced hypotension [5]. Up to now also endothelin receptor antagonists have been used in high doses. Experiments evaluating the lower threshold of activity in restenosis need to be done and are currently under way in our laboratory. In addition, due to the lack of a potent inhibitor of the endothelin converting enzyme (ECE), studies investigating a possibly better effect of enzyme inhibition as compared to receptor antagonism have not been possible so far. Such studies will be of great interest to clarify if blockade of ET synthesis, which, if anything, could best be compared with inhibition of both ET receptors, is superior to ET\textsubscript{A} receptor antagonism alone. It has been shown experimentally [50] and very recently also clinically [51] that ET receptor antagonists seem to lower blood pressure with a slow onset of action [50], thereby possibly avoiding some of the problems encountered in all clinical trials with antagonists of the renin–angiotensin system. Despite the promising experimental results discussed above, one can currently not be certain that ET receptor blockade will be as efficacious in preventing human restenosis after PTCA or stenting as one would wish. Only a large scale clinical trial can test the hypothesis that this new therapeutic principle could significantly reduce restenosis after systemic application.

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References


