Eur J Clin Pharmacol (2002) 57: 751–758
DOI 10.1007/s00228-001-0392-7

REVIEW ARTICLE

J. Hauptmann

Pharmacokinetics of an emerging new class of anticoagulant/antithrombotic drugs

A review of small-molecule thrombin inhibitors

Received: 25 June 2001 / Accepted in revised form: 24 September 2001 / Published online: 20 November 2001

Abstract Small-molecule direct thrombin inhibitors represent a new class of anticoagulants and are emerging as antithrombotic drugs with a range of indications. The tripeptide type or peptidomimetic compounds, including argatroban, efegatran, inogatran and napsagatran, hitherto clinically studied represent a first generation of thrombin inhibitors that are pharmacokinetically characterised by relatively rapid hepato-biliary clearance and short half-lives necessitating their administration as intravenous infusion. They are not orally bioavailable because of poor enteral absorption and presystemic hepatic extraction. Melagatran can be administered subcutaneously, and a prodrug form of melagatran, ximelagatran, is at present the only oral thrombin inhibitor available. Direct thrombin inhibitors produce predictable, stable and rapidly reversible anticoagulation measurable by common coagulation assays. Significant pharmacokinetic drug–drug interactions have not been reported. Possible pharmacodynamic interactions, in terms of prolongation of plasma clotting times, with other anticoagulant drugs must be taken into account when monitoring direct thrombin inhibitors using coagulation assays.

Keywords Thrombin inhibitors · Pharmacokinetics · Anticoagulation

Introduction

Among the various new antithrombotic drugs developed and introduced into clinical practice in the last decade, small-molecule direct inhibitors of the coagulation enzyme thrombin have an important position because of the qualities of their anticoagulant effects and their potential for oral administration. Such novel oral anticoagulants are expected to meet the needs for differentiated treatment of various cardiovascular disorders connected with thromboembolic events, thus valuably complementing the therapeutic armamentarium or representing alternatives to current anticoagulant drugs [1, 2, 3, 4]. Various aspects of the design, preclinical evaluation and development of small-molecule direct thrombin inhibitors have already been extensively reviewed.

This review will focus on the pharmacokinetics of those representative potent and selective thrombin inhibitors already studied in humans, regardless of their developmental or approval state: argatroban [inhibition constant (K_i) 0.040 μmol/l], efegatran [apparent association constant (K_{ass}) 2.4×10^9 l/mol], napsagatran (K_i 0.0003 μmol/l), inogatran (K_i 0.015 μmol/l), melagatran (K_i 0.002 μmol/l) and its prodrug ximelagatran (proposed name, previously known as H376/95). There have been further compounds in phase-I studies, the results of which, however, have not yet been available publicly.

According to the present state of development of this new class of anticoagulants/antithrombotics, most of the human pharmacokinetic data reported are from studies in healthy volunteers. This applies in part also to the data on their pharmacodynamics, i.e. their anticoagulant effects. Nevertheless, the therapeutic efficacy of these direct thrombin inhibitors, i.e. their antithrombotic effects, have been proven in a variety of clinical settings, such as short-time anticoagulation during interventional coronary procedures, adjunctive treatment during thrombolysis, heparin-induced thrombocytopenia, prevention of deep-vein thrombosis after orthopaedic surgery and prevention of thrombotic stroke in cardiac fibrillation.

It must be stressed that the pharmacodynamics of the thrombin inhibitors are directly correlated to their pharmacokinetics since circulating blood is the ''compartment of effect'' of a coagulation enzyme inhibitor, in

J. Hauptmann
Centre for Vascular Biology and Medicine,
Friedrich Schiller University Jena,
Nordhäuser Strasse 78,
99089 Erfurt, Germany
E-mail: hauptman@zmkh.ef.uni-jena.de
Tel.: +49-361-7411480
Fax: +49-361-7411471

contrast to those drugs with targets in tissue/organ compartments. Therefore, all determinants governing the concentration of free drug in plasma are of prime importance.

Deduced from longstanding clinical experience with the classical anticoagulant heparin as an informative parameter of the in vitro anticoagulant potency, the thrombin inhibitor concentration doubling the activated partial thromboplastin time (APTT) of citrated plasma, i.e. giving an APTT-ratio of 2.0, has been established. The following relationship having a certain experimental base is commonly accepted also for the clinic: thrombin inhibitor potency (reciprocal of K_i) $\sim$ anticoagulant effect (APTT) $\sim$ antithrombotic action. The K_i, however, does not linearly translate into anticoagulant potency. However, there is an ongoing debate whether APTT is suited for ex vivo monitoring in various clinical settings at all [5, 6]. The results of several clinical studies did not show a clear relationship between the dose-related anticoagulant response in terms of APTT and the clinical outcome. It has been clearly shown, however, that the anticoagulant effects of direct thrombin inhibitors are more predictive and more stable than the effects of (unfractionated) heparin.

Most anticoagulant drugs have a rather narrow margin of safety with regard to doses and ensuing plasma levels: the inherent side effect on overdosing is bleeding, whereas other possible adverse reactions would likely not be related to the mechanism of action of thrombin inhibitors. During the phase of the introduction into broader clinical use of a new class of drugs various aspects are of importance, first of all proper dosage and safety, both prerequisites for therapeutic benefit. When several drugs of the same new class are emerging the most marked differences to be expected in their individual characteristics are potency and pharmacokinetics, both determining the clinical dosage.

An unfavourable pharmacokinetic profile often precludes the progression of newly developed drug candidates or is the reason for their failure. The field of synthetic thrombin inhibitors presents numerous examples for this experience; the development of several compounds has been discontinued after preclinical or phase-I studies. First-generation thrombin inhibitors, which have been used clinically so far, are mainly products of the development in the 1980s and early 1990s. They are characterised by relatively uniform pharmacokinetics limiting their practical use; they are not orally bioavailable and must be administered via intravenous infusion. The problems with regard to pharmacokinetics, especially oral bioavailability, are shared with various other peptidomimetic drugs recently developed and extensively studied, such as human immunodeficiency virus-proteinase inhibitors, metalloproteinase inhibitors and platelet fibrinogen receptor antagonists. There is more structural diversity among newly synthesised small-molecule thrombin inhibitors allowing the assumption that compounds might be developed with physicochemical properties endowing them with more suitable pharmacokinetic characteristics.

Preclinical studies

Preclinical studies in various species showed that the pharmacokinetics of most of the thrombin inhibitors derived from arginine, benzamidine or similar scaffolds, being hydrophilic tripeptide-derivatives or peptidomimetics with a strongly basic group targeting the so-called specificity pocket of the active site of thrombin, are characterised by high systemic clearance, short plasma half-life and low oral bioavailability [7, 8]. Several representatives of these first-generation thrombin inhibitors are characterised by extensive hepatic extraction which governs their overall elimination. Hepato-biliary elimination was first found for *N*α-[(2-naphthylsulfonyl)glycyl]-4-amidinophenylalanine piperidide (NAPAP), a prototypical benzamidine-derived peptidomimetic, and argatroban [9, 10, 11]. Moreover, efegatran and related tripeptides [12], inogatran and napsagatran are biliary excreted to a marked extent. Biliary excretion was also reported for napsagatran in humans [13]. Hepatic uptake and biliary excretion are active processes: the uptake of the benzamidine-type thrombin inhibitor, CRC 220, into isolated rat hepatocytes proceeds via the multispecific basolateral organic anion transporter, Oatp1 [14]. The marked differences in inogatran half-lives in rats after intravenous administration of various doses may be explained by saturation of elimination processes at higher doses [15]. Hepatic uptake and biliary excretion of these thrombin inhibitors may be subject to interactions with other hepato-biliary eliminated drugs resulting in increased plasma levels and lowered biliary clearance [16, 17].

The pharmacokinetic parameters of napsagatran in four animal species showed large interspecies differences for liver and kidney excretion because of the involvement of active transport in both organs [18]. The values for total clearance, non-renal clearance and distribution volume for man were overpredicted by allometric scaling from the four species. However, the values observed for man were fairly well predicted by allometric scaling from cynomolgus monkeys [18]. Argatroban, inogatran and napsagatran with total clearances in the range of 40–80 ml/min/kg in rats, indicating blood flow-limited hepatic extraction, show a clearance of about 4–7 ml/min/kg in man only [13, 19, 20, 21]. Total clearance in man is lower than one would expect referring only to the respective liver blood flow (in rats$\approx$60 ml/min/kg, in man$\approx$20 ml/min/kg).

Clearance in man

Pharmacokinetic parameters (Table 1) were mainly calculated from plasma levels in individuals receiving the

Table 1 Pharmacokinetic parameters of small-molecule thrombin inhibitors in man. *HPLC* high-performance liquid chromatography, *sc* subcutaneous, *po* oral

Drug	Total clearance (ml/min/kg)	Renal clearance (ml/min/kg)	Half-life (min)	Distribution volume V_{ss} (l)	Remarks	Reference
Argatroban	4.7		46			[21]
	5.0		24	11.7	HPLC assay	[19]
	4.4		21	9.7	Coagulation assay	[19]
	5.8		61			[34]
			30–45			[24]
	5.1		39–51			Manufacturer
Efegatran	5.9–6.4[a]					[47]
	6.4		35–150	32.2[b]	Half-life depends on infusion	[25]
Napsagatran	5.5[b]		40			[13]
	6.2[b]		124	25.8		[35]
	6.6[b]	2.2[b]	102	24		[18]
Inogatran	5.4[a]					[20]
Melagatran	1.4[b]		150	19		[33]
	1.3[b]		120	16	Melagatran sc	[57]
	5.4[b]		288	159	Ximelagatran po	[57]

[a]Read or calculated from the data (dosage, plasma level) published
[b]Referred to 70 kg body weight

respective thrombin inhibitor via intravenous infusion. Differences presented in the values for half-life of a given thrombin inhibitor may be due to varying sensitivity, precision and detection limits of the assays used for determining the plasma level and by various sampling regimens in the terminal elimination phase.

In Table 1, for comparison several numerical values for total body clearance have been calculated by the author of this review dividing infusion rates by plasma levels at steady state. Thus, the total body clearance of argatroban, calculated from the original data, ranged from 2.4 ml/min/kg to 17.8 ml/min/kg [22, 23, 24].

With the other thrombin inhibitors showing similar clearances and half-lives, their volumes of distribution at steady state are rather uniform, too. The non-renal clearance of napsagatran amounted to about two-thirds of total clearance [18]. It is noteworthy that melagatran has in animals and man a total clearance only one-fourth that of argatroban, efegatran, inogatran and napsagatran. The poor enteral absorption of the compounds will render the biliary secretion process a definitive elimination pathway without significant entero-hepatic circulation taking place.

There are only few data published on the metabolism of synthetic thrombin inhibitors in animals and humans. Argatroban is metabolised in various species, including man, to several metabolites (Fig. 1). The primary metabolite (M 1) occurring in plasma is the product of aromatisation of the tetrahydroquinoline ring; it has about 20–30% of the antithrombin activity of the parent compound [21]. Metabolites M2–M4 are hydroxylated products found in very low quantities in the urine only. Efegatran is in part metabolised in rats at the aldehyde group to the corresponding acid and alcohol. In plasma, the epimeric DLD-efegatran is also found [25]. For napsagatran, inogatran and melagatran, no metabolisation has been reported so far. The melagatran prodrug ximelagatran, a derivative with a hydroxyamidino instead of the amidino group and an esterified carboxyl group, is readily converted to melagatran [26] (Fig. 1). This conversion proceeds via hydrolysis of the ethyl-ester bond and reduction of the hydroxyamidine, a reaction already described for the hydroxyamidine (amidoxime) derivative of the thrombin inhibitor NAPAP [27]. The enzyme system responsible for this reaction has been characterised as an oxygen-insensitive liver microsomal reduced nicotinamide adenine dinucleotide-benzamidoxime reductase [28].

Oral bioavailability

The low oral bioavailability of the first-generation synthetic thrombin inhibitors has various reasons, first of all the poor permeation across the intestinal membrane barrier due to low lipophilicity of the compounds bearing strongly basic guanidino or amidino groups [7, 8]. In the preclinical development increased lipophilicity of new compounds has, on the one hand, positively influenced the intestinal absorption, but, on the other hand, it has sometimes negatively affected the pharmacodynamics, i.e. the anticoagulant activity decreased by extensive plasma–protein binding. The consequence is that in the design of thrombin inhibitors the hydrophobicity of a molecule must be balanced in order not only to be highly active in plasma, but also to provide suitable pharmacokinetic characteristics.

A second point is of equal importance in limiting the oral bioavailability, the hepatic first-pass effect brought about by extensive hepatic extraction of the compounds and consecutive biliary excretion (see above). Structural moieties and properties of the compounds determining the hepatic first-pass effect have not yet been fully evaluated.

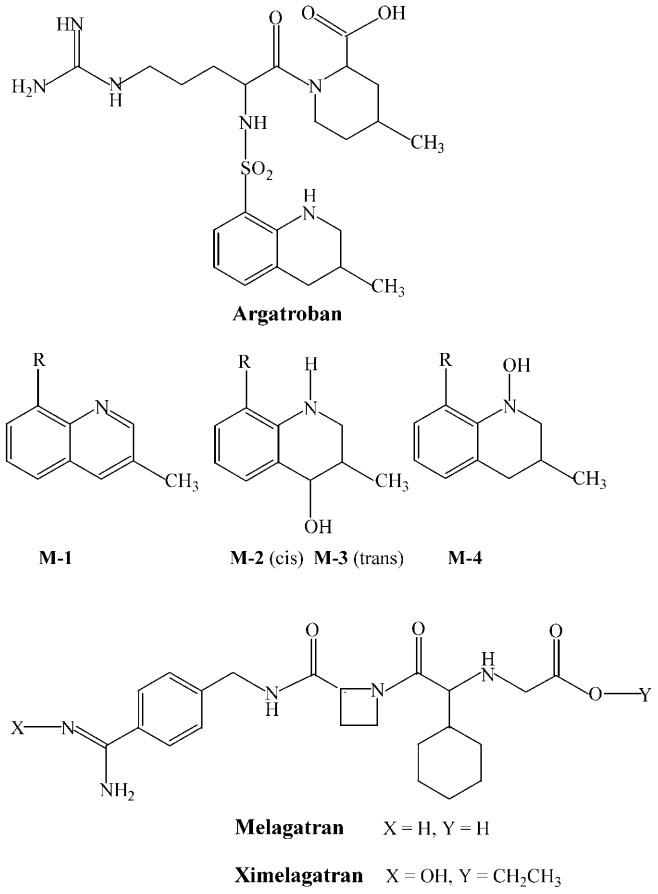

Fig. 1 Structures of the thrombin inhibitors argatroban and melagatran, of the argatroban metabolites and of the melagatran prodrug, ximelagatran

For the thrombin inhibitors showing insufficient oral bioavailability in preclinical studies, no data on oral administration in humans have been published. With respect to oral bioavailability, the only promising thrombin inhibitor under clinical investigation is the lipophilic melagatran prodrug, ximelagatran (170-fold higher octanol/water partition coefficient than melagatran). It has an oral bioavailability of 18–24% compared with only 3–7% for melagatran, with the latter showing a non-linear increase in oral bioavailability upon higher doses [26]. Low variability in plasma levels and lack of influence of food have been reported for ximelagatran, whereas the oral bioavailability of melagatran was lowered to only 0.9% after food intake. In the clinical setting, the comparatively lower clearance and longer half-life of the parent drug, melagatran, obviously, allows for twice-daily dosing of the oral prodrug. However, there seems to be the need for further proof of sustained plasma levels and anticoagulant responses with this dosage regimen since the coagulation-rebound phenomenon, reported after cessation of thrombin inhibitor infusions, requires caution to avoid plasma-level troughs.

Factors influencing pharmacokinetics

Among the various factors known to possibly influence the pharmacokinetics of a given drug, the following have been evaluated in studies with the presented thrombin inhibitors:

1. Age: there was no distinct effect of age on the pharmacokinetics of argatroban [29, 30]. The lowered renal clearance of melagatran in elderly people resulted in somewhat higher bioavailability after oral and subcutaneous administration of its prodrug [31].
2. Gender: most studies in volunteers were conducted in males. In a study in both sexes, argatroban produced the highest anticoagulant effect in young healthy males and the lowest in elderly females [21]. From the clinical studies no significant differences between female and male patients with regard to pharmacokinetics or clinical outcome have been reported.
3. Hepatic impairment: hepatic insufficiency decreased the total clearance of argatroban to 1.5 ml/min/kg compared with 5.8 ml/min/kg [21, 32]. Consequently, the dose adjustment in hepatic impairment recommended by the manufacturer takes the fourfold decrease in clearance into account (initial dose of 0.5 μg/kg/min in moderate hepatic impairment compared with a commonly used initial dose of 2 μg/kg/min). Corresponding observations have not yet been published for the other thrombin inhibitors with predominant hepatic clearance.
4. Renal impairment: argatroban clearance was not markedly dependent on renal function, as studied in persons with various degrees of renal impairment [30]. For the compounds eliminated mainly via the kidneys, as expected, an influence of renal function was seen; the total body clearance of melagatran was linearly correlated to the creatinine clearance [31, 33].

Drug interactions

Adverse drug reactions may arise from drug–drug interactions. Potential pharmacokinetic interactions with co-administered drugs would directly influence the effect of inhibitors of coagulation enzymes via alterations of the plasma level after a given dose. The low to intermediate plasma-protein binding of the small-molecule thrombin inhibitors reported, less than 15% for melagatran (manufacturer's information), 20–30% for inogatran [15] and 54% for argatroban (manufacturer's information) would not give rise to interactions with drugs binding strongly to plasma proteins.

For obvious reasons, the effects of co-administered anticoagulant and antithrombotic drugs have been of special interest: the cyclooxygenase inhibitor, aspirin, did not affect the pharmacokinetics of argatroban or melagatran [19, 34]. There was also no influence of the vitamin K antagonist, warfarin, on the elimination of

napsagatran [35]. However, there are interactions between the vitamin K antagonists and the thrombin inhibitors at the level of the anticoagulant effect that must be taken into account in case of monitoring the latter by coagulation assays (see also below): co-administration of argatroban and warfarin gives combined effects on the prothrombin time (PT) or the international normalised ratio (INR) calculated from the PT [36, 37]. In this study warfarin was given daily for 10 days combined with a 5-h infusion of argatroban per day. Argatroban increased the INR during the infusion with the increase being dependent on the thromboplastin reagent used. The authors assumed that the predictable effect of argatroban at doses up to 2 μg/kg/min on INR during warfarin co-therapy allows for prediction of the level of oral anticoagulation in this situation. A cross-over study in volunteers who received warfarin just prior to the start of a 24-h napsagatran infusion came to other conclusions [22]. In accordance with the delayed onset of the anticoagulant action of the vitamin K antagonists, there was at about 12 h an additional increase in PT and APTT with warfarin plus napsagatran, compared with napsagatran alone. The different sensitivity of PT and APTT for the vitamin K antagonists and direct thrombin inhibitors, respectively, was the reason for a greater percent increase in PT than in APTT. From this study it was concluded that the PT cannot be used to monitor the effect of oral anticoagulants during the transition from intravenous direct thrombin inhibitors to oral anticoagulants. These problems would not be encountered in case of an oral thrombin inhibitor, the use of which would overcome the need for switching from infusions of a short-acting anticoagulant to an oral drug suited for long-term treatment.

Whether there might be an interference at the level of hepatic metabolism with other drugs was studied with the hepatically metabolised argatroban. Co-administration of the potent CYP3A4 inhibitor erythromycin did not alter the pharmacokinetic parameters of argatroban or the anticoagulant response [38]. The oxidative metabolism by CYP3A4 was, therefore, not seen as an important elimination pathway. Obviously, hepatic uptake and biliary excretion per se govern the overall clearance of argatroban.

Dosage and plasma levels

Some of the data presented in Table 2 are not explicitly given in the papers referred to, instead they were read from the original graphs. An initial bolus dose, if given, is omitted in the table for the sake of clarity.

The doses of the parenteral thrombin inhibitors used so far in various studies, including therapeutic ones, in man were in general between about 0.5 μg/kg/min and 3 μg/kg/min. Efegatran with a lower efficacy had to be administered at higher doses. In the earlier reports on argatroban, the doses used and the plasma levels reached showed a relatively broad range. The data given in Table 2 for argatroban are only examples from the various reports on the use of this drug in various clinical settings. In phase-1 studies, argatroban was dosed up to 40 μg/kg/min in volunteers (manufacturer's recommendation: clinical dose 2–10 μg/kg/min) [23]. The data reported for inogatran are from a study in unstable coronary disease using three dosages [20].

It is clearly seen that upon an infusion with similar doses of argatroban, efegatran, inogatran and napsag-

Table 2 Dosage, plasma level and ex vio anticoagulant effect of small-molecule thrombin inhibitors in man. *APTT* activated partial thromboplastin time

Drug	Dosage (μg/kg/min)	Plasma level (μg/ml)	APTT ratio at plasma level indicated	Plasma level (μg/ml) for APTT ratio 2.0	Remarks	Reference
Argatroban	1.0		1.8			[19]
	2–3	0.70 ± 0.30	≈2.0			[24]
	4.8[a]	2.0				[22]
	10	1.0				[23]
Efegatran	3.5–14	≈0.60–2.35	≈1.3–2.2	≈2.0		[25, 47]
	1.75–20					[59]
Napsagatran	1.14[a]	0.20				[35]
	1.19–2.14[a]			≈0.80		[13]
	1.14[a]	0.1–0.2				[18]
Inogatran	0.48–2.38					[5, 20]
	0.48–2.38	0.066–0.35	≈1.3–1.8			[60, 61]
	various			0.55	in volunteers	[45]
	various			0.64	in patients	[45]
Melagatran	0.083–0.25	0.073–0.23	1.3–1.7	0.43		[33]
	[b]			0.17	start of treatment	[62]
	[b]			0.39	end of treatment	[62]

[a]Referred to 70 kg body weight
[b]Oral administration of ximelagatran

atran similar plasma levels are achieved. However, in light of the similar elimination kinetics of these drugs, the different doses (and the ensuing different plasma levels) needed for the same anticoagulant effect, i.e. an APTT ratio of 2.0, are an expression of the various potencies of the individual thrombin inhibitors. For melagatran the higher potency and lower clearance than the predecessor inogatran must be taken into consideration.

The infusions, in part with preceding loading bolus doses, lasted at maximum up to 6 days. From the pharmacokinetic point of view, taking the rather short half-lives into account, a bolus dose seems not to be necessary regularly, rather in short-time interventional procedures in order to rapidly reach an appropriate plasma level. Dosages, plasma levels, the pharmacokinetic parameters determined and anticoagulant effects were in all cases linearly related. In most studies, there were comparatively low intraindividual variations in plasma levels and anticoagulant response.

The short half-lives of the parenteral thrombin inhibitors may be seen as a relative advantage regarding the control of plasma levels using infusions: steady-state levels (also the anticoagulant effects) are rapidly achieved and plasma levels decline rapidly after cessation of infusion, which might represent a safety aspect in cases of bleeding complications. The mean melagatran plasma levels measured 6–8 h after oral doses of 8–24 mg of ximelagatran in patients undergoing total knee arthroplasty were proportional to the doses and amounted to 0.026–0.086 μg/ml [39].

Monitoring

Clinical monitoring of anticoagulants is primarily aimed at following parameters or effects (in a stricter sense pharmacodynamics) closely related to the plasma level rather than at measuring the plasma levels directly. So one must distinguish between the assessment of plasma levels of thrombin inhibitors in the course of pharmacokinetic and dose-finding studies on the one hand and the monitoring for clinical purposes on the other hand. Synthetic thrombin inhibitors were assayed in plasma (and in body fluids) using functional chromogenic thrombin inhibition assays [40, 41], enzyme-linked immunosorbent assay [42] and, in most experimental and clinical studies, high-performance liquid chromatography [19, 25, 26, 27, 35, 43, 44]. The concept of monitoring anticoagulants by plasma coagulation assays has been transferred to this new class of anticoagulants from the common practice used over decades for vitamin K antagonists and heparin. Thrombin inhibitors prolong plasma-clotting times in assays with the target enzyme either added exogenously (TT) or generated from plasma prothrombin (APTT and PT). Noteworthy, the TT is the most- and the PT the least-sensitive assay.

Pooled data from several phase-I and phase-II studies with inogatran were used for population modelling of the effects of inogatran on APTT [45]. Patients with unstable angina pectoris appeared to have a less pronounced effect on APTT than healthy young volunteers. The predicted plasma concentrations needed to double APTT are shown in Table 2. Correlations between plasma levels and APTT were presented for napsagatran [13], argatroban [46], efegatran [47], melagatran [32] and inogatran [5, 45]. The relationship was described using various models, including combination with nonlinear ones.

Besides the APTT, the activated clotting time has been used for monitoring, especially in cardiovascular surgery with extracorporeal circulation [48, 49, 50, 51, 52]. Also the ecarin clotting time, giving linear concentration-effect relationships over a broader range, may be suited [6, 51]. Recently, with PT assays it was found for melagatran that the results are not only dependent on the plasma concentration of melagatran but also on the sensitivity of the PT reagent, so that PT assays cannot be used for monitoring [53].

Perspectives

The potent and selective direct thrombin inhibitors, either recombinant protein or small-molecule synthetic ones, have not yet fulfilled all expectations with respect to therapeutic efficacy and safety, of which the pharmacokinetic drawbacks are only one aspect. At present, the restriction of the small-molecule direct thrombin inhibitors, except melagatran and its orally active prodrug, to intravenous infusion limits their use to the hospital setting.

The suitability of direct thrombin inhibitors as alternatives to heparin for several indications, including short-term interventional cardiovascular procedures, has been unequivocally proven. Other indications necessitating long-term treatment are still under study. Several items regarding the action as well as the pharmacokinetics merit further attention: a coagulation-rebound phenomenon, known also for heparin, has been observed after withdrawal of effective anticoagulant treatment with various thrombin inhibitors, i.e. after cessation of infusion [20, 24, 54, 55]. It is not directly related to the mode of action of the reversible thrombin inhibitors or to their pharmacokinetics, but rather to the duration of treatment in relation to the progression of the underlying disease. Moreover, it is anticipated that in further studies in patients with various disease states additional factors influencing the pharmacokinetics of the present generation of small-molecule thrombin inhibitors and of a new generation to come will be discerned. The same might be true of co-administered hepatically eliminated drugs. For two other new classes of antithrombotic drugs, low-molecular-weight heparins and platelet fibrinogen receptor antagonists, which have already been used for several years, the impact of renal failure on their pharmacokinetics has been reviewed recently [56]. Quite importantly, the question as to the

best method of monitoring thrombin inhibitors and whether monitoring should be performed or is necessary at all must be answered.

Further evaluation of efficacy and safety in various indications will be required for a final assessment of the therapeutic value of this new class of anticoagulant/antithrombotic drugs. For long-term treatment only orally active compounds will finally gain clinical acceptance and patients' compliance. The desirable once- or twice-a-day dosage regimen requires small-molecule thrombin inhibitors with high oral bioavailability and appropriate elimination half-life, the pharmacokinetics of which will ease their administration for the profit of the cardiovascular patient.

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