SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures which have been finalised before 1 September 2003. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Rapilysin 10 U contains the active substance reteplase, an unglycosylated recombinant mutant of tissue type plasminogen activator (t-PA) produced in *Escherichia coli*.

This medicinal product is intended to be used in the treatment of acute myocardial infarction (AMI).

Plasminogen activators catalyse the cleavage of endogenous plasminogen to generate plasmin, an unspecific proteinase that degrades the fibrin matrix of a thrombus. The natural t-PA is a serine protease synthesised by endothelial cells and secreted as a single or double chain molecule. Each 527 amino acid chain has a molecular mass of 65000. The natural t-PA is a poor plasminogen activator in the absence of fibrin. t-PA binds to fibrin via lysin binding sites and activates bound plasminogen several hundred-fold more rapidly than it activates plasminogen in the blood circulation. Clearance of t-PA is primarily made by hepatic metabolism and its half-life is about 3 minutes.

Reteplase is a "fibrin-selective" recombinant plasminogen activator derived from human t-PA and containing a 355 amino acid chain. Three major domains of the molecule have been removed: the amino terminal finger domain which is involved in the high affinity binding to fibrin, the epidermal growth factor domain and the kringle 1 domain. Only the kringle 2 domain which is involved in fibrin selectivity and the catalytic site are preserved. Removal of 3 major domains in the t-PA molecule and the lack of glycosylation would be expected to modify the pharmacodynamics and pharmacokinetic characteristics.

The rationale behind the development of reteplase was to find a faster thrombolytic agent compared to approved dosage regimens of standard thrombolytics without increasing the risk of thrombolysis. The second aim was to find a substance with a longer effective half-life as compared to approved dosage regimens of standard thrombolytics in order to facilitate the method of administration.

Thrombolysis is the first choice therapy of AMI. Placebo-controlled studies have shown that thrombolytic therapy reduces mortality in AMI patients with symptoms onset of less than 12 hours as compared to non-thrombolytic therapy.

The pharmaceutical company initially responsible for this medicinal product, Boehringer Mannheim GmbH, transferred the MA to Roche Registration Limited (United Kingdom) on 8 April 1999.

2. Chemical, pharmaceutical and biological aspects

Rapilysin is presented in lyophilised form in a 20 ml glass vial with a chlorobutyl rubber closure and an aluminium cap. The new formulation for the finished product comprises:

- 2 vials containing each 10 U reteplase as active substance and tranexamic acid, di-potassium-hydrogen phosphate, phosphoric acid and polysorbate 80 as excipients,
- 2 pre-filled syringes with 10 ml of water for injection,
- 2 reconstitution devices and 2 needles.

The TAPS-formulation contains tranexamic acid instead of arginine as solubilizing agent. The abbreviation stands for tranexamic Acid, Phosphate, Sucrose.

The new formulation exhibits new properties in comparison with the current one: on stability, dissolution time and higher concentration thus optimizing the manufacturing process.

As a consequence of this change, the SPC, PIL and the labels have been revised. For the finished product, the specifications and routine methods, the manufacturing process including validation, and the stability report had to be adapted accordingly

Manufacturing process and process validation

The proposed (TAPS) formulation has been considered acceptable. The consequential changes to in-process controls and specifications for the finished product are justified and acceptable. The validation/revalidation of the analytical methods was done according to relevant guidelines and is acceptable. The changes in the manufacturing process resulting from the new formulation are justified, validated and acceptable. A shelf-life of 12 months at 25°C is covered by the submitted stability data and should be granted unless stability studies for a longer period of time are provided.

The reconstituted drug solution is chemically stable for 4 hours. Immediate use after reconstitution is recommended.

The manufacturing process essentially remains the same. Three major adaptations are introduced:

- Transfer of the arginine/phosphate drug substance solution into the TAPS-formulation via diafiltration over 10kD crossflow membranes, after addition of polysorbate 80 and pH adjustment.
- Omission of the additional filtration step before preparing the drug product solution (please refer to the Variation type I adopted in September CPMP 1997)
- Adaptation of the freeze-drying conditions to the physico-chemical properties of the new formulation.

The entire manufacturing process is monitored by appropriate in-process controls and in-process-specification limits and, where appropriate, validated.

At the time of the submission of the variation Type II "New TAPS-Formulation"-EMEA Procedure No. EMEA/H/C/II/04-, only one year stability data for the final product were available. In the meantime the 24-month stability data to support the extension of the shelf life of the final product to 24 months were established and have been accepted by the CPMP.

Original dossier submitted in 1995

The key manufacturing steps for Rapilyisin as described in the original dossier submitted by the applicant are summarised as follow:

Cell Line Production - To produce a modified recombinant t-PA molecule (reteplase) harboring the domains K2 and P, the host cell E. coli K12 is used. The coding sequence was integrated into the expression vector and the cell was cotransformed with the helper vector which improves the yield of level of reteplase and reduces the risk of translation errors.

The plasmids were introduced into E. coli by the calcium chloride technique. The expression of the protein is induced by addition of lactose. It leads to a non-glycosylated protein which accumulates inside the prokaryotic cells as inactive aggregates or inclusion bodies.

The rationale behind the construction of the plasmids and the production strain is convincing. Selection and characterisation of the host cell, the expression vector and the helper vector have been described satisfactorily. The characterisation is adequate and the stability throughout the fermentation process has been demonstrated.

Cell Banks - The description of the preparation and characterisation of the master cell bank (MCB) and the manufacturer's working cell bank (MWCB) have been provided. The identities of both cell banks are verified by analysing the genetic markers, antibiotic resistances, restriction enzyme analysis and expression of reteplase. The nucleotide sequences of the two independent colonies of the MWBC have been determined. Using Eur. Ph. methods, the absence of bacteria, yeast and fungi are demonstrated. The absence of bacteriophages is confirmed by using the plaque assay.

The preparation of the cell banks, their size and their storage ensure the continuous production of reteplase.

Fermentation and Harvesting - Fermentation process takes place on a scale of 1000 L production fermentor. A vial of the MWCB is successively expanded into 100 ml and 10 L cultures (both containing ampicillin and kanamycin) for inoculation of the production fermentor. At the end of fermentation, harvesting and isolation of inclusion bodies are performed and then followed by the refolding of the protein.

According to the Opinion of the CPMP on the granting of the MA dated May 23, 1996, Boehringer Mannheim agreed to submit to the EMEA experimental evidence for the need for the antibiotics ampicillin and kanamycin in the preculture. The justification provided was found satisfactory. However, the company announced the submission of a variation in order to produce the active substance without using antibiotics. On the basis of the data shown that the precultures of the E. coli host cells can be cultivated without antibiotics and the supporting documentation this variation was accepted by the CPMP.

Refolding Process - It is a crucial step of the production procedure to transform the protein into its active state. The inclusion bodies are solubilised and denatured under reducing conditions. Then the reducing agent is removed under denaturing conditions. Cysteine residues are modified with glutathione, producing a mixed disulfide solution which is used for the refolding reaction. The renaturation and purification process is shown to be consistent. The validation of the process is described in sufficient detail and the rationale behind the development is clearly documented.

Purification Procedures - They are essential for isolation of the active form of reteplase and elimination of incorrectly folded structures. They consist mainly of 4 steps: acidification plus filtration, affinity chromatography on Erythrina trypsin inhibitor (ETI)-Sepharose and two ion-exchange chromatographies.

Boehringer Mannheim applied for a minor change in the main purification step in the manufacturing process of the acive substance reteplase, the chromatography by the Erythrina trypsin inhibitor (ETI) as ligand purified from seeds of several Erythrina species. The change accepted by the CPMP refers to the use recombinant produced ETI (recSerETI) using the same E. coli production strain as is used to produce reteplase.

The equivalence of the recSerETI and ETI Sepharose column is demonstrated. The reteplase bulk drug substance purified on the new recSerETI Sepharose column is shown to be equivalent as compared to reteplase purified on the ETI Sepharose column.

Due to the need of additional full-scale fermentation runs the company has provided a time schedule for submission.

The active ingredient is obtained after concentration, diafiltration and sterile filtration. The bulk drug substance contains reteplase in an arginine buffer.. As mentioned before following the new formulation there is a transfer of the arginine/phosphate drug substance solution into the TAPS-formulation via diafiltration over 10kD crossflow membranes, after addition of polysorbate 80 and pH adjustment.

The specification and routine control tests are satisfactory. Reteplase has been well characterised. Validation of the production has been provided and it assures consistency of the yield, degree of purity and quality of the active ingredient. For potency testing, the determination of amidolytic activity and the clot lysis assay were adequately validated.

Finished Product - Release specifications for reteplase 10U lyophilisate are in reasonable accordance with the specifications for the bulk drug substance regarding the protein parameters.

The development of the formulation was well documented and justified. The manufacturing process is well validated and its consistency has been demonstrated. All methods used have been adequately validated.

Following the Marketing Authorisation of Rapilysin, a new improved formulation for the finished product with Tranexamic Acid intended to improve the manufacturing process was introduced by the MAH in March 1998.

Stability - Based on the full results obtained to date of the first Marketing authorisation, which cover 18 months of stability testing, a shelf life of 2 years at a temperature of 2° C to 25° C has been

requested and accepted. The company provided additional satisfactory data justifying the accepted shelf-life on a post-authorisation phase.

On the basis of the new formulation the stability is referred in the initial part of the pharmaceutical section.

The reconstituted product is chemically stable for 4 hours at 30° C but immediate use after reconstitution is recommended.

After the granting of the Marketing Authorisation the MAH claimed for an extension of the shelf-life of the active substance reteplase from 24 to 36 months with the currently accepted storage conditions at -70 ° C. On the basis of the data presented the variation applied was accepted.

3. Toxico-pharmacological aspects

Additional studies in this section have been provided by the MAH after the Marketing authorisation to prove the equivalence of the TAPs formulation vs the arginine-phosphate formulation.
The experiments undertaken to demonstrate local tolerance following intravenous injections and to prove identical fibrinolytic efficacy without any side effects upon hemostatic variables were performed in animals. The test strategies used were very closely comparable to thrombolytic treatment of patients suffering from AMI. Several animal experiments demonstrated equivalence to the standard arginine-phosphate-formulation in every aspect tested. It may be concluded from the animal experiments, that there is no difference between the two formulations with respect to efficacy and variables of adverse clinical outcome. The new TAPS-formulation has shown to be as safe and effective as the current formulation in the animals studied.

Based on the submitted data the CPMP has considered that the new formulation could be accepted without further clinical studies and an unfavourable effect of tranexamic acid is extremely improbable. The CPMP has considered that sufficient assurance has been given to prove that TAPS formulation does not alter the risk/benefit ration for Reteplase. However, the company should provide two additional in vitro studies as an undertaking measure after the adoption of this variation.

Original dossier submitted in 1995

The definition of the dose used during the preclinical and clinical development of reteplase was MU/kg or kU/kg. However, unit definition is in the process of being changed as follows: 1MU (old)=1U (new), 1kU (old)= 1mU (new). Throughout this report, old values have been used.

Pharmacodynamics

Primary pharmacology

The pharmacodynamic studies demonstrated that reteplase is a fibrin selective plasminogen activator, possibly interacting with fibrin via the lysine binding site. It had a relative, i.e. dose-dependent, fibrin selectivity similar to that of alteplase.

The in vitro fibrin binding was 30% of that of alteplase. In the absence of fibrin, similar enzyme kinetics towards plasminogen were observed for reteplase and alteplase. In the presence of fibrin, the catalytic activity of reteplase was enhanced (approximately 300 times) although being lower than that of alteplase during the same experimental conditions.

The in vitro clot lysis activity of reteplase was lower than that of alteplase but a higher potency of reteplase was demonstrated in ‘in vivo’ models (jugular vein thrombosis model in rabbits, canine model of coronary artery thrombosis), possibly due to its lower clearance rate.

In the canine model of coronary artery thrombosis, the double bolus injection regimen was shown to be superior to a single bolus dose.

Safety pharmacology

Studies monitoring the major organ systems revealed no unexpected effects. In conscious dogs, hypotension occurred at 1.4 MU/kg. Prolongation of bleeding time occurred at doses above 0.2 MU/kg, possibly related to inhibition of platelet aggregation. As expected for a variant of a human protein, guinea pigs and dogs formed specific antibodies to reteplase.

No relevant drug interaction studies have been performed with reteplase.

Pharmacokinetics

Tissue distribution was investigated in rats with ^{125}I-reteplase. Out of the total radioactivity, 16% (36% when using a lysosome specific label) was detected in liver and 20% in kidneys although only marginal amounts of radiolabel were recovered in bladder and urine. No studies of the distribution of reteplase in pregnant animals or into breast milk have been performed.

In rats, liver and kidneys were the main organs of active uptake and degradation of reteplase but about 30% of the compound was inactivated by protease inhibitors in plasma. Studies in rats with impaired renal and/or hepatic function indicated that the renal elimination was of more importance than the hepatic.

The single dose pharmacokinetics of reteplase were studied in different animal species including rat, dog, rabbit and non-human primate by monitoring changes of enzymatic activity in plasma. These studies demonstrated that clearance rates and $t_{1/2}$ were similar in all animal species (3-8 ml/min/kg and 7-15 min; respectively). Furthermore, these parameters were comparable to data from healthy volunteers and AMI patients (clearance: 5-6 ml/min/kg; $t_{1/2}$: 11-13 min). In animals, reteplase had a 3-8 fold lower total plasma clearance and thus, a longer $t_{1/2}$ than alteplase.

Pharmacokinetics following repeated doses have not been investigated in animals.

Toxicology

The toxicological programme included studies of single and 14 days repeat dose toxicity, reproductive toxicity, local tolerance and a complete battery of genotoxicity tests. Studies were performed in accordance with Good Laboratory Practice.

Single dose toxicity studies have been performed in rabbits (intravenous doses up to 4.2 MU/kg) as well as in rats and cynomolgus monkeys (intravenous doses up to 8.4 MU/kg). In these studies, no mortalities occurred. Major signs of toxicity were transient sedation.

Repeated dose toxicity was studied in rats, dogs and monkeys during 14 days administration of intravenous doses up to 3-5 fold the therapeutic dose. Main findings were hypotension and haemorrhages at the injection site which both possibly were related to the pharmacological effect of reteplase. Furthermore, as predicted for this type of product, dogs and monkeys showed immunogenic reactions.

Reproductive toxicity was studied in rats, monitoring effects on fertility and reproductive performance (Segment I) and following administration during the organogenetic period (Segment II). These studies did not reveal significant effects on fertility or signs of embryotoxicity. In rabbits, only a limited dose-range finding segment II study was conducted which resulted in vaginal bleeding, reduced pregnancy rate and abortions. Perinatal and postnatal toxicity (Segment III) was not investigated.

Based on these data, adequate information has been included in the Summary of Product Characteristics.

Genotoxicity was monitored in an adequate battery of in vitro (gene mutations in bacteria and mammalian cells, chromosomal aberrations in mammalian cells) and in vivo tests (micronucleus test in mice and rats). Reteplase showed no genotoxic potential in these studies.

Carcinogenicity studies were not performed, which is acceptable considering the intended use of reteplase

Local tolerance in rabbits given intravenous injections of clinically relevant concentrations was acceptable while local irritation was observed after paravenous injection.

A phase 1 environmental risk assessment revealed a negligible risk to the environment and therefore no further studies were required.

The pharmacological studies seemed to support an effect of reteplase relevant for the claimed indication. Enzymatic characteristics and fibrin binding potential were elucidated.

In the canine model of coronary artery thrombosis, the double bolus injection regimen was shown to be superior to a single bolus dose. Furthermore, in vivo data indicated a higher thrombolytic potency of reteplase with respect to alteplase, possibly due to its lower clearance rate.

Major findings in the toxicity studies were hypotension, related to the pharmacological effect of reteplase and formation of antibodies, which is expected for this type of product.

Safety factors based on dose comparisons were in the same range as those established for alteplase.

Questions raised regarding product interactions, in vivo inhibition, effects on platelet function, lack of repeated dose pharmacokinetic data and regarding reproductive toxicity were sufficiently addressed by the applicant.

4. Clinical aspects

The clinical trials with reteplase were performed in compliance with the European Good Clinical Practice guideline. As the use of placebo in AMI studies would be unethical the studies on reteplase were either uncontrolled or active controlled with streptokinase or alteplase (rt-PA).

Human pharmacology

The pharmacodynamic and pharmacokinetic properties of reteplase have been evaluated predominantly in open uncontrolled studies involving 70 humans.

Pharmacodynamics

Study M1 was an open trial involving 3 healthy volunteers per dose. The trial evaluated tolerability and effects on the haemostatic system of single bolus intravenous injections of reteplase (doses ranged from 0.1125 MU to 5.5 MU).

Study M2 was an open label trial involving 6 healthy volunteers per dosage group -5 allocated to treatment and 1 allocated to placebo - evaluating the effects of several single bolus intravenous injections of reteplase. Doses ranged from 0.9 mg to 7.2 mg (17 mg are equivalent to approximately 10 MU).

Study N1 was a single-blind cross-over trial involving 7 healthy volunteers. The trial evaluated tolerability and effects on the haemostatic system of a 6 MU single intravenous reteplase injection.

The results of these pharmacodynamic studies showed that single bolus doses of reteplase up to 6 MU produced only minor changes in the hemostatic system. Laboratory parameters did not show any specific changes which could be attributed to reteplase. No formation of antibodies was observed up to 4 weeks after reteplase administration. No effects on blood pressure, heart rate, cardiac rhythm or ECG-intervals were observed. Adverse events in healthy volunteers were rare and not clinically relevant.

Studies P1 (single bolus dose of 10 MU and 15 MU), **P2** (10 MU +5 MU double bolus dose) and **P3** (15 MU single bolus dose, 10 MU +5 MU double bolus dose, 10 MU +10 MU double bolus dose) aimed to provide information on pharmacodynamics, pharmacokinetics and dose response in AMI patients. A description of each study is provided in the dose finding studies.

Reteplase showed a dose-dependent reduction in plasma concentrations of fibrinogen, plasminogen as well as a dose dependent increase in fibrin degradation products, measured as D-Dimers, and fibrinogen degradation products. Reteplase was well tolerated with regard to effects on vital signs and laboratory parameters.

The influence of reteplase on the hemostatic and fibrinolytic system suggests that reteplase is a moderate fibrin-selective plasminogen activator which should be used with aspirin and heparin since

its administration results in a procoagulant state which lasts at least 2 days after the reteplase dose regimen claimed for.

The pharmacodynamic effects of reteplase in patients with AMI were considered to be sufficiently evaluated in order to conduct further trials with the 10 MU + 10 MU dosage regimen. Furthermore sufficient data were available for doses which might represent the upper limit (15 MU as a bolus) and the lower limit (10 MU) of the therapeutic dose.

Pharmacokinetics

Plasma concentrations of reteplase were measured by using both enzymatic plasminogen activity assay and immunological ELISA antigen technique. Plasma activity concentrations were lower than the measurements of the respective antigen concentrations probably due to complex formation with plasma inhibitors. This finding has been reported for rt-PA as well.

The two measurements methods resulted in two different plasma concentration-time curves. The plasma enzymatic activity concentration showed a monoexponential pharmacokinetic profile whereas the plasma concentrations of reteplase antigen revealed a biexponential pharmacokinetic profile.

The conventional calculated pharmacokinetic parameters were as follows: peak concentration (Cmax, U/ml), area under the curve (AUC, U.ml.h), volume of distribution (litres), alpha half-life (t½, minutes), beta half-life (t ½, hours), clearance (ml/min).

The above-mentioned **M1**, **M2** and **N1** pharmacodynamics studies supplied also pharmacokinetic data in healthy volunteers.

With respect to the 6 MU dosages, clearance values were 371 ml/min for reteplase enzymatic activity and 183 ml/min for reteplase antigen. The respective alpha t ½ were 11 minutes and 14 minutes.

A dose dependent linear increase of AUC as measured by the enzymatic activity was observed after single intravenous bolus doses ranging between 2.2 MU and 5.5 MU reteplase. The Cmax values showed a trend to a linear increase for doses between 2.2 MU and 6.6 MU.

These studies demonstrated a lower total clearance and a longer activity half-life for reteplase as compared to alteplase.

Study P1 and **P3** described below supplied also pharmacokinetics data in AMI patients.

The AUC and Cmax values increased with doses between single bolus 10 MU and double bolus 10 + 10 MU. The values of clearance as well as the respective t ½ did not show any relevant differences within the dose range of 10 MU to 10 MU + 10 MU. The elimination pattern remained the same within the same dose range.

With respect to the 10 MU + 10 MU dosage regimen used in further therapeutic trials, clearance values were 275 ml/min for reteplase enzymatic activity and 115 ml/min for reteplase antigen. The respective alpha t ½ was 12.4 minutes and 17 minutes. The beta t ½ for reteplase antigen was 5.5 hours.

Urinary elimination of reteplase was less than 4%.

Two days after thrombolysis reteplase antigen and activity had virtually returned to baseline.

The pharmacokinetic properties of reteplase have been sufficiently evaluated in Caucasian and Asian healthy volunteers and Caucasian patients with AMI. The pharmacokinetic results obtained in humans are consistent with the pharmacokinetic animal results.

The pharmacokinetic studies confirmed reteplase has a lower clearance and a longer t ½ than t-PA. Studies showed that patients with AMI generally have a lower clearance and a longer t ½ than the healthy volunteers as a function of their clinical condition.

The pharmacokinetic profile has not been investigated in patients with severely impaired liver function and renal dysfunction. Since the experimental data suggest that both organs are involved in the metabolism and excretion of reteplase, these patients should be excluded from reteplase until additional data are available.

Interactions between reteplase and acetylsalicylic acid and/or heparin respectively were investigated in preclinical studies. No clinical studies aimed specifically to evaluate interactions between reteplase and other medicinal products.

On the basis of experimental findings and clinical experience gathered from other thrombolytic agents, the potential and relevant interactions with respect to risk of bleeding are mentioned in the Summary of Product Characteristics.

Dose finding studies

Four clinical studies were performed in order to optimise the reteplase dosage regimen in patients with AMI. Major evaluation criteria were angiographic changes of coronary perfusion using the TIMI criteria (Thrombolysis in Myocardial Infarction).

These are defined as follows: TIMI flow 0 (no perfusion), flow 1 (penetration without perfusion), flow 2 (partial perfusion), flow 3 (complete perfusion).

Efficacy endpoints were patency (TIMI 2- or TIMI 3 flow) and TIMI 3 flow at 90 minutes after administration of the first reteplase bolus. Based on the results of the GUSTO I angiographic substudy, TIMI 3 flow has been shown to be correlated with survival rate and favourable prognosis in patients receiving thrombolytic therapy for AMI.

A total of 219 patients with suspected myocardial infarction were enrolled in 3 open uncontrolled studies (**P 1, 2, 3**) and treated with an intravenous single or double bolus regimen of 10 MU, 15 MU, 10 + 5 MU or 10 + 10 MU:

Study P1 was a sequentially designed multicentre trial evaluating 10 MU and subsequently 15 MU reteplase administered as single bolus dose in a total of 142 patients with AMI (symptom onset ≤ 6 hours). The initial dose of 10 MU was selected on the basis of previous experimental and human pharmacology data which suggested efficacy and acceptable safety with respect to bleedings.

The primary endpoint was 90-minutes patency, secondary target criteria included patency assessed at 30, 60 minutes and at day 14-21.

The results with respect to the primary and secondary target criteria showed that reteplase 15 MU produced a 9 % higher patency rate at 90 minutes than the 10 MU dose (76% versus 67%)

If only the TIMI 3 rates were considered the results were 69% and 52% respectively.

There was a trend to more early reocclusions and serious adverse events in the 15 MU dose group but these results did not seem to be different as compared to other thrombolytic agents. Tests for antibodies against reteplase were found to be negative.

In summary, the results showed that 15 MU reteplase dose seemed to have a better benefit-risk ratio than the 10 MU dose.

Study P2 was a sequentially designed multicentre study evaluating an intravenous 10 + 5 MU double bolus dose regimen of reteplase in 52 patients with AMI (symptom onset ≤ 6 hours).

Based on the results of study P 1, the double bolus regimen was chosen in order to increase efficacy and decrease the risk of adverse events, in particular bleedings.

On the basis of the pharmacokinetic characteristics (e.g. the half-life; furthermore, the Cmax value of the 10+5 MU dose was lower than that of the 15 MU dose, whereas the AUCs were comparable), the second reteplase bolus was administered 30 minutes after the first bolus.

The results of this trial showed that the 10 + 5 MU regimen produced comparable patency - and reocclusion rates to those of the 15 MU dose.

Since there was no indication for an increased risk of haemorrhages for the 10 + 5 MU dosage regimen, study P 3 was conducted with a higher dose of the second bolus i.e. the 10 + 10 MU double bolus regimen of reteplase.

Study P 3 evaluated the effects of 3 different doses and regimens of reteplase (15 MU, 10 + 5 MU, 10 + 10 MU) in 25 patients with AMI (onset of symptoms ≤ 6 hours) in a randomised fashion.

In this study the 15 MU dose seemed to be most effective regarding angiographic variables but due to the small sample sizes these results were difficult to interpret. Thus, all three dosage regimens were included in the subsequent larger RAPID I trial to better define the optimal dosage regimen of reteplase.

No antibodies against reteplase were detected at the time of discharge.

RAPID I was an open, randomised, controlled, parallel group multicentre study involving 606 AMI patients (onset of symptoms ≤ 6 hours). The angiograms were read blinded by two core laboratories. The trial compared the aforementioned (see P 3) dosage regimens of reteplase (15 MU, 10 + 5 MU, 10 + 10 MU) with the former standard regimen of rt-PA (3 hours infusion).

The primary efficacy endpoint was 90- minute patency of the infarct-related coronary artery.

Secondary endpoints were patency and TIMI 3 rates at 30 and 60 minutes after thrombolysis and at follow-up (≥ 7 days). Other secondary efficacy- and safety variables included reocclusions, coronary interventions, ejection fraction, clinical events and outcome, strokes, bleedings, antibodies to reteplase and survival status (monitored at short-term and up to 6 months after thrombolysis).

Of the 3 reteplase dosage regimens, only 10+10 MU produced a statistically significant higher TIMI 3 rate than alteplase, but no significant difference was seen in patency rates at 90 minutes.

During follow-up, patency- and TIMI 3 rates were significantly better for the 10+10 MU reteplase group as compared to alteplase.

The reocclusion rates (patent artery at 90 minutes after thrombolysis but occluded artery at follow-up angiography) and coronary intervention rates were comparable in the 10+10 MU reteplase and alteplase groups.

During follow-up the 10+10 MU group had a comparable incidence of short-term clinical events than the other reteplase regimens or alteplase, as well as a statistically significant better ejection fraction than the latter.

The number of serious adverse events and the respective drop-out rate were comparable between 10+10 MU reteplase and alteplase.

The rate of patients suffering from bleedings and receiving transfusions was comparable between alteplase and the 10+10 MU reteplase group.

The short-term and long-term mortality rates were comparable between the reteplase regimens and the alteplase group.

Three deaths considered positively related to thrombolytic therapy (e.g. due to intracranial haemorrhage or hemopericardium) occurred in the alteplase group whereas one of such death was observed in each reteplase dosage regimen.

During the short-term follow-up, 3.9% of the alteplase treated patients suffered strokes 2.6 % of which were haemorrhagic. Patients treated with the 10 + 10 MU and the 10 + 5 MU reteplase dosage regimen did not suffer any stroke but one patient in the 15 MU group had intracranial bleeding. The differences in stroke rates between alteplase and the 10 + 10 MU (as well as the 10 + 5 MU) dosage regimen were statistically significant.

No clinically relevant differences were observed between the four treatments with respect to non-cerebral bleedings.

No antibody formation to reteplase could be detected during short-term and long-term follow-up.

Based on the angiographic results this study showed that the dosage regimen of 10 + 10 MU reteplase was most effective. The overall safety profile and especially the incidence of intracerebral bleedings suggest that the benefit/risk ratio of the 10 + 10 MU double bolus regimen was at least equal to that of the former alteplase standard regimen.

Consequently the 10 +10 MU reteplase dosage regimen was selected for the subsequent confirmatory trials.

Confirmatory trials

Two randomised active-controlled, parallel group, multicentre trials implying a statistically confirmatory approach were performed in 6334 patients with AMI (symptom onset less or equal to 12 hours) in order to evaluate efficacy in terms of and angiographic and clinical outcome measures and to determine the safety of reteplase (RAPID II, INJECT).

RAPID II trial was an open, randomised, alteplase controlled parallel group multicenter study involving 324 AMI patients. Though the study was conducted in an open label design, the reading of the angiograms was performed by a central core laboratory blinded to all treatment groups.

This trial compared reteplase 10+10 MU with the accelerated alteplase dosage regimen (15 mg bolus injection followed by a 0.75 mg/kg infusion over 30 minutes and a subsequent 0.50 mg/kg infusion over 60 minutes).

The primary efficacy endpoint was patency of the infarct-related coronary artery at 90 minutes after the initiation of thrombolytic therapy.

Secondary endpoints were patency- and TIMI 3 rates at 30, 60 minutes, day 5 after thrombolysis and at angiographic follow-up (mean value 6.4 days).

Other secondary efficacy and safety variables evaluated at short-term included ejection fraction, reocclusions, coronary interventions, relevant clinical events, strokes, net clinical benefit, whereas survival status was assessed 35 days and 6 months after thrombolysis.

The 10+10 MU reteplase group had statistically significant higher 90-minute patency and TIMI 3 rates than the alteplase group (differences of 10 % and 15% respectively). As already observed in the RAPID GUSTO and I I trials, patients with TIMI 3 flow had a significantly lower mortality rate as compared to patients with TIMI 0-1.

Though not statistically significant, the in-hospital coronary reocclusion rate was 2% higher but the rate of coronary interventions within 35 days was significantly lower for reteplase.

No significant differences between the two treatments were found with regard to ejection fraction, relevant clinical events including shock, reinfarction, ischaemia/angina, congestive heart failure, malignant arrhythmias and hypotension.

The rates of patients suffering adverse events and bleedings requiring transfusions were comparable in the alteplase and reteplase groups.

The percentage of patients who suffered stroke within 35 days of follow-up was 3 % in the reteplase group and 4.5% in the alteplase group. This difference, as well as that for haemorrhagic strokes (1.2 % and 1.9%) was not statistically significant.

The differences in 35-day mortality rates (4.1% for reteplase and 8.4% for alteplase) and 6-month mortality rates (6.1% for reteplase and 10.3% for alteplase) were not statistically significant.

The death of 3 patients in the alteplase group was judged to be positively related to the medication but this was not the case for any death in the reteplase group.

The difference in the “net clinical benefit” (retrospectively defined combined endpoint of death or continuing disability induced by stroke at day 35 after thrombolysis) was not statistically significant (5.3 % for reteplase and 9% for alteplase).

Hence, the RAPID II study resulted in a significant better benefit risk ratio of 10+10 MU reteplase as compared to the accelerated rt-PA regimen regarding patients with AMI (symptom onset less or equal to 12 hours).

INJECT was a randomised, double-blind, streptokinase-controlled parallel group multicentre trial involving 6010 patients with AMI. The trial was designed to determine whether reteplase was at least “equivalent” (within 1 %) to a standard regimen of streptokinase with regard to all cause mortality at day 35 after thrombolysis (primary efficacy criterion).

All cause mortality at 6 months after thrombolytic therapy as well as reinfarction, heart failure, stroke and coronary interventions during the first 35 days following thrombolysis were investigated as major secondary efficacy criteria.

Other secondary efficacy and safety variables included cardiovascular events like for instance intracerebral bleedings, cardiogenic shock, clinically relevant arrhythmia, hypotension, as well as bleedings, allergic reactions, antibody formation to reteplase and "net clinical benefit".

The majority of the patients -and approx. the same proportion in both treatment groups- received i.v. heparin (48-72 hours) and low dose ASA (until discharge) concomitantly with thrombolytic therapy.

The results concerning the primary endpoint 35-day all cause mortality were 9.53 % in the streptokinase group (285 deaths) and 9.02 % (270 deaths) in the reteplase group.

The difference of -0.51 % in favour of reteplase was not statistically significant (95 % CI: -1.98 % to 0.96 %). On the basis of the statistical approach these results indicated, that reteplase was at least equivalent to streptokinase with respect to the 35-day all cause mortality rate.

Mortality rates at 6 months were 11.02 % for reteplase and 12.05% for streptokinase. The difference of 1.03% was not statistically significant as well.

The in-hospital stroke rate was 1.21% for reteplase and 1.01% for streptokinase. The difference in haemorrhagic strokes (reteplase 0.78%, streptokinase 0.37%) was statistically significant. Furthermore the reteplase treatment was associated with a significantly higher stroke rate in patients with an admission systolic blood pressure of greater than 160 mm Hg (10% of total study population).

However, the "net clinical benefit" (retrospectively defined combined endpoint of death by day 35 day or continuing disability from an in-hospital stroke) was 9.19 % for reteplase and 9.79% for streptokinase, although this difference was not statistically significant.

Reteplase resulted in lower cardiovascular event rates than streptokinase in-hospital as well as at day 35 after thrombolysis.

The incidence of recurrent myocardial infarction was similar in the two treatment groups but statistically significant differences in favour of reteplase were observed with regard to the frequencies of atrial fibrillation, asystole, cardiac shock, hypotension and heart failure (this difference was also reflected in a reduced need for heart failure drugs).

Significantly fewer reteplase treated patients than streptokinase treated patients (22.5% versus 24.9%, p=0.02) reported serious adverse events.

The rates of bleeding events either serious or non serious were comparable in the two treatment groups. 15.21% of the reteplase treated patients and 15.48% of those treated with streptokinase reported bleedings in hospital. 1.11% and 1.35% respectively were considered to be serious and 0.64% of the reteplase group as well as 0.98% of the streptokinase group needed transfusions.

Formation of antibodies to reteplase could not be detected in 1934 patients whose blood samples were examined up to 5 weeks after thrombolysis.

Fewer allergic reactions were observed for reteplase as compared to streptokinase (1.13% for reteplase and 1.77% for streptokinase).

3 patients in the reteplase group and 10 patients in the streptokinase group reported fatal or life threatening which were classified as being unexpected.

At present, one patient enrolled in the ongoing GUSTO III trial has experienced an anaphylactoid/ anaphylactic reaction which was judged to be positively related to reteplase and of which the outcome was fatal in the setting of AMI.

In summary, the safety profile of reteplase was satisfactory and seemed to be at least comparable to that of streptokinase. On the basis of the INJECT data the benefit/risk ratio of reteplase can be considered to be at least equal to that of streptokinase.

5. Conclusion

Reteplase is a new recombinant thrombolytic agent produced by recombinant DNA technique.

The manufacturing and production process has been shown to be acceptable in terms of quality and reproductibility. A batch-to batch consistency has been shown for the active ingredient and the

finished product. After the granting of the Marketing Authorisation the company has submitted satisfactory results of additional studies including stability data.

The extent and quality of the preclinical programme were considered acceptable for the use of reteplase in the treatment of AMI.

The dose finding for reteplase has been performed thoroughly and adequate efforts were made to optimise the dosage regimen applied for, as well as to estimate the benefit-risk ratio of doses higher and lower than that recommended.

Clinical efficacy and safety of reteplase were established on the basis of angiographic measurements and all cause mortality rate (at day 35 after thrombolysis) from adequate confirmatory randomised active controlled trials using the currently acknowledged standard thrombolytic agents alteplase and streptokinase used as comparators. Both aforementioned studies showed a benefit risk ratio of reteplase at least as equal to alteplase based on angiographic data, and streptokinase based on 35-day all cause mortality results.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Rapilysin was favourable in the treatment of thrombolytic therapy of acute myocardial infarction within 12 hours after the onset of acute myocardial infarction symptoms.

Rapilysin is presented as a powder for injection administered as a 10 U + 10 U double bolus intravenous injection. Each bolus is administered as a slow intravenous injection over 2 minutes whereas the second bolus is administered 30 minutes after the first bolus.

6. Post-Authorisation

The *GUSTO III clinical trial* in approximately 15,000 patients comparing reteplase with the accelerated dose regimen of alteplase (2:1 randomisation reteplase:alteplase) did not show statistically different results for the primary endpoint of 30-day mortality (reteplase: 7.47%, alteplase 7.23%, p = 0.61) or for the combined endpoint of 30-day mortality and non-fatal disabling stroke (reteplase: 7.89%, alteplase 7.88%, p = 0.99). Overall stroke rates were 1.64 % for reteplase and 1.79% for alteplase. In the reteplase group, 49.4% of these strokes were fatal and 27.1% were disabling. The corresponding figures for the alteplase group were 33.0% and 39.8%, respectively.

In December 1999 the MAH informed the EMEA of the need to trigger an Urgent Safety Restriction (USR) due to a risk to public health, based on the reports of flocculation of Rapilysin when given concomitantly with heparin in the same intravenous line. As a result, sections 4.2, 4.4, 4.5, 6.2, 6.3 and 6.6 of the SPC were updated, in order to highlight the incompatibility of Rapilysin with heparin.

In March 2001 the wording of the therapeutic indication for all fibrinolytic agents was changed following a recommendation from CPMP In October 2000. The new wording states that Rapilysin is indicated for the thrombolytic treatment of suspected myocardial infarction with persistent ST elevation or recent left Bundle Branch Block within 12 hours after the onset of AMI symptoms".

In August 2003 the contraindication in diabetic patients (section 4.3 of the SPC) was deleted following the publication of a CPMP position statement concerning the use of iv fibrinolytics in diabetic patients.

Reports of adverse drug reactions

Frequently reported sequelae of MI and/or thrombolytic administration are recurrent ischemia/angina, hypotension and heart failure/pulmonary oedema. Arrhythmias, cardiac arrest, cardiogenic shock and reinfarction have been ocassionally reported and there have been rare reports of mitral regurgitation, pulmonary embolism, other systemic embolism/cerebral embolism and ventricular septal defect. These cardiovascular events can be life-threatening and may lead to death.

Adverse events related to the nervous system (e.g. epileptic seizure, aphasia, speech disorder, delirium, acute brain syndrome, agitation, confusion, depression, psychosis) have been reported in isolated cases. Ischaemic or haemorrhagic cerebrovascular events may be contributing or underlying conditions.

Hypersensitivity reactions have been uncommonly reported, and there have been isolated reports of serious anaphylactic/anaphylactoid reactions. Available evidence does not indicate an antibody-mediated origin of these hypersensitivity reactions.