2/27/96

Review of the Pharmacokinetics of BM 06.022

PLA 95-1167

Sponsor: Boehringer Mannheim Pharmaceutical Corporation

Product: recombinant human plasminogen activator; reteplase

Indication: Lysis of thrombi following AMI

Abbreviations: Cl = clearance; t-PA = tissue plasminogen activator; CI = confidence interval

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Introduction

BM 06.022 is a simple deletion variant of t-PA which retains the essential structures for fibrinolytic activity and possess a decreased rate of clearance. The protease domain is retained for enzymatic activity and the kringle 2 domain for fibrin selectivity.

The therapeutic advantage of BM 06.022 was established relative to tissue-type plasminogen activator (t-PA). t-PA is widely used as a thrombolytic drug for the treatment of thromboembolic disease. t-PA contains domains involved with plasma clearance and biological activity. These include finger domain, growth factor domain, 2 kringle domains and the protease domain. t-PA has a rapid clearance resulting from an initial t1/2 of about 3. min. The rapid clearance of t-PA appears due to at least 2 different receptor mediated mechanisms in the liver. One pathway involves the mannose receptor located mainly on liver endothelial cells, and the other is dependent on receptors on hepatic

parenchymal cells. These pathways appear to depend on the high mannose-type oligosaccharide on kringle 1 of t-PA and on a second structure which is not the carbohydrate moiety. To prolong the plasma t1/2, BM 06.022 was produced using E. Coli. which precluded gylcosylation. Similarly, clearance decreased because the oligosaccharide side-chains have not been expressed. Fibrin selectivity for BM 06.022 was retained after the deletion of the finger domain because deglycosylation was shown to increase fibrin binding of the kringle 2 domain.

Pharmacokinetics Review

Clinical Studies:

Study of the pharmacokinetics of BM 06.022 and of its effects on coagulation parameters. . Jan 28, 1991

The pharmacokinetics, hemostatic properties, and tolerability of BM 06.022 were studied in a single-blind, randomized, cross-over study using healthy male adults (N = 7). The washout period was 7 days. A dose of 6 MU vs vehicle was administered over a 2 min period in a standard volume of 10 ml. Lots 821 563 A and 821 564 A for BM 06.022 and 821 560 A and 821 561 C for vehicle (0.5 arginine-phosphate buffer, pH 7.1) were used in the study. No meaningful changes were found in hematological, clinical chemistry or urinalysis following administration of B. Using an ELISA technique, no antibodies were found in plasma samples taken 2 and 4 weeks after administration of BM 06.022. Regarding coagulation parameters, the lowest resultant concentration as a per cent age of preinjection levels for fibrinogen, plasminogen, and alpha2-antiplasmin were 97.82 ± 9%, 83.2 ± 1%, 63.5 ± 3.3%. Fibrinolysis was observed as an increase in fibrin D-dimers to a maximum concentration of 1016 ± 216 ng/ml. Fibrinogen degradation products reached a maximum concentration of 555 ± 152 ng/ml. Activated partial thromboplastin time, prothrombin time, and thrombin time were unchanged. Ninety minute bleeding time levels remained within normal limits. Platelet count was unaffected.

Pharmacokinetic analysis supported by a pharmacodynamic

analytical assay[1] yielded an AUC of 272 ± 9 IU h/ml, clearance of 371 ± 13 ml/min, and a t1/2 of 11.2 ± 0.4 min. Half-life was computed from 0.11 h. Analysis of pharmacokinetics through an antigen based immunoassay revealed an AUC of 994 ± 102 ng h/ml, clearance of 183 ± 15 ml/min and a terminal t1/2 of 173 ± 33 min (computed from 1.9 h). An initial t1/2 was reported as 13.9 ± 0.7 min. The large differences found for the various pharmacokinetic endpoints is due to the different analytes being measured. The pharmacodynamic analytical assay measures the activity of BM 06.022 whereas the immunoassay determines levels of BM 06.022 and BM 06.022 complexed to an endogenous inhibitor, plasminogen activator inhibitor (PAI-1). Therefore, the pharmacokinetics of the immunoassay may reflect a mixture of assayed substances.

Decrease of plasminogenolytic activity over time after addition of BM 06.022 to human blood or plasma in vitro. L 14. Aug 28, 1991.

BM 06.022 and tPA were incubated at a concentration of [ ], and in human citrate-blood and -plasma over 6 hours. The concentration of BM 06.022 remained constant over time but at lower than nominal concentration. The activity of BM 06.022 decreased in EDTA-blood and -plasma as well as in citrate-blood and -plasma. Compared to tPA, the decrease of plasma activity of BM 06.022 was more rapid and achieved statistical significance in some instances. These findings suggest that blood and plasma components inactivate BM 06.022 at a more rapid rate than tPA.

In vitro stability of BM 06.02 in human plasma. L 18. May 23, 1994.

Varying concentrations of BM 06.022 were incubated in plasma for 1 to 150 min. A dose dependent decline was observed in BM 06.022 activity. Half-life decreased from 72 min at 0.3 μg/ml to 38 min at 10 μg/ml. SDS-polyacrylamide gel electrophoresis followed by fibrin autography showed the generation of several BM 06.022 complexes which were precipitated with antibodies against C1-

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For specific methods see vol 46 page 05 005228 of the PLA.

inactivator, alpha2-antiplasmin and alpha1-antitrypsin. SDS page and immunoblotting showed that single-chain BM 06.022 was rapidly (<45 min) converted into its

Changes in t1/2 with increased concentrations of BM 06.022 may be the result of generating a 2 chain form which is inactivated at a faster rate than the single chain form.

Study on the tolerability of BM 06.022 it effects on hemostatic parameters and determination of pharmacokinetic parameters. Jan 18, 1991.

An open label phase 1 study was conducted in healthy male subjects (N = 18). Pharmacokinetics, hemostatic parameters, bleeding time, tolerability and platelet aggregation were measured. Injections were made iv over 2 minutes in a volume of 10 mol at doses of 0.1125, 0.55, 2.2, 3.3, 4.4 and 5.5 MU or 0.19, 0.95, 3.81, 5.71, 7.61, and 9.52 mg (N = 3/dose). Residual plasma concentrations of fibrinogen, plasminogen, and alpha2-antiplasmin 2 h after iv injection of 5.5 MU of BM 06.022 was 100 ± 1%, 87 ± 3% and 79 ± 3%. A slight dose dependent effect was observed on reduction of alpha2-antiplasmin. Plasminogen activator inhibitor was reduced after injection of BM 06.022 indicating an interaction. The plasma concentration of fibrin D-dimers increased in a dose dependent fashion after iv administration of BM 06.022. Ninety minute bleed time were within normal limits for all except 1 volunteer dosed at 5.5 MU (12 min). Platelet aggregation induced by 1 μg/ml collagen or ADP was slightly inhibited at doses of 3.3 and 4.4 MU by 6 to 8% or 14 to 17%, but was not reduced at 5.5 MU of BM 06.022. At all doses studied, no proportional effects of BM 06.022 on fibrinogen was observed. At the higher doses, a slight reduction in the plasma concentration of plasminogen was observed. A slight dose dependent decrease was observed on plasma alpha2-antiplasmin. Plasma concentrations of anti-thrombin III and alpha2-macroglobulin did not decrease. Partial thromboplastin, prothrombin, and thrombin time remained unchanged when compared to preinjection levels. PAI activity was clearly reduced after injection of BM 06.022 without any evidence of a rebound effect at 24 h. Measurement of PAI-antigen was inconclusive. Fibrin D-dimers increased in a dose dependent manner after BM 06.022, but normalized after 24 h and only slightly exceeded the normal range

(125 - 425 ng/ml) following BM 06.022. A slight, transient reduction of platelet aggregation was observed. Except for one individual at the highest dose (normally <10 min in the one individual 12 min), platelet aggregation returned to normal as measured by 90-min bleeding times. Platelet count did not decreased after BM 06.022. Nor antibodies were detected against BM 06.022 at 2 and 4 weeks and 6 months. No meaningful effects were observed on ECG, heart rate, and blood pressure after iv bolus injection over 2 minutes.

AUC values for tPA like activity showed a dose dependent linear increase starting a 2.2 MU of BM 06.022, but were nonlinear if lower doses were considered. Lower dose (0.1125 and 0.55 MU) demonstrated loss of tPA like activity presumably through binding to plasminogen activator inhibitors. Individual plasma concentration and an analysis of the data are listed in the accompanying tables (10.35, 10.36, 10.37. Plasma clearance for antigen dependent measurements were 33% lower when compared to the clearance as measured by activity. The time-plasma profile for antigen exhibited a biphasic character which was not observed for activity dependent measurements.

Pharmacokinetics were measured for activity using an indirect spectrophotometric assay for tPA activity. Antigen levels were measured with a ELISA method. A non-compartmental method was used to determine pharmacokinetic endpoints. AUC was computed using the trapezoidal rule for the period from 0 to 24 h and extrapolated to infinity. The limit of detection for the antigen assay was insufficient to determine pharmacokinetic endpoints which required extrapolation to infinity since a terminal elimination rate could not be computed. Therefore, the sponsor elected not to report a number of pharmacokinetic endpoints Cl, t1/2, and Vz) based on the antigen assay at the 2 lower doses.

Although these values would have some utility in understanding the overall pharmacokinetics of BM06.022, they are not essential in deriving a therapeutic dose. The following pharmacokinetic endpoints were determined: Cmax, Tmax, AUC, Cl, t1/2, and the volume of distribution associated with the terminal elimination rate (Vz). Vz was determined as the apparent volume of distribution when computed as Cl/lamba$_z$ (lamba$_z$ is the elimination rate constant calculated by log-linear regression using the terminal data points). Although the sponsor maintains

Table 10.35

BOEHRINGER MANNHEIM GMBH KLINISCHE PHARMAKOLOGIE - KINETIK
PROGRAM: RDFCL DATE OF COMPUTATION: 18-MAY-90

PROJECT: BM 06.022 ; ; DIFFERENCE TO BLANK VALUE
BATCH : 821 563 A (1 MU-AMP.) / 821 564 A (5 MU-AMP.)
DOSE : VARYING MU (3 VOL./ DOSE) i.v.INJECT. OVER 2 min.
ASSAY : t-PA ACTIVITY IN PLASMA ; CUT AT 2 hours

NUMBER OF SUBJECTS: 18

A U C BY TRAPEZOIDAL RULE , EXTRAPOLATION WITH RATE CONSTANT lambda z
LOG.-LIN.REGRESSION; COMPUTATION BEGINNING AT 0.9 (h)

	SUBJECT	DOSE	C max	t max	A U C 0.- 2.[h]	A U C	CL	lambda z	t 1/2	V z
		MU	IU/ml	[h]	[IU*h/ml	[IU*h/ml	ml/min	[1/h]	[h]	[l]
1	BA.F. M	0.11	3.00	0.250	1.09	2.42	756.09	0.40547	1.710	112.00
2	SO.K. M	0.11	12.20	0.083	2.24	< < <	< < <	< < <	< < <	< < <
3	MU.M. M	0.11	9.00	0.083	2.31	< < <	< < <	< < <	< < <	< < <
4	SC.W. M	0.55	73.50	0.083	8.73	8.80	1041.91	1.09861	0.631	56.90
5	HA.M. M	0.55	35.10	0.083	9.09	10.01	915.32	0.69315	1.000	79.21
6	GE.S. M	0.55	32.50	0.033	10.16	10.24	895.07	2.07944	0.333	25.83
7	RI.G. M	2.20	252.90	0.167	68.85	69.05	531.04	1.38629	0.500	22.98
8	RO.R. M	2.20	422.90	0.050	96.66	96.66	379.33	3.69651	0.190	6.51
9	AL.H. M	2.20	350.00	0.083	91.95	92.03	398.43	4.06044	0.171	5.89
10	ST.B. M	3.30	328.50	0.083	114.31	114.44	480.59	3.36730	0.206	8.56
11	MA.S. M	3.30	212.70	0.083	61.87	61.95	887.83	2.26060	0.306	23.40
12	SC.K. M	3.30	453.60	0.083	110.16	110.43	464.01	2.19722	0.315	12.68
13	MU.R. M	4.40	1016.30	0.083	252.43	253.31	289.50	2.21469	0.313	7.84
14	DI.K. M	4.40	789.10	0.083	213.86	213.98	342.71	3.52636	0.197	5.83
15	KN.B. M	4.40	548.00	0.083	172.52	172.67	424.71	3.28091	0.211	7.77
16	MA.L. M	5.50	680.30	0.083	257.23	257.57	355.89	2.78148	0.249	7.68
17	SE.C. M	5.50	1290.60	0.083	405.32	405.51	226.01	3.39451	0.204	3.99
18	AR.J. M	5.50	958.00	0.083	272.55	272.86	335.94	2.58714	0.268	7.79

0 0 0 = AT 2.0 [h] THE THEORETICALLY CONCENTRATION FROM THE REGRESSION LINE IS USED
< < < = INVALID HALF-LIFE < 0. NO EXTRAPOLATION OF AREA

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appendices

Table 10.36

PROJECT: BM 06.022 ; ; DIFFERENCE TO BLANK VALUE
BATCH : 821 563 A (1 MU-AMP.) / 821 564 A (5 MU-AMP.)
DOSE : VARYING MU (3 VOL./ DOSE) i.v.INJECT. OVER 2 min.
ASSAY : t-PA ACTIVITY IN PLASMA ; CUT AT 2 hours

A U C BY TRAPEZOIDAL RULE , EXTRAPOLATION WITH RATE CONSTANT lambda z
LOG.-LIN.REGRESSION; COMPUTATION BEGINNING AT 0.9 [h]

MEAN - VALUES (N=3) (EXCEPT DOSE 0.11: AUC, CL, t 1/2, V z: N=1)

DOSE [MU]	C max [IU/ml]	t max [h]	A U C [IU*h/ml] # # #	CL [ml/min]	t 1/2 [h]	V z [l]
0.11	8.33	0.139	2.42	756.89	1.710	112.00
0.55	47.03	0.066	9.68	950.77	0.655	53.99
2.20	342.20	0.100	85.91	436.27	0.290	11.79
3.30	331.60	0.083	98.27	610.94	0.276	14.91
4.40	784.47	0.083	213.32	352.31	0.240	7.15
5.50	976.30	0.083	312.01	305.95	0.240	6.49

= AT 2.0 [h] THE THEORETICALLY CON

Table 10.37

BOEHRINGER MANNHEIM GMBH KLINISCHE PHARMAKOLOGIE - KINETIK
PROGRAM: RDSER. DATE OF COMPUTATION: 1-JUN-90

PROJECT: BM 06.022 : : 1 MU = 1.73 mg
BATCH : 821 563 A (1 MU-AMP.) / 821 564 A (5 MU-AMP.)
DOSE : VARYING MU (3 VOL./ DOSE) i.v.INJECT. OVER 2 min.
ASSAY : BM 06.022 - ANTIGEN " E L I S A "

TABLE OF CONCENTRATIONS [ng/ml]

SUBJECT	DOSE [mg]	TIME [hours] 0.0333	0.0500	0.0833	0.1667	0.2500	0.5000	0.7500	1.0000
1 BA.F. M	0.1946	7.0		21.0	16.0	12.0	@ 0.0	@ 0.0	@ 0.0
2 SO.K. M	0.1946	@ 0.0		22.0	6.0	11.0	@ 0.0	@ 0.0	@ 0.0
3 HU.M. M	0.1946	@ 0.0		24.0	15.0	17.0	@ 0.0	@ 0.0	@ 0.0
4 SC.W. M	0.9515	6.0		117.0	93.0	77.0	38.0	17.0	9.0
5 HA.M. M	0.9515	27.0		170.0	118.0	66.0	21.0	4.0	@ 0.0
6 GE.S. M	0.9515	82.0		81.0	94.0	69.0	13.0	@ 0.0	@ 0.0
7 RI.G. M	3.8060	499.0		787.0	466.0	274.0	128.0	83.0	29.0
8 RO.R. M	3.8060	372.0	525.0	494.0	348.0	231.0	107.0	48.0	26.0
9 AL.H. M	3.8060	117.0	416.0	546.0	448.0	368.0	152.0	80.0	37.0
10 ST.B. M	5.7090	257.0	982.0	1460.0	1103.0	750.0	338.0	186.0	93.0
11 MA.S. M	5.7090	353.0		791.0	507.0	378.0	169.0	78.0	39.0
12 SC.K. M	5.7090	331.0		960.0	790.0	559.0	332.0	176.0	108.0
13 MU.R. M	7.6120	1181.0		1158.0	951.0	714.0	384.0	191.0	145.0
14 DI.K. M	7.6120	1060.0		1675.0	869.0	510.0	288.0	183.0	126.0
15 KN.S. M	7.6120	848.0		1138.0	708.0	419.0	196.0	156.0	101.0
16 MA.L. M	9.5150	677.0 ✓		1701.0	1202.0	766.0	336.0	171.0	114.0
17 ZE.C. M	9.5150	1160.0 ✓		2543.0	1530.0	1229.0	504.0	285.0	228.0
18 AR.J. M	9.5150	1010.0 ✓		1075.0	1101.0	835.0	270.0	146.0	83.0

SUBJECT	DOSE [mg]	TIME [hours] 1.5000	2.0000	3.0000	4.0000	6.0000	8.0000	24.0000
1 BA.F. M	0.1946	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *	@ 0.0	@ 0.0	@ 0.0
2 SO.K. M	0.1946	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *
3 HU.M. M	0.1946	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *
4 SC.W. M	0.9515	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *
5 HA.M. M	0.9515	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *
6 GE.S. M	0.9515	@ 0.0	@ 0.0	@ 0.0	@ 0.0	@ 0.0 *	@ 0.0	@ 0.0 *
7 RI.G. M	3.8060	16.0	8.0	2.0	@ 0.0	@ 0.0 *	@ 0.0	@ 0.0 *
8 RO.R. M	3.8060	12.0	10.0	5.0	1.0	@ 0.0 *	@ 0.0	@ 0.0
9 AL.H. M	3.8060	16.0	10.0	7.0	5.0	2.0	@ 0.0	@ 0.0 *
10 ST.B. M	5.7090	28.0	13.0	13.0	9.0	1.0	@ 0.0	@ 0.0
11 MA.S. M	5.7090	21.0	11.0	8.0	6.0	@ 0.0	@ 0.0	@ 0.0
12 SC.K. M	5.7090	55.0	38.0	25.0	8.0	7.0	3.0	@ 0.0 *
13 MU.R. M	7.6120	57.0	39.0	32.0	22.0	11.0	6.0	@ 0.0 *
14 DI.K. M	7.6120	52.0	35.0	22.0	17.0*	7.0	2.0	@ 0.0
15 KN.S. M	7.6120	50.0	38.0	22.0	19.0	13.0	12.0	1.0
16 MA.L. M	9.5150	49.0	33.0	25.0	14.0	9.0*	6.0	@ 0.0 *
17 ZE.C. M	9.5150	88.0	55.0	35.0	17.0	4.0	4.0	@ 0.0 *
18 AR.J. M	9.5150	49.0	47.0	40.0	36.0	19.0	@ 0.0	@ 0.0 *

* = DIFFERENCE BETWEEN TIME SCHEDULED BY EXPERIMENTAL DESIGN AND
TIME OF ACTUAL SAMPLING > TOLERANCE VALUE = 120 [sec]

@ = CONCENTR. LESS THAN 0.9 [ng/ml] = DETECTION LIMIT

that the AUC was linear with dose, an inspection of the accompanying Figure 10.6 demonstrates that a more complex curve is more comprehensive in describing the relationship between dose and activity, eg. an asymptotic curve. Urinanalysis was performed for active BM 06.022 or its metabolites and antigen levels. Since urine contains urokinase which is active in the spectrophotometric method, the ELISA method was first performed. Subsequently urokinase activity was removed by washing the plate and the spectrophometric assay performed. The ELISA method utilized a wall-coated monoclonal antibody against _____ A number of coagulation parameters were measured which are listed in the attached table. PAI-antigen was measured with an enzyme-linked immunosorbent assay from free, complexed, and latent PAI-1. Ex vivo platelet aggregation was also measured using platelet rich plasma.

No urinary excretion of BM 06.022 was detected. The validity of the urinalysis was supported by positive controls.

Studies of tolerability, pharmacokinetics and pharmacological effects of BM 06.022 administered intravenously to healthy adults (Single administration)[2]. M2. June 12, 1992.

The tolerability, effect on blood coagulation and fibrinolysis, pharmacokinetics and the time course of plasminogen activase (PA) activity in serum were studied in a randomized, placebo controlled trial. The following dosages were investigated after bolus iv administration over 2 minutes: 0.9, 1,8, 3.6 and 7.2 mg. At each dose 5 individual were administered BM 06.022 and 1 placebo. Only males subjects were studied. Measurements of coagulation and fibrinolysis included bleeding time, PT, APTT, ALT, fibrinogen, FDP, FDP-D-dimer, plasminogen, alpha2-antiplasmin, PAI, alpha2-macroglobulin, PIC, protein C, and platelet aggregation to collagen or ADP. Also measured were antibody titer to BM 06.022, E. coli proteins, and erythrinotrypsin inhibitor (ETI). Plasma levels of BM 06.022 by an ELISA method and PA activity by plasminogen activation of the euglobulin distribution test were determined. Urinary level of BM 06.022 were also investigated.

Study was translated from Japanese.

The following coagulation parameters were determined:

PARAMETER		NORMAL VALUES	METHOD
PTT (kaolin-activated)	s	30- 40	Clotting rate
PT (Quick test)	%	70-120	"
Thrombin time	s	16- 20	"
Fibrinogen	mg/dl	150-450	"
"	mg/dl	"	
"	mg/dl	"	
Plasminogen	%	80-120	
α_2-Antiplasmin	%	80-120	"
α_2-Macroglobulin	mg/dl	120-380	"
Antithrombin III	%	80-120	"
Fibrinogen-fibrin degradation products	µg/ml	<10	
D-dimers	ng/ml	<400	ELISA
Fibrin degradation products	ng/ml	40-420	"
Fibrinogen degradation products	ng/ml	125-425	"

4.2 Safety variables

Subjective and objective adverse reactions were recorded and an adverse event (AE) form was completed for further processing.

ECG:

Limb lead I, II, and III, and aVR, aVL, and aVF, and chest leads V_1 to V_6 were recorded. ECGs were examined for changes in shape and rhythm throughout the study. QRS-, QT-, and PQ-time intervals were determined. Heart rate was derived from the ECG. Blood pressure was measured on the arm of the injection site.

表－41 健常人にBM06.022を単回急速静注した後の血漿中プラスミノーゲン・アクチベーター（PA）活性の薬物動態学的パラメーター

用量	C_{max}	$t_{1/2}$	Vd**	$AUC_{0-\infty}$	CL_{tot}*	MRT
0.9mg	118±13	9.29±0.55	4.62±0.20	28±2	323±18	0.238±0.008
1.8mg	215±15	9.69±0.29	4.56±0.10	57±2	309±10	0.258±0.008
3.6mg	521±46	10.44±0.62	3.75±0.29	152±17	244±30	0.301±0.031
7.2mg	1139±87	10.52±0.94	3.42±0.18	332±36	222±25	0.277±0.021

（n＝5）Mean±S.E. *:p<0.01 **:p<0.05（一元配置分散分析）

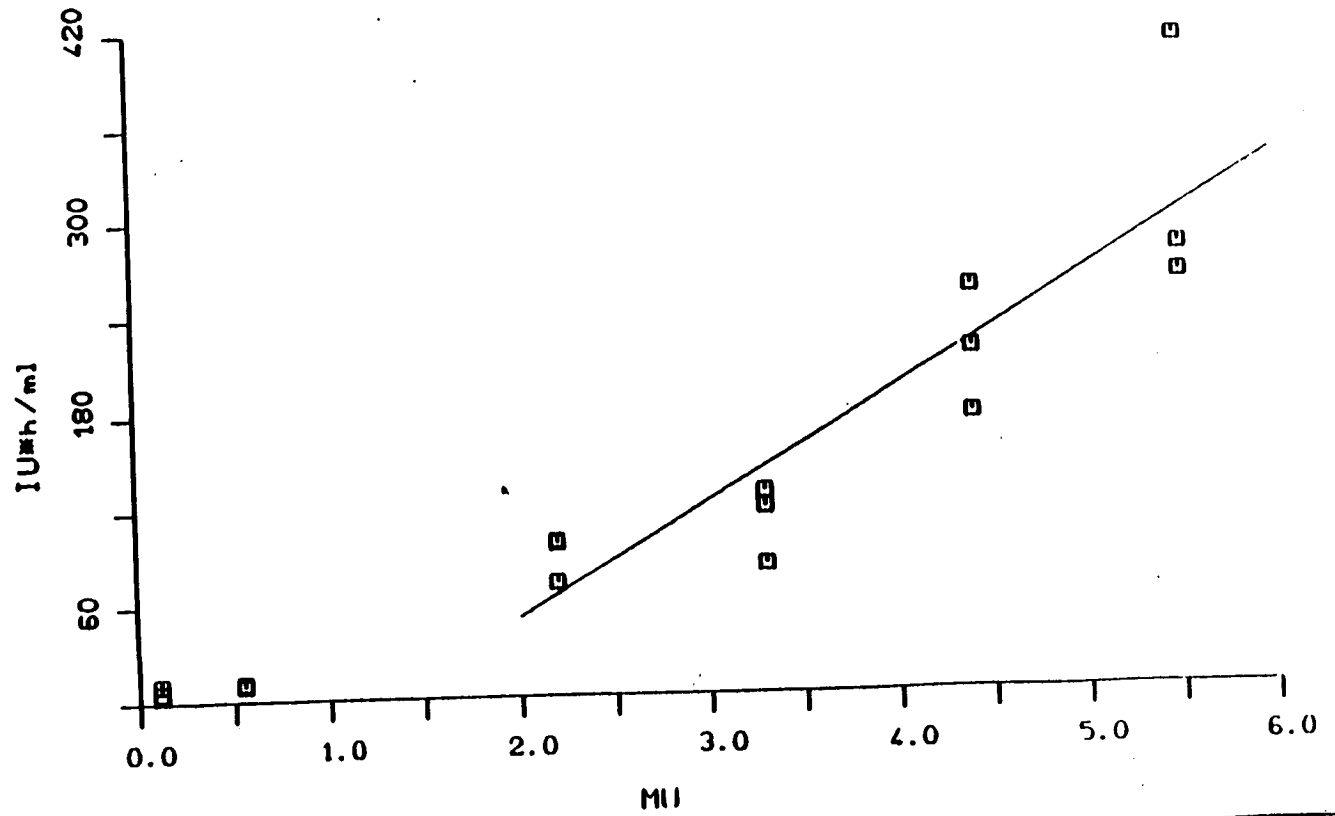
C_{max} C：最高血漿中濃度［BMU/ml］
$t_{1/2}$ D：血漿中濃度半減期［min］
Vd t：分布容積［l］
$AUC_{0-\infty}$ F：血漿中濃度－時間曲線下面積［BMU·hr/ml］
CL_{tot} G：全身クリアランス［ml/min］
MRT H：平均滞留時間［hr］

DRUG NAME: STUDY NO.: MF 7076
DRUG CODE: BM 06.022 REPORT NO.: M1 PAGE: 27f

Fig. 10.6

PROJECT: BM 06.022 , , DIFFERENCE TO BLANK VALUE
BATCH : 821 563 A (1 MU-AMP.) / 821 564 A (5 MU-AMP.)
DOSE : VARYING MU (3 VOL./ DOSE) (...INJECT. OVER 2 min.
ASSAY : t - PA ACTIVITY IN PLASMA
□ = ABSC. : DOSE [MU]
ORDIN.: A U C [IU*h/ml] 0 - 24 h

DATE: 18-MAY-90
B = 71.169 A = -93.478 R = 0.891 (R=0) P < 0.001
(LIN) P = 0.313



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Tables/Figures

Values for plasminogen and alpha-antiplasmin were decreased in a dose dependent fashion after administration of BM 06.022. Levels were statistically decreased for plasminogen at 7.2 mg of BM 06.022 and for alpha-antiplasmin at 3.6 and 7.2 mg. Levels of PAI were also deceased by approximately 55% at 1.8 mg; however, changes were not always consistent with dose. Platelet aggregation achieved a level of 85% in general after injection of BM 06.022. PT was significantly prolonged in the 7.2 mg group shortly after administration (5 min) and returned to normal. Tests for APTT, bleeding time, FDP, alpha2-macroglobulin and protein C were not changed in any biologically meaningful manner. No antibodies were detected to BM06.022, E. coli proteins or ETI.

Data from pharmacokinetic studies of plasminogen activase activity and antigen levels in plasma revealed linear kinetics within the dose range investigated (0.9, 1.8, 3.6 and 7.2 mg). Data for PA activity was best fit using a 1-compartment model and that for antigen levels a 2-compartment model. For PA activity, t1/2 ranged from 9.29 to 10.52 min, Vd from 3.42 to 4.62 L, Cl 222 to 323 ml/min across all doses. For antigen levels, t1/2alpha varied from 9.18 to 11.06 min, t1/2beta from 113 to 135 min, Vc from 3.59 to 3.68 L, and Cl min/min from 144 to 170 across all doses. There were no statistical difference between any pharmacokinetic endpoint among the different doses. AUC appeared linearly proportional to dose for PA activity as well as antigen levels. (See attached table) No urinary excretion of BM 06.022 was detected.

Preclinical Studies:

Pharmacokinetic Evaluation of BM 06.022 (r-PA) Derived from Different Production Scales in Conscious Dogs, Report No L 16, Jan 20, 1993.

The pharmacokinetic properties of 2 batches of BM 06.022 from different production scales were evaluated in a randomized, parallel group design. Dogs (n = 29) were implanted with arterial and venous catheters to administer drugs and remove blood samples. Originally, 15 dogs per groups were planned for study, but 1 dog was inadvertently given the same preparation

twice and dropped from the study. Dogs were heparinized[3] to maintain catheter patency. Two batches (large production scale (50 gram) - batch no. 780 514 80A and pilot scale - batch no 780 244 80A) of BM 06.022 were injected iv over 1 min at a dose of 200 ku/kg(349,650 ng/kg and 343,053 ng/kg, respectively). The pilot scale produced material was considered the reference standard. Plasma concentration were determined by both an enzyme immunoassay (ELISA) and an assay for specific activity[4]. Arterial blood samples (1.35 ml) were taken prior to drug administration, 2, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 180 min post injection. Various pharmacokinetic parameters were determined (Cmax, Tmax, AUC, Cl, and terminal half-life). These values were determined using both the ELISA and specific activity methods for the reference and test article as follows: for specific activity - Cmax, IU/ml - 2120 ± [5]877 vs 1885±887; Tmax, h - 0.04 ± .02 vs .04 ± .02; AUC, IU h/ml - 547 ± 211 vs 444 ± 213; Cl, ml/min kg - 7.5 ± 4.2 vs 9.7 ± 5.7; and t1/2, h - 0.21 ± .06 vs .23 ± .06; for ELISA Cmax, ng/ml - 3960 ± 995 vs 4306 ± 872; Tmax, h - 0.03 vs .03; AUC, ng h/ml - 2012 ± 560 vs 2200 ± 488; Cl, ml/min kg - 9.7 ± 5.7 vs 7.5 ± 4.2; and t1/2, h - 0.23 ± .5 vs 1.7 ± .4. AUC was computed by the trapezoidal method from time 0 to the time of the last sample collected and extrapolated to infinity by using the terminal rate constant. Less than 20% of the AUC was extrapolated. It is of interest to note that differences were found in Cl and t1/2 as determined by the ELISA and specific activity methods. This may be a function of sensitivity, but a difference in the analytes being detected cannot be ruled out. Using Student's t test for unpaired data, no statistical differences were found between the 2 production batches. The relative bioavailability by ELISA was 92% and 81% by specific activity method and is not significant given the experimental variation. It was determined that the study had at 80% power for AUC, the minimal detectable difference of 26% of ELISA and 46% by specific activity assay. The lack of power in the specific activity assay is likely reflective of a higher coefficient of

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. Sodium heparin was administered iv bolus (100 IU/kg) just prior to the injection of BM 06.022.

. Verheijen, et al, Thromb. Haemostas 48: 266 -269 1982.

. values reported as mean ± standard deviation

variation (40%).

A randomized, cross-over study in conscious dogs assessing the pharmacokinetic bioequivalence of BM 06.022 derived from pilot scale and large scale production lots. Study report L 17. Aug 2, 1993.

The pharmacokinetic bioequivalence of pilot plant lot (5 gram, batch 780 244 80A) as reference article and large scale lot (100 gram, batch 780 787 80A) as test article were assessed. Dogs (n=31) were studied in a cross-over design with a 3[6] day washout period. Dogs were heparinized during the course of the study. BM 06.022 was injected iv as a 30 second bolus at a strength of 200 kU/kg which corresponded to 343, 222 ng/kg for the test article and 343,053 ng/kg for the reference article. Blood samples were taken prior to injection and at 2, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 min after injection. Both antigen (ELISA, see Appendix 11.3) and specific activity (spectrophotometric, Verheijen et al., 1982[7]) were measured and used to determine pharmacokinetic endpoints including AUC, Cmax, Tmax, and terminal half-life. AUC was determined using the trapezoidal rule and extrapolated to infinity using the terminal rate constant. AUC between 0 and 120 min was used as the primary pharmacokinetic determinant of pharmacokinetic equivalence. The AUC for 0 to 2 h computed from data taken from the ELISA assay was 1951.4 ± 249.5 ng h/ml for pilot plant lot and 1930.1 ± 400.7 for the large scale lot. A two sided 90% confidence interval (CI) was used to calculate statistical equivalence. Using this technique, the 90% CI was found to 0.912 to 1.026 for the antigen based assay and 0.882 to 1.075 for the activity assay. Pharmacokinetic equivalence was demonstrated by both the antigen and activity assay (limits of acceptability are .8 to 1.25). A slight, but not significant residual effect (P=0.07) was observed

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. The 3 day washout period was based on the number of terminal half-lives needed to avoid a cross-over effect and the window of time before the development of antibodies.

Verheijen JH, Mullaart E, Chang GTG, Kluft C and Wijngaards G. A simple sensitive spectrophotometric assay for extrinsic (tissue-type) plasminogen activator applicable to measurements in plasma. Thromb Haemostas 48: 266-269, 1982.

due the inclusion of a single animal (No. 752). Inclusion or exclusion of the animal from the analysis for pharmacokinetic equivalence did not alter the conclusion of the study. Animal No. 752 was identified as an outlier by the Grubbs/Beck test[8]. The CI after exclusion of the outlier was 0.952 to 1.037. Based on the activity assay and using a slightly shorter period (0 to 1.25 h) for AUC, an additional CI (0.882 to 1.075) was calculated for pharmacokinetic bioequivalence which was within acceptable limits.

The CV for AUC between studies L 16 above and L 17 were notable for both the antigen (12.8 and 20.8%, respectively) and activity based assay (26.4 and 36.2, respectively). Improvements in the assay performance are, in part, attributed to changes in the reagents used and improved sample pretreatment.

Pharmacokinetic profile of BM 06.022 after single and double bolus administration in anesthetized dogs. Report No. L 12, July 29, 1991.

The pharmacokinetics of BM 06.022 from two different batches (Lot No. 821403 and lot No. 821564) were compared in anesthetized dogs (n=6/group) used in conjunction with a pharmacodynamic study of coronary arterial thrombosis. Dogs were infused iv with sodium heparin 10 min post occlusion of the left circumflex coronary artery and subsequently injected with BM 06.022 1 h after thrombus formation. BM 06.022 was administered as either a single iv bolus or double bolus. Single bolus doses were 140 kU/kg (0.24 mg/kg) or 280 kU/kg (0.49 mg/kg). Double bolus doses were either 140 kU/kg injected twice or 140 kU/kg followed by 50 kU/kg (0.09 mg/kg). Venous blood samples were collected and analyzed for activity using an indirect spectrophometric assay. One and 2 compartment models were used to analyze the pharmacokinetic data. No differences were found in the pharmacokinetics between the 2 batches of BM 06.022. pharmacokinetics with regard to dose were linear. For example, doubling the dose also doubled Cmax and AUC, but did not influence t1/2 or Cl. No accumulation was noted between doses given 44 min apart.

. Grubbs, FE and Beck G, Technometrics 14: 847, 1972.

Pharmacokinetics of antigen of BM 06.022 in conscious dogs after i.v. bolus injection. Report No. L 7, Jan 2, 1991.

The pharmacokinetics of BM 06.022 were compared to those of t-PA. Dogs chronically implanted with arterial and venous catheters were used in a cross-over design with a 2 day washout period. Either BM 06.022 (0.388 mg/kg) or t-PA (0.25 mg/kg) were administered iv over 1 min. Both doses represent 200 kU/kg of activity; however, doses were normalized to 0.4 mg/kg for the calculation of pharmacokinetic endpoints. Plasma samples were taken over a 2 h period of time, analyzed using an ELISA assay and fitted to either a 1 or 2 compartment model. BM 06.022 was eliminated with a t1/2 of 25.3 ± 3.3 min in a 1 compartment model and t-PA with t1/2s of 2.5 ± 0.6 min and 23.7 ± 1.9 min. For t-PA, the initial phase accounted for 70 ± 3.7% of the total AUC. The AUC for BM 06.022 was 3.6 fold higher than that of t-PA (1328 ± 231 ng h/ml vs 374 ± 166 ng h/ml). Hence, the total plasma Cl of BM 06.022 (5.8 ml/min/kg) was 3.7 fold slower than that of t-PA (21.2 ± 4.5 ml/min/kg).

Pharmacokinetic properties of BM 06.022 in conscious dogs following intravenous bolus injection of 200 kU/kg. Report No. L 4, Oct 19, 1990.

The pharmacokinetics of BM 06.22 (iv bolus over 1 min, 200 kU/kg) were compared with those of t-PA in conscious mongrel dogs (2F, 3M) with chronically implanted arterial and venous catheters. A crossover design was used with a 2 day washout period. BM 06.022 was eliminated with a t1/2 of 12.7 ± 0.7 min; t-PA had an elimination t1/2 of 1.7 ± 0.4 min which corresponded to 79.4 ± 2.7% of the total AUC. t-PA also had a terminal or beta-phase t1/2 of 16.8 ± 4.2 min. Cmax's were similar for BM 06.22 (2351 ± 237 IU/ml) and t-PA (2313 ± 496 IU/ml). However, the AUC for BM 06.22 (676 ± 50 IU h/ml) was 5.1-fold higher than that of tPA (132 ± 24 IU h/ml) which yielded correspondingly lower rates (5.9-fold) of clearance for BM 06.22 (5 ± 0.4 ml/min/kg) vs t-PA (29.5 ± 6.2 ml/min/kg). Pharmacokinetic endpoints which were reported above were calculated using a non-compartmental (AUC, Cmax, Cl) or compartmental (t1/2s) models.

Pharmacokinetic profile of BM 06.22 following intravenous bolus injection in anesthetized dogs. Report L 2, Oct 12, 1990.

The pharmacokinetics of BM 06.22 and t-PA were characterized following iv bolus injection (1 min) in beagle dogs with coronary artery thrombosis. BM 06.22 was administered at various doses (50, 100, 140, 200 kU/kg with an N = 6/dose) as was t-PA (200, 800, 1130, 1600 kU/kg with an N = 6). Cmax and AUC of BM 06.22 increased linearly with dose whereas Cl did not and ranged from 4.1 to 6.6 ml/min/kg over the various doses. t-PA showed a strong inverse dose dependency with Cl going from 12.6 at 1600 kU/kg to 42.3 ml/min/kg at 200 kU/kg, ie. showed saturable clearance. At a dose of 200 kU/kg, BM 06.22 had a Cl rate 10.3-fold slower than tPA. Similarity using equipotent dose a 4.6-fold decrease was revealed when BM 06.22 (140 kU/kg) was compared to tPA (800 kU/kg).

For pharmacokinetic profiles best consistent with a 1-compartment model, 13 of 24 dogs injected with BM 06.22 demonstrated t1/2s which ranged from 10.2 to 11.8 min and for tPA 12/24 dogs yielded t1/2s of 3 to 4.9 min. For pharmacokinetic profiles best consistent with a 2-compartment model, the initial t1/2 for BM 06.22 (N=11) ranged from 5.0 to 7.2 min which accounted for 53.6% of the AUC and the terminal t1/2 ranged 14.3 to 18 min. For tPA (N=12) the initial t1/2 ranged from 1.6 to 3.7 min and accounted for 82.7% of the total AUC, and the terminal t1/2 ranged from 10.2 to 22.1 min.

Pharmacokinetic endpoints which were reported above were calculated using a non-compartmental (AUC, Cmax, Cl) or compartmental (t1/2s) models.

The following observations may be made in comparing the pharmacokinetics of BM 06.22 and tPA at a dose of 200 kU/kg in conscious mongrel dogs to unconscious beagle dogs with coronary artery thrombosis. For BM 06.22, AUC was increased as was Cmax, initial t1/2 decreased and Cl remained unchanged. These results suggest that a decrease in elimination rate or increased volume of distribution had occurred. For tPA, AUC was decreased as was Cmax, t1/2 unchanged, and Cl increased. These suggest that the volume of distribution was decreased. Altogether, the data suggests different mechanism of distribution and elimination between BM 06.22 and tPA. The saturable, dose dependency of tPA underscores this difference.

Pharmacokinetics of activity of BM 06.22 compared with alteplase

at an infusion regimen in anesthetized dogs. Report L 9. Jan. 7, 1991.

At doses considered equipotent, the pharmacokinetic properties of BM 06.22 (140 kU/kg) and tPA (800 kU/kg) were compared when a 90 min infusion was followed by an initial iv bolus injection of 10% of the total dose. Studies were conducted in anesthetized dogs and blood samples for activity were taken before, during and after infusion. Peak concentrations at the end of the bolus injection (1 min) were 217 ± 48 IU/ml for BM 06.22 and 555 ± 89 IU/ml for tPA. The Cmax was 372 ± 40 IU/ml at 61 ± 11 min for BM 06.22 and 718 ± 92 IU/ml at 26 ± 16 min for tPA after initiation of the infusion. AUC at 2 h were 442 ± 58 IU h/ml for BM 06.22 and 491 IU h/ml for tPA. Plasma Cl of BM 06.22 (5.6 ml/min/kg) was 5.5-fold lower than that of tPA (30.5 ± 4.2 ml/min/kg). The terminal t1/2s were 11.1 ± 1.4 min for BM 06.22 and 5.2 ± 0.8 for tPA.

Pharmacokinetic profile of BM 06.22 at four different dose levels in anesthetized male rabbits following intravenous bolus injection. Report L 1. Jan 30, 1990.

In anesthetized, male rabbits (New Zealand White, N = 5-6/dose) with jugular vein thrombi, the pharmacokinetics of BM 06.22 were characterized at 4 doses (50, 100, 200, and 400 kU/kg) and tPA at two doses (200 and 400 kU/kg) following an iv injection. For BM 06.22, both Cmax and AUC increased in a linear fashion with dose; Cl ranged from 2.5 to 3.0 ml/min/kg and was not dose dependent. The Cl of BM 06.22 in comparison to alteplase was 4.6 to 8.5-fold slower. The pharmacokinetics of 18/23 rabbits injected with BM 06.22 and 10/23 for tPA were best consistent with a 1-compartment model. The t1/2 for BM 06.22 (11.6 to 15.4 min) was about 5-fold longer than tPA (2.3 to 4.5 min). In animals those pharmacokinetics was best fitted to a 2-compartment model, the initial t1/2 for BM 06.22 were longer being 3.6 to 7.2 min as compared to 1.5 to 4.2 min for tPA and terminal t1/2 were approximately the same 12.1 to 15.1 and 10.2 to 16.1 min. The plasma Cl of tPA was found to be dose dependent and inversely related with successive decreases ranging from 22.9 to 8.5 ml/min/kg. pharmacokinetic endpoints which were reported above were calculated using a non-compartmental (AUC, Cmax, Cl) or compartmental (t1/2s) models.

Pharmacokinetic properties of BM 06.022 in anesthetized male rats following intravenous bolus injection of 200 kU/kg. Report L 5. Jan 14, 1992.

The pharmacokinetic properties of BM 06.22 compared with those of tPA in anesthetized male rats. Plasminogen activators were injected iv at a dose of 200 kU/kg (over 1 min, vol 1 ml/kg). BM 06.22 was eliminated from plasma in 4/6 rats with an initial t1/2 of 4.3 ± 0.9 min which accounted for 49.6 ± 13.4% of the total AUC and a terminal t1/2 of 12.3 ± 1.7 min. In the remaining 2 rats, BM 06.22 was eliminated from plasma with a t1/2 of 9.7 min. t-PA had an initial half-life of 1.0 ± 0.1 min which accounted for 77.3 ± 4.9% of the total AUC; terminal t1/2 was 14.7 ± 3 min. Cmax's were comparable between BM 06.022 and t-PA (2322 ± 335 vs 2175 ± 438 IU/ml). However, AUC value for BM 06.022 was 5 fold higher than that of t-PA (456±40 IUh/ml vs 92 ± 14 IUh/ml). Correspondingly, the plasma clearance of BM 06.022 was 5.7 fold slower than that of t-PA (7.6±0.6 ml/min/kg vs 43 ± 7.8 ml/min/kg).

Pharmacokinetic properties of BM 06.022 compared with alteplase after nephrectomy or ligation of the portal vein in anesthetized rats. L 13. Aug 6, 1991.

The pharmacokinetics of BM 06.022 were studied in rats after iv bolus injection of 200 kU/kg and compared to t-PA. Rats were anesthetized and underwent sham surgery, bilateral nephrectomy, or ligation of the portal vein. The data indicate that bilateral nephrectomy vs sham surgery induced elevated plasma concentrations of BM 06.022 (1130 ± 107 vs 549 ± 42 IUh/ml) whereas levels of t-PA were unaffected (115 ± 20 vs 106 ± 18 IUh/ml). Ligation of the portal vein produced liver malperfusion as indicated by retention of bromosulphalein in plasma, and was accompanied by severe hemodynamic compromise. With portal ligation, the AUC for t-PA increased 7.3 fold relative to that of BM 06.022.

Influence of hepatic failure on pharmacokinetic properties of BM 06.022 in rats. L 15. Jul 3, 1992.

The pharmacokinetics of BM 06.022 were compared to those t-PA in anesthetized rats experiencing hepatic failure or combined

hepatic and renal failure. Hepatic insufficiency was chemically induced with carbon tetrachloride 1 day prior to the study. Renal insufficiency was induced by acute bilateral, surgical nephrectomy. Pharmacokinetics as measured by the AUC of BM 06.022 was not modified by hepatic failure vs control(717 ± 84 vs 611 ± 61 IUh/ml) after iv administration in contrast to t-PA (125 ± 26 vs 47.5 ± 10.8 IUh/ml). The combination of renal and hepatic failure vs control resulted in a 2.2 fold increase in the AUC of BM 06.022 (1415 ± 405 vs 638 ± 90 IUh/ml). For t-PA, the combination of hepatic and renal failure vs control resulted in a 3 fold increase in AUC (181 ± 15 vs 60.5 ± 8.5 IUh/ml). These data indicate that the liver plays a minor role in the catabolism of BM 06.022 even in after combined renal and hepatic dysfunction.

Pharmacokinetic properties of BM 06.022 following intravenous infusion over 30 min in anesthetized rabbits. L 6. Oct 31, 1990.

The pharmacokinetics of BM 06.022 were compared to t-PA in anesthetized male rabbits (N = 4) after iv infusion of 200 kU/kg over 30 min. At the end of the period of infusion, BM 06.022 was 7 fold higher than that of t-PA (1892 ± 429 vs 271 ± 31 IUh/ml). BM 06.022 exhibited monophasic clearance in 3/4 rabbits with a t1/2 of 12.2 ± 1.4 min; additionally, 1 animal yielded biphasic kinetics (t1/2s 9.5 and 21.4 min). t-PA exhibited an initial t1/2 of 2.5 ± 0.2 min which accounted for 84 ± 4.5% to the total AUC. The terminal t1/2 was 10.8 ± 1.0 min. The AUC of BM 06.022 was 8.9 fold higher than that of t-PA (1064 ± 222 vs 120 ± 15 IUh/ml) and clearance was correspondingly slower (3.5 ± 0.5 vs 29.4 ± 3.4 ml/min/kg).

A randomized, cross-over study in conscious dogs assessing the pharmacokinetic bioequivalence to two BM 06.022 batches after a process change: new seed lot with a different promotor. L 20. May 17, 1994.

The pharmacokinetic equivalence of 2 batches of BM 06.022 were determined. The reference compound (batch 780 835 80 A, TA) was produced using a seed lot containing the ——— whereas the test compound used ——————— (batch 780 911 80 A, TB). Thirty one adult, heparinized beagle dogs of either sex (15 female, 16 males) were tested in a randomized, cross-over design

with a washout period of 3 days. BM 06.022 was administered as a double bolus injection of 140 and 140 kU/kg (approximately 231 microgm/kg per bolus). Activity and plasma antigen level of BM 06.022 were assayed, the latter with an ELISA technique. Pharmacokinetic endpoints were obtained using a non-compartmental analysis. The primary endpoint was AUC from zero to 3 hour. Data were analyzed after a logarithmic transformation in a generalized linear model. Pharmacokinetic equivalence was determined using a two-sided 90% confidence interval (CI). The point estimator and 90% CI were reported to be 1.0214 and 0.950 to 1.100 for antigen based assay and 1.018 and 0.956 to 1.084 for activity based assay. No carrier over effect was found in the analysis of pharmacokinetic equivalency; however, for activity equivalency, a carry over effect was observed ($p<0.005$; period 2 > period 1). Based on the pharmacokinetic study, the 2 versions of BM 06.022 are considered equivalent.

Pharmacokinetic properties of BM 06.022 in anesthetized, female non-human primates following intravenous bolus injection of 200 kU/kg. L 3. Oct 19, 1990

The pharmacokinetics of BM 06.022 were compared with those of tPA in anesthetized, female non-human primates (Macaca arctoides, N = 6). Both biologics were injected iv over 1 min at a dose of 200 kU/kg. BM 06.022 was eliminated with a t1/2 of 7.2 ± 0.5 min. tPA was eliminated in 3 animals with an initial t1/2 of 1.6 ± 0.5 min and a beta-phase t1/2 of 5 ± 1.3 min. The initial phase accounted for 68.3 ± 11.3% of AUC. In the remaining 3 animals, tPA was eliminated with a single t1/2 of 2.6 ± 0.03 min. Although administered equal doses on a unit basis, the AUC value for BM 06.022 was 3 fold higher that of tPA. Correspondingly the clearance was 3.2 fold slower.

Pharmacokinetics of antigen of BM 06.022 in anesthetized, female non-human primates after i.v. bolus injection. L 8. Jan 2, 1991.

The pharmacokinetics of BM 06.022 were compared with those of tPA in anesthetized, female non-human primates (Macaca arctoides, N = 6). Both biologics were injected iv over 1 min at a dose of 0.402 mg/kg or 0.25 mg/kg. BM 06.022 was eliminated in 4/6 animals with an initial t1/2 of 6.9 ± 0.2 min, the subsequent beta-phase t1/2 was 59.4 ± 14.6 min. The initial phase accounted

for 68.8 ± 10% of AUC. In the remaining 2 animals BM 06.022 was eliminated with a t1/2 of 8.7 min. tPA was eliminated in 5 animals with an initial t1/2 of 3.0 ± 0.2 min and a beta-phase t1/2 of 25 ± 2.7 min. The initial phase accounted for 68.8 ± 10% of AUC. In the remaining 1 animal, tPA was eliminated with a single t1/2 of 3.5 min. Although administered equal doses on a unit basis, the AUC value for BM 06.022 was 4.9 fold higher that of tPA. Correspondingly the clearance was 4.6 fold slower.

ADME

Plasma clearance, organ distribution and liver interaction of BM 06.022. L 10. Jan 14, 1992.

This study examines the catabolism of BM 06.022 in the rat. Total clearance of BM 06.022 is much slower than t-PA. Thirty per cent of BM 06.022 is cleared in the initial or alpha phase of its pharmacokinetics. Clearance in the initial phase appears to be consequence of uptake of BM 06.022 in the kidney and liver. Nephrectomy led to a prolonged t1/2 and smaller contribution of the initial phase to the overall elimination of the drug. Most of BM 06.022 is cleared in the beta phase. Clearance during this phase is associated with distribution to a wide range of tissues. Liver is the major organ responsible for the clearance of BM 06.022 followed by the kidney, skin, bone and muscle; however, uptake besides that into the liver may be nonspecific. Following uptake into the liver, degradation occurs at a slow, linear rate. Hepatic degradation is sensitive to the effect of inhibitors of proteolysis such as leupeptin, but is sensitive to other inhibitors such as chloroquine. These findings suggest that liver degradation is extra-lysosomal in contrast to t-PA. Among the cells contributing to the clearance of BM 06.022 are endothelial, parenchymal and Kupffer cells. The amount of BM 06.022 taken up into the various cells is a reflection of the time dependency of their kinetics. At 10 min after injection parenchymal cells accounted for 47.3%, endothelial cell 35.7% and Kupffer cell 16.3% whereas at 30 min parenchymal cells accounted for 14.9%, endothelial cell 15.0% and Kupffer cell 70.1%. Uptake into Kupffer cells may be mediated by the formation of complexes between BM 06.022 and protease inhibitors. Binding of BM 06.022 to liver parenchymal cells did not demonstrate saturation and binding was not displaceable suggesting that the binding was of

low affinity. Binding was not Ca2+-dependent which is consistent with the lack of an EGF binding domain. Additionally, binding of BM 06.022 was displaceable by melanoma-derived t-PA which suggests BM 06.022 and t-PA may share a low affinity site. The binding of BM 06.022 to liver parenchymal cells was not dependent on carbohydrate-specific receptors and no competition was found with carbohydrates or glycoproteins.

Autoradiography of 125I-BM 06.022 in Rats after Intravenous Administration. L 11. April 15, 1991

The distribution of radiolabeled BM 06.022 was determined after iv administration in rats by autoradiography. 125I-B was distributed to almost all tissues. Ten minutes after administration radioactivity was localized to the kidney > liver > heart, lung, spleen > bone marrow > gastric and intestinal mucosa, nasal mucosa and hair follicle. After 1 hour localization was observed in gastric mucosa, urinary bladder > intestinal mucosa > kidney > liver ,lung > heart > bone marrow , eye, feces > skin. After 4 hours localization was observed as to thyroid > gastric mucosa, oesophagus, urinary bladder >> kidney > eye, heart, liver, lung, intestinal and nasal mucosa, skin > bone marrow. The distribution of BM 06.022 to various organs appeared to be blood flow dependent. Radiolabelling at later times is likely to be due to the disassociation of the label, ie iodine, and its uptake into tissues like the thyroid.

Uptake, Internalization, and Degradation of the Novel Plasminogen Activator BM 06.022 in the Rat. L 19. May 9, 1994.

A series of studies were performed with BM 06.022 using rat isolated tissues (liver and kidney) to characterize dose and time dependent uptake and metabolism in relationship to pharmacokinetics using radiolabelling with 125I. The terminal t1/2 ranged from 20.6 to 42 min and did not appear dose dependent over the range of 30 to 800 microgm/kg. Liver uptake did appear to be dose dependent. Hepatic uptake was considered slow and occurring at a low rate. At 6 min post injection, liver uptake was 14.7% and at 30 min was 13 to 16%. Organ distribution showed no dose dependency at 10 min post injection. Tissue uptake at 10 min was liver > kidney > skin > bone. At 90 min radioactivity in the liver decreased to 4.7% and that of kidney to 1.5% but increased for skin to 20.9%, intestine 11.3% and bladder 3.8%.

Regarding hepatic metabolism, parenchymal cells account for 65%, whereas endothelial cells 15% and Kupffer cells 23%. Liver uptake of 125I-tyramine cellobiose modified BM 06.022 was different exhibiting an uptake at 30 min of 36.2%. This label accumulates in the lysosomes as re-circulation of the label is precluded. This finding suggests that over time degradation products occur and may account for various sites of radioactivity. Use of 125I-TC BM 06.022 revealed that only 2 organs take up and degrade BM 06.022 which are the kidney and liver. Binding of BM 06.022 to liver parenchymal cells could be inhibited by monensin, an inhibitor of receptor recycling, which indicates the dependence of a receptor mediated event. Initial uptake into skin and muscle may be result of extra vascularization.

Conclusions:

1. Lack of glycosylation prolongs half-life and increases apparent pharmacodynamic activity.

2. Pharmacokinetics of BM 06.022 are governed by multiple parallel processes. Different processes may be characterized as saturable or non-saturable. Saturable processes include binding to endogenous inhibitors, eg. PAI; whereas, non-saturable processes are associated with organ uptake.

3. The degree of unspecific (not thrombus related) activation of plasminogen and fibrinogen degradation will depend on the dose injected and endogenous levels of inhibitors found in plasma.

Martin D. Green 2/27/96

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