New NO-Donors with Antithrombotic and Vasodilating Activities, X:

Antiplatelet and Antithrombotic Effects of 3-Methylsydnone-5-nitrosimine (RE 2047) in Combination with ASA, Pentoxifylline, and Ticlopidine

Klaus Rehse*,**) and Thomas Ciborski+)

Institut für Pharmazie der Freien Universität Berlin, Königin-Luise-Str. 2+4, D-14195 Berlin

Received May 27, 1994

The combined effects of the NO-donor RE 2047 with ASA, pentoxifylline, ticlopidine or BM 14515 were determined *in vitro* (*Born*-test) and *in vivo* (rat thrombosis model). The inhibitory effects on platelet aggregation as well as the inhibition of thrombus formation *in vivo* were over additive and over independent. The combination of 10 mg/kg RE 2047 with the same dose of ASA in arterioles (A) showed 70% inhibition of thrombosis (venoles (V): 40%). The respective values for 10 mg/kg RE 2047 and 10 mg/kg pentoxifylline are 70% (A) and 35% (V). It is concluded that NO-donors in principle are compounds suitable for the combination with antithrombotic drugs of different mechanism of action.

Neue NO-Pharmaka mit antithrombotischen und gefäßerweiternden Eigenschaften, 10. Mitt.:
Hemmung von Thrombozytenaggregation und Thrombusbildung durch 3-Methylsydnon-5-nitrosimin (RE 2047) in Kombination mit ASS, Pentoxifyllin und Ticlopidin

Die Wirkung der Kombination des NO-Donators RE 2047 mit ASS, Pentoxifyllin, Ticlopidin oder BM 14515 wurden *in vitro* (*Born*-Test) und *in vivo* (Thrombosemodell, Ratte) bestimmt. Sowohl die Hemmung der Thrombocytenaggregation als auch die Thrombusbildung waren überadditiv und überunabhängig. Die Kombination von je 10 mg/kg RE 2047 und ASS hemmte die Thrombusbildung in Arteriolen (A) zu 70% und in Venolen (V) zu 40%. Die Werte für RE 2047/Pentoxifyllin (je 10 mg/kg) waren 70% (A) bzw. 35% (V). Die Ergebnisse zeigen, daß NO-Donatoren vorteilhaft mit weiteren Antithrombotika kombiniert werden können, sofern diese über einen anderen Wirkungsmechanismus verfügen.

We have shown[1] the advantages of the combined application of antithrombotic drugs with different mechanisms of action. In general over additive effects were seen and a combination of a new antithrombotic oligoamine (RE 1492C) with the title NO-donor had turned out to be especially potent and useful. These results encouraged us to investigate the combination of this NO-donor with compounds already used as single drugs for therapeutic purposes in men, *i.e.* acetylsalicylic acid (ASA), pentoxifylline (P), ticlopidine (T) and molsidomine (M) (Scheme 1). For *in vitro* experiments SIN 1 instead of M was used. The inhibitor of red blood cell aggregation BM 14515 was included because its mechanism of action is different from that all other drugs.

In vitro experiments (Born-test with collagen)

The results of the *Born*-test are summarized in Tab. 1. Exp. 1-6 show the antiplatelet effects of the single drugs. For the combination exp. 7-10 the second compound was added in a concentration which alone has only a small effect (~ 20% inhibition of platelet aggregation). It is obvious that the action of RE 2047 is strongly reinforced by all compounds used. A combination of 0.976 µmol/L RE 2047, *e.g.*, which itself has no effect and 125 µmol/L ASA which itself shows an 18% inhibition of platelet aggregation only, together shows an 83% inhibition. The same effect is seen together with 468 µmol/L of T while P was somewhat less efficient. The addition of a third compound (exp. 10) only gives an appreciable increase in inhibition if the effect of the first two compounds is still rather small (compare exp. 8 with exp. 10).

The type interaction was determined by calculating the theoretical additive effect by the method of *Pöch* and *Holzmann*[2]. The left panel in Fig. 1 shows that 125 µmol/L ASA (18% inhibition) are equieffective to 2.92 µmol/L RE 2047 (trace **a**). This concentration is (theoretically) added to each concentration of RE 2047 and gives rise to trace **c**. Trace **b** - the theoretical independent effect - is calculated by means of the *Ariens* equation[3]. Trace **d** represents the results of exp. 7. It is striking that the effect of the combination by far is over independent and over additive at all concentrations tested. This proves that a massive potentiation occurs. The right panel of Fig. 1 shows, that this behaviour is not at all self evident for the combination of antithrombotic drugs. When ASA is combined with pentoxifylline, at least *in vitro* an over independent but supra additive effect is seen. The addition of the NO-donor SIN 1 to this combination (Tab. 1, exp. 12) markedly increases the inhibitory

+) Part of the PhD thesis *T. Ciborski*, FU Berlin 1991.

**)Cordially dedicated to Prof. Dr. Dr. *W. Schunack* on the occasion of his 60th birthday.

  0366-6233/95/0101-0071 $ 5.00 + .25/0

effect. The addition of P to the combination of RE 2047/ASA only had a marginal effect (compare exp. 7 and 10).

In vivo experiments

The *in vivo* inhibition of thrombus formation in mesenteric vessels of rats induced with a laser beam is shown in Tab. 2 for the single drugs. At high doses of 30-60 mg/kg all compounds markedly inhibit the formation of thrombi, the effect again[1] being more prominent in arterioles than in venoles. The NO-donors RE 2047 and M appear to be most active.

The effect obtained with twelve combinations are compiled in Tab. 3. The data indicate that all drug combinations used strongly inhibit the formation of thrombi at doses when the single drugs only have a small inhibitory effect or even are ineffective. The best results were obtained with a combination of 10 mg/kg RE 2047 and 10 mg/kg ASA. Here a 70% inhibition of thrombus formation in arterioles and a 40% inhibition in venoles was observed (Tab. 3, exp. 2). A nearly equal result was seen for the combination of RE 2047 with P (exp. 4) while the combination of ASA with P was slightly less active (exp. 10). The combinations of RE 2047 with T or BM 14515 gave somewhat smaller inhibitory results (see exp. 5-8). The comparison of exp. 11 and 12 where three compounds were administered in combination suggests that 5 mg/kg RE 2047 and 1 mg/kg M are equieffective.

Fig. 2 gives two examples for the fact that all combined effects *in vivo* have to be classified as over additive and over independent.

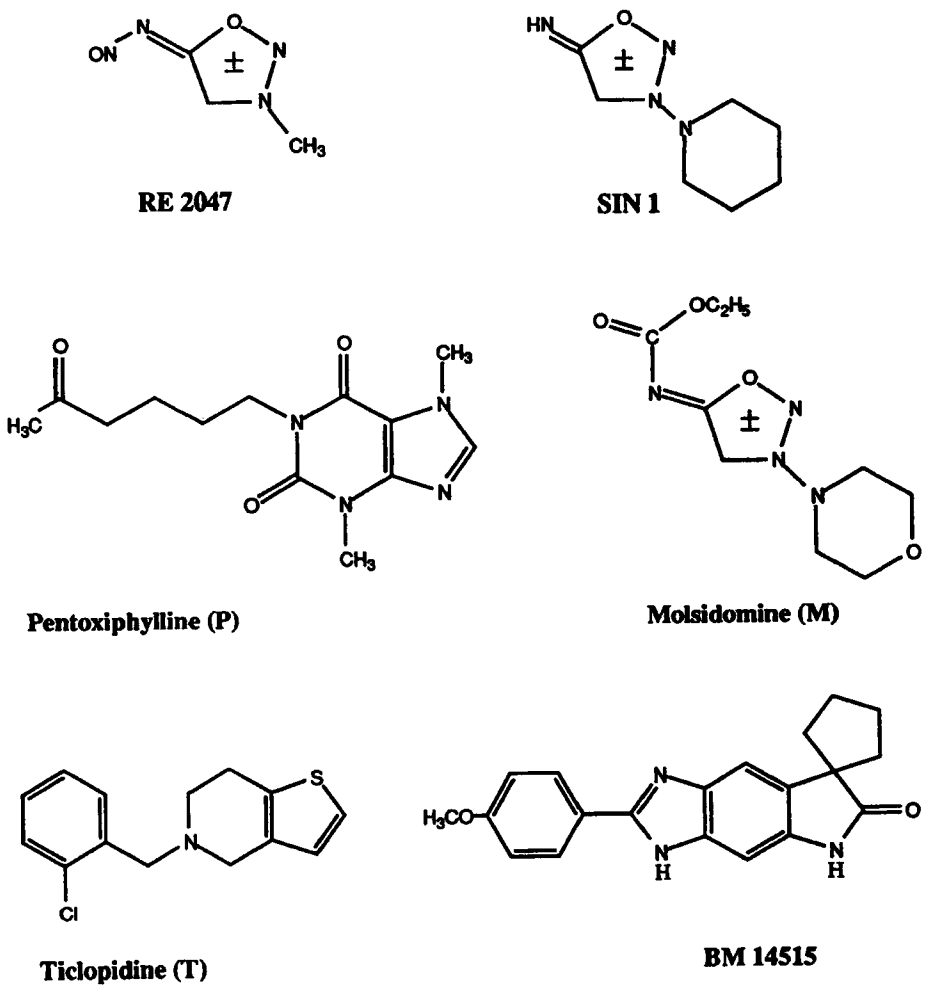
In conclusion it turns out that NO-donors are very suitable and effective antithrombotic drugs which show potentiated effects when applied together with other drugs used for antithrombotic purposes.

Experimental Part

All experiments have been carried out as communicated[1] recently.

Tab. 1: *In vitro* interaction between RE 2047 and other antithrombotic compounds; *Born*-test (ASA = acetylsalic acid; P = pentoxifylline; T = ticlopidine). Effects: • supra additive, • • additive, • • • over additive, + independent, ++ over independent

No exp.	Compound	c[µmol/L] (% inhibition)				
1	RE 2047	0.976 (0)	1.46 (5)	2.92 (18)	3.9 (32)	5.85 (50)
		7.81 (82)	11.7 (100)			
2	ASA	62.5 (0)	93.7 (6)	125 (18)	187 (40)	250 (100)
3	Pentoxiphylline (P)	234 (7)	625 (13)	1250 (44)	1870 (88)	2500(100)
4	Ticlo-	312 (0)	468 (13)	625 (20)	937 (30)	
5	pidine (T)	1250 (51)	1870 (83	2500 (100)		
6	SIN 1	0.122 (0)	0.244 (15)	0.366 (38)	0.976 (83)	1.46 (100)
7		++•••	++•••	++•••	++•••	++•••
	RE 2047 / ASA (125 µmol/L)	0.976 (83) ++•••	1.46 (86)	1.95 (91)	2.92 (95)	3.9 (94)
		5.85 (100)				
8		++•••	++•••	++•••	++•••	++•••
	RE 2047 / P (625 µmol/L)	0.976 (39) ++•••	1.46 (76)	1.95 (82)	2.92 (95)	3.9 (96)
		5.85 (100)				
9		++•••	++•••	++•••	++•••	
	RE 2047 / T (468 µmol/L)	0.976 (83)	1.46 (92)	1.95 (93)	2.92 (100)	
10	RE 2047 / ASA (125 µmol/L) P (625 µmol/L)	0.976 (93)	1.46 (97)	1.95 (100)		
11		++••	++•	++•	++••	
	ASA + P (625 µmol/L)	62.5 (53)	93.7 (66)	125 (88)	187 (96)	250 (100)
12	ASA + P (625 µmol/L) + SIN 1 (0.244 µmol/L)	62.5 (86)	93.7 (97)	125 (100)		



Scheme 1: Chemical structures of antithrombotic compounds

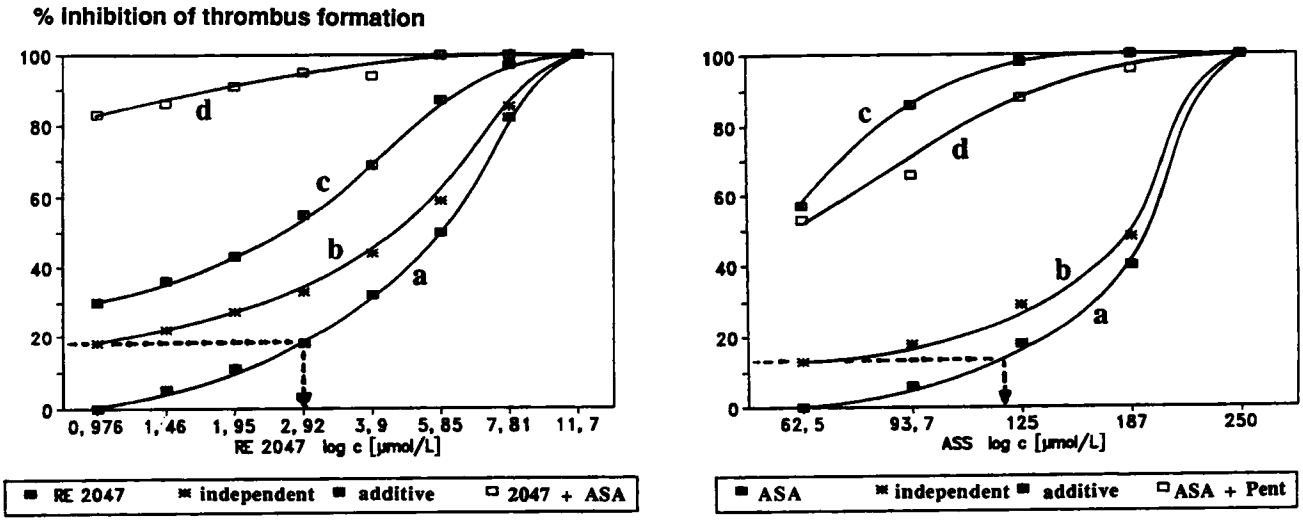


Fig. 1: Classification of the type of synergism between RE 2047 and 125 µmol/L ASA (left panel), or ASA and 625 µmol/L pentoxifylline (right panel)

Tab. 2: *In vivo* inhibition of thrombus formation in rats after oral administration of drugs stated (m-TFI = 1.28 (V); 1.96 (A); n = 5; p.o.; U-test: α^{*} = 0.1-0.5, α^{**} = 0.01-0.05, α^{***} = 0.001-0.005

	Arterioles						Venoles					
mg/kg	0.5	2	5	10	30	60	0.5	2	5	10	30	60
ASA (1h)												
m-TFI	1.96	2.13	2.37	2.70	3.50	3.90	1.27	1.30	1.17	1.43	1.80	2.23
% inhib.	0	4	10	18	38	48	0	0	0	3	11	20
α	n.s.	n.s.	*	**	***	***	n.s.	n.s.	n.s.	n.s.	n.s.	***
RE 2047 (2h)												
m-TFI	-	1.97	2.57	3.13	4.17	4.73	-	1.3	1.23	1.37	2.2	3.23
% inhib.	-	0	15	29	55	69	-	0	0	2	19	41
α	-	n.s.	**	**	***	***	-	n.s.	n.s.	n.s.	***	***
Pentoxiph.(1h)												
m-TFI	-	-	2	2.3	2.7	3.07	-	-	1.17	1.47	1.67	1.97
% inhib.	-	-	1	8	18	27	-	-	0	4	8	15
α	-	-	n.s.	n.s.	*	**	-	-	n.s.	n.s.	*	*
Molsidomine (1h)												
m-TFI	-	-	2.57	3.7	4.47	-	-	-	1.67	2	2.73	-
% inhib.	-	-	15	43	62	-	-	-	8	15	31	-
α	-	-	*	**	***	-	-	-	*	*	***	-
Ticlopidine (3h)												
m-TFI	-	-	-	-	2.33	3.4	-	-	-	-	1.73	2.33
% inhib.	-	-	-	-	9	36	-	-	-	-	10	22
α	-	-	-	-	n.s.	***	-	-	-	-	**	**

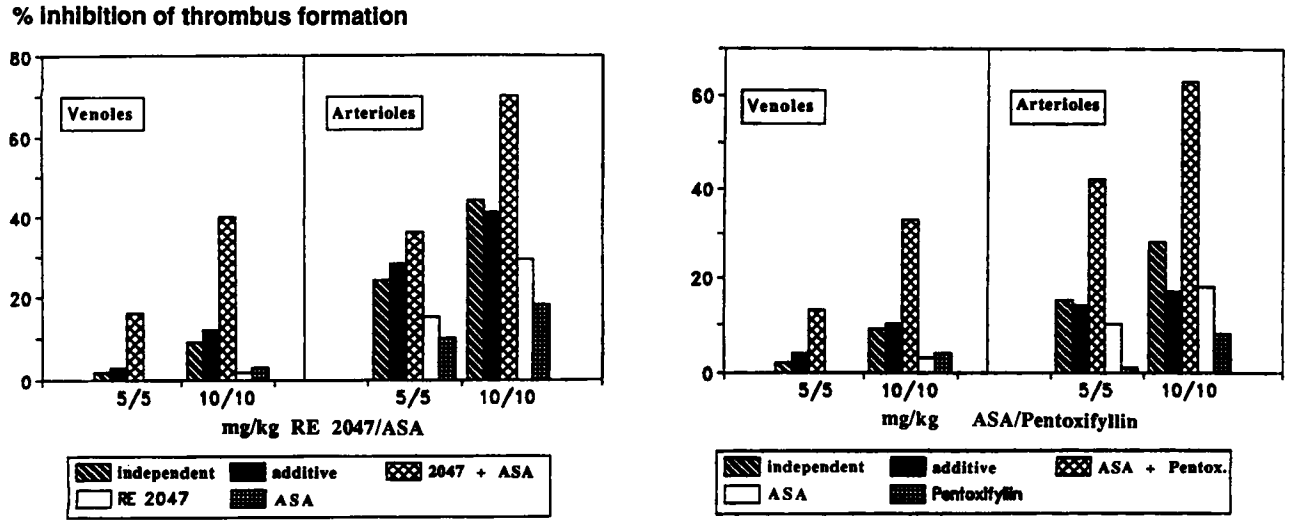


Fig. 2: Inhibition of thrombus formation in rats by RE 2047/ASA (left panel) or ASA/P (right panel)

Tab. 3: Antithrombotic effects of drugs combinations. T was given p.o. at time zero, RE 2047 or M 1 h later, P, ASA or BM 14515 2 h later, so that in all combinations peak activities could be measured (U-test: $\alpha^{*} \leq 0.5$; $\alpha^{**} \leq 0.05$; $\alpha^{***} \leq 0.005$)

Exp	Combination (mg/kg)	Arterioles			Venoles		
No		m-TFI	% inhib.	α	m-TFI	% inhib.	α
1	RE 2047 (5) ASA (5)	3.4	36	**	2.03	16	*
2	RE 2047 (10) ASA (10)	4.77	70	***	3.17	40	***
3	RE 2047 (5) P (5)	3.5	38	***	1.77	10	*
4	RE 2047 (10) P (10)	4.77	70	***	2.93	35	**
5	RE 2047 (5) T (15)	2.77	20	**	1.77	10	**
6	RE 2047 (10) T (30)	3.5	38	***	2.13	18	**
7	RE 2047 (5) BM 14515 (5)	2.77	20	*	1.9	13	***
8	RE 2047 (10) BM 14515 (10)	4	50	*	2.56	27	***
9	ASA (5) P (5)	3.67	42	***	1.87	13	**
10	ASA (10) P (10)	4.5	63	***	2.83	33	*
11	RE 2047 (5) ASA (5) P (5)	3.83	56	***	2.57	27	***
12	ASA (5) P (5) M (1)	4.2	55	***	2.5	26	***

References

1 K. Rehse, T. Ciborski, *Arch. Pharm. (Weinheim)*, submitted for publication (oligoamine: 26. Mitt.).
2 G. Pöch, S. Holzmann, *J. Pharmacol. Methods* **1980**, *4*, 179-188.
3 E.J. Ariens, J.M. van Rossum, A.M. Simonis, *Arzneim. Forsch.* **1956**, *6*, 737-746.

[Ph251]