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Brief definitive report

Bleeding time and antiplatelet agents in normal volunteers

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Summary. Clinical trials have shown that antiplatelet agents are effective in the prevention of thrombosis in arterial diseases and increase bleeding time. To compare the effects of three such drugs [acetylsalicylic acid (ASA) at two dose levels, ticlopidine and indobufen] on bleeding time, we performed a randomized cross-over study on 12 normal subjects. All received the four treatments (ASA 300 mg daily and 500 mg twice daily, ticlopidine 250 mg twice daily and indobufen 200 mg twice daily, each for 6 days plus one dose on day 7) in a sequential manner with a washout period of 15 days between the treatments. Bleeding time was measured using a Surgicut device (Ortho, Milan, Italy) before treatment, 2 and 24 h after the first administration, and before and 2, 24, 48 and 72 h after the last administration. ASA (at both doses) and indobufen quickly induced a significant prolongation of bleeding time, but the effect of indobufen soon wore off after the treatment was stopped, unlike that of ASA. In contrast, ticlopidine treatment prolonged bleeding time only after the first 24 h, and after 7 days the mean value was significantly higher than with ASA (both doses) and indobufen. This significant difference in bleeding time between ticlopidine and the other drugs was still present 48 h after the end of treatment.

Key words: Antiplatelet agents – Bleeding time – Acetylsalicylic acid – Indobufen – Ticlopidine

Introduction

Clinical trials have shown that antiplatelet drugs are useful in the treatment of arterial diseases. For instance, acetylsalicylic acid (ASA) prevents thromboembolic disease both in patients with transient ischemic attacks and in those with unstable angina, and may reduce the incidence of death and reinfarction in survivors of myocardial infarction [1]. Ticlopidine seems effective in preventing thrombosis in neurological patients [7, 8] and perhaps also in unstable angina patients [14]. Indobufen, a reversible inhibitor of thromboxane-dependent platelet aggregation [10–12], has been shown in recently completed randomized trials to produce antithrombotic effects [4, 13]. Monitoring during administration of these drugs revealed hemorrhagic complications, and antiplatelet agents do increase bleeding time. We performed an open, randomized, comparative study on 12 normal volunteers to evaluate the effects on bleeding time, the duration of the effects, and the tolerability of ASA (at two dose levels), ticlopidine and indobufen after repeated administrations.

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Methods

Twelve healthy volunteers, 6 males and 6 females aged between 18 an 30 years, were admitted to the study. All were nonsmokers and all had normal blood chemistry values, blood pressure and physical examinations. Each of the following treatments was given in sequence to all subjects according to a Latin square design: indobufen 200 mg b.i.d. (Ibustrin; Farmitalia-Carlo Erba, Milan, Italy), ASA 300 mg/day and 500 mg b.i.d. (Bayer, Leverkusen, FRG) and ticlopidine 250 mg b.i.d. (Tiklid, Midy, Italy). Each treatment was administered for 6 days plus one dose on day 7. These drug doses are those usually used in controlled and uncontrolled clinical studies. A 15-day washout was allowed between the treatments to obtain optimal clearance and to avoid treating women with antiaggregants during menstruation. Bleeding time was measured by applying a 40 mm Hg pressure on the upper arm and making an incision on the anterior surface of the forearm using a Surgicut device (Ortho, Milan, Italy). The time required for bleeding to stop was evaluated and the results expressed in minutes and seconds. Bleeding time was determined before treatment, 2 and 24 h after the first administration, and before and 2, 24, 48 and 72 h after the last administration of each drug. All bleeding times were measured by the same physician, blinded as to treatment.

The significance of differences in bleeding time at each time point were evaluated by analysis of variance for a Latin square design [3]. When analyzing bleeding time we processed reciprocals of values observed in order to normalize the distribution of data. When the analysis of variance revealed a statistically significant difference between the means of the various comparisons among all the possible pairs, tables were computed according to Bonferroni [2].

Table 1. Bleeding time during the four treatments

Treatment		Study day							
		1			7				
		Hours			Hours				
		0	2	24	0	2	24	48	72
A	*n*	12	12	12	12	12	12	12	12
	Mean	32.17	17.00	16.72	20.56	16.31	20.51	25.15	29.38
	SD	14.23	6.33	6.80	4.09	3.75	6.49	10.28	17.78
B	*n*	12	12	12	12	12	11	11	11
	Mean	24.24	17.12	15.86	15.62	15.60	14.65	17.26	18.44
	SD	6.64	3.88	3.35	4.57	5.96	4.68	6.59	4.70
C	*n*	11	11	11	11	11	11	11	11
	Mean	28.12	14.88	15.55	14.54	14.89	15.25	18.05	22.14
	SD	7.51	6.87	5.27	4.51	3.09	5.21	9.32	5.18
D	*n*	12	12	12	12	12	12	12	12
	Mean	25.27	23.08	20.67	8.92	8.44	9.61	8.74	10.74
	SD	7.08	8.56	9.80	4.27	4.54	5.59	3.90	3.68

[a] Reciprocals of the values were analyzed in order to normalize distribution of data
A, Indobufen; B, ASA 300 mg/day; C, ASA 500 mg b.i.d.; D, ticlopidine

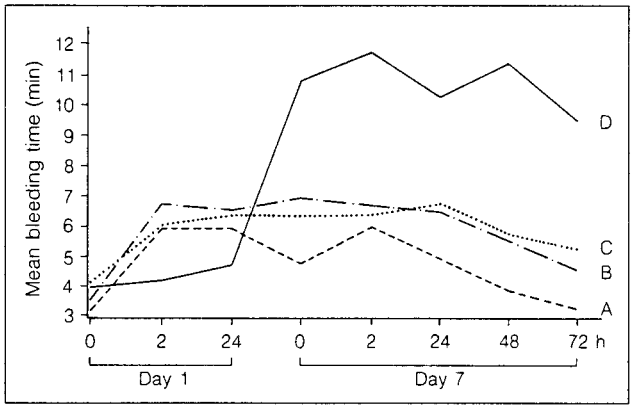

Fig. 1. Bleeding time during the four treatments (A, indobufen; B, ASA 300 mg/day; C, ASA 500 mg b.i.d.; D, ticlopidine)

Results

The bleeding time pattern observed with the four treatments is shown in Fig. 1. ASA (at both doses) and indobufen caused a rapid modification of bleeding time, whereas with ticlopidine the values remained constant for the first 24 h (Table 1). During this period the only significant difference was between ticlopidine and the higher ASA dose (Table 2).

After 7 days of treatment, the effect of indobufen disappeared more rapidly than that of ASA. With ticlopidine treatment, bleeding time showed a considerable increase at 7 days and values remained high after discontinuation of the drug. The mean value 48 h after treatment was significantly higher with ticlopidine than all the other treatments, and it was still significantly higher than after indobufen and the higher ASA dose at 72 h (Table 2).

Tolerability

During treatment with the higher ASA dose episodes of heartburn and mild epigastric pain were observed in 5 and 4 subjects respectively, which resolved spontaneously at the end of treatment. One episode of mild menometrorrhagia occurred in 1 woman at the end of treatment with the lower ASA dose, which resolved spontaneously. Ticlopidine treatment was associated with mild diarrhea in 60% of subjects, and this side effect also resolved spontaneously when treatment ended. No fall in the leukocyte count was observed with the ticlopidine treatment. Indobufen was well tolerated on the whole.

Discussion

This cross-over trial confirmed that prolongation of bleeding time is a common side effect of the antiaggregating drugs used in clinical practice for the prevention of arterial thromboembolic disease. No difference was observed between the two doses of ASA investigated (300 mg/day and 500 mg b.i.d.) as reported in the literature, although not in cross-over trials on normal volunteers [9]. However, 30 mg ASA are known to prolong bleeding time [9–11].

In our study ASA and indobufen showed a similar effect on bleeding time, which rose significantly over the first 24 h of treatment with both drugs. In contrast as expected, the administration of ticlopidine did not affect bleeding time during the first 24 h. However, ticlopidine did increase bleeding time after the first 24 h with a two- to threefold increase on the 7th day of treatment with respect to baseline.

This significant increase persisted for the 72 h following drug suspension when the mean value was 11 min. In

Table 2. Statistical analysis, according to Bonferroni tables, was performed on the basis of significant differences between the means of bleeding time

	Bleeding time hour	Treatment times		Bleeding time (min)				Significant differences	
				A	B	C	D	Treatments	*P*
Day 1	0	$s_e^2 = 94.987$ (29)							
		0.208	0.966	32.17	24.24	28.47	25.27		
	2	$s_e^2 = 35.234$ (29)						C–D	0.0035
		0.017	0.136	17.00	17.11	15.11	23.08		
Day 7	24	$s_e^2 = 21.519$ (29)							
		0.047	0.79	16.72	15.86	15.65	20.66		
	0	$s_e^2 = 11.208$ (29)						A–B	0.0011
								A–C	0.0002
								A–D	0.0001
								B–D	0.0001
		0.0001	0.187	20.56	15.61	14.65	8.92	C–D	0.0003
	2	$s_e^2 = 14.613$ (29)						A–D	0.0001
								B–D	0.0001
		0.0001	0.157	16.31	15.60	14.85	8.44	C–D	0.0004
	24	$s_e^2 = 15.908$ (28)						A–B	0.0034
								A–C	0.0059
								A–D	0.0001
								B–D	0.0029
		0.0001	0.144	20.51	15.11	15.48	9.61	C–D	0.0017
	48	$s_e^2 = 37.766$ (28)						A–D	0.0001
								B–D	0.0009
		0.0001	0.288	25.15	18.36	18.68	8.74	C–D	0.0007
	72	$s_e^2 = 86.927$ (28)						A–D	0.0001
		0.0006	0.353	29.36	18.88	22.50	10.74	C–D	0.0058

contrast, bleeding time returned to normal 24 h after the suspension of indobufen, and more slowly after the end of both ASA treatments. Forty-eight hours after suspending ASA, bleeding time was significantly less than after ticlopidine. The different pattern with indobufen and ASA is attributable to the persistent and irreversible effect of ASA on primary hemostasis (platelets/endothelium), whereas the effect of indobufen is reversible. Nevertheless, it seems possible that ASA exerts a higher degree of inhibition on platelets than bleeding time.

The persistent prolongation of bleeding time induced by ticlopidine even after its suspension is interesting. Similar findings were reported by Ellis et al. [6] many years ago. It has been suggested that ticlopidine's activity could be related to one or more unstable metabolites that, after each oral administration, exert their action at the intestinal level [5]. When considering the use of antiplatelet drugs in clinical practice, this specific characteristic of ticlopidine must be borne in mind. It has been reported in most patients included in recent clinical trials using this drug to treat arterial thrombotic disease [7]. In our study indobufen was the easiest drug to handle in terms of effects on bleeding time, with the same antiplatelet effect as the other drugs studied. Thus this drug may be clinically very useful in the future. Recently two controlled clinical studies (performed in Italy) demonstrated for the first time that indobufen is able to reduce aortocoronaric bypass reocclusion as effectively as the Chelsbro classic protocol, but with fewer side effects and higher tolerance, and to improve the patency rate in patients who have undergone percutaneous transluminal coronary angiography after acute myocardial infarction [4, 13]. The data obtained in our study, which was performed according to strict pharmacological criteria, should be considered carefully when planning treatment of arterial thrombotic disease, also in terms of patient compliance with a particular kind of antiplatelet drug.

References

1. Antiplatelet Trialists' Collaboration, Secondary prevention of vascular disease by prolonged antiplatelet treatment. BMJ p 296, 1988
2. Bailey BJR, Tables of the Bonferroni "t" statistics. J Am Stat Assoc 72:469, 1977
3. Cochran WG, Cox GM, Experimental design. Wiley, New York, p 17, 1950
4. Dalla Volta S, Antiplatelet treatment in the prevention of reocclusion after PTCA: an Italian multicenter study. Blood 74 [Suppl 1]:1769, 1989
5. Defreyn G, Bernat A, Delebrassee, Maffraud P, Pharmacology of ticlopidine: a review. Semin Thromb Hemost 15:159, 1989

6. Ellis DJ, Roerl Bruno JJ, Cranston BJ, McSpadden MM, The effects of ticlopidine hydrochloride on bleeding time and platelet function in man. Thromb Haemost 46: 1973, 1981
7. Gent M, Easton JD, Hachinski VC, Sicurella J, Blakel JA, Ellis DJ, Harbison JW, Roberts RS, Turpie AGG, and the CATS group, The Canadian American ticlopidine study (CATS) on thromboembolic stroke. Lancet p 1217, 1989
8. Hass WK, Easton JD, Adams HP, Pryse PW, Molony BA, Anderson S, Kamm B, A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. N Engl J Med 321: 501, 1989
9. Kallmann R, Nieuwenhuis HK, De Groot PG, Van Gijn J, Sixma JJ, Effects of low doses of aspirin, 10 mg and 30 mg daily, on bleeding time, thromboxane production and 6-keto-PG F1a excretion in healthy subjects. Thromb Res 45: 355, 1987
10. Mannucci L, Maderna P, Colli S, Lavezzari M, Sirtori CR, Tremoli E, Indobufen is a potent inhibitor of whole blood aggregation in patients with a high atherosclerotic risk. Thromb Res 48: 417, 1987
11. Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A, Satta MA, Peskar BA, Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. Thromb Res 17: 317, 1980
12. Pogliani E, Corvi G, Mandelli V, Fuccella LM, Preliminary human pharmacology studies on the inhibition of platelet aggregation by Indobufen (K 3920). Haematologica (Pavia) 66: 1, 1981
13. Rovelli F, Indobufen versus aspirin + dipyridamole in preventing early aortocoronary graft occlusion: a double blind randomized multicenter study. Blood 74 [Suppl 1]: 1769, 1981
14. Violi F, Scrutini D, Ciminiello C et al., S.T.A.I. (Study of Ticlopidine in Unstable Angina) (abstract). J Am Coll Cardiol 13 [Suppl A]: 238 A, 1989