ESTIMATION OF THE PLASMA CONCENTRATION AND COURSE OF ACTION OF PHENTOLAMINE BASED ON ITS INHIBITORY EFFECT ON ADRENALINE-INDUCED PLATELET AGGREGATION

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1 Orally administered phentolamine retards the first phase of adrenaline-induced platelet aggregation. The extent to which aggregation was inhibited was compared in blood samples from six healthy volunteers treated with phentolamine and samples to which various concentrations of the drug were added *in vitro*.

2 These experiments permitted conclusions to be drawn concerning the magnitude and time-course of the plasma concentrations reached after the administration of phentolamine. A dose of 40 mg of the standard formulation (SF) produced a maximum effect after 30 min corresponding to that of a phentolamine concentration of approximately 12×10^{-8} M (≈ 34 ng/ml). After 3 h about one-third of the maximum effect persisted, and after 6 h only a slight degree of activity was still detectable. The slow-release formulation (SR) (100 and 200 mg) had a rapid onset of action (1 h); the maximum effect was observed after 4 h, corresponding to an estimated plasma concentration of phentolamine of 11 and 42 ng/ml respectively, and the duration of action was well in excess of 12 h.

3 The time-course of the increase in heart rate and the increase in myocardial contractility (as determined from the pre-ejection period) was largely consistent with the calculated time-course of the plasma concentrations: A dose of 40 mg SF produced its maximum effect after 30–60 min and had a duration of action of 3 h. The maximum effect of the SR formulation was reached after 4 h, and the duration of action was more than 10 h.

Introduction

Phentolamine has a marked and readily reproducible inhibitory effect on the aggregation of platelets induced by adrenaline (Pfister & Imhof, 1977). This action is attributable to competitive inhibition of the effect of adrenaline on the platelet membrane. As has been demonstrated *in vitro*, the consequent retardation of the initial phase of aggregation depends on the concentration of phentolamine.

On the basis of these findings, we have elaborated a test system permitting conclusions to be drawn with respect to the plasma concentrations after the administration of the standard and slow-release formulations of phentolamine. Simultaneous measurements were made of heart rate and the systolic time-intervals.

Methods

The study was performed on six healthy male volunteers of ages ranging from 26–51 years and

weighing 64–88 kg after they had given their informed consent in writing. The subjects were given a placebo, 40 mg of the standard formulation (SF) of phentolamine (Regitine®, Ciba-Geigy Limited, Basle, Switzerland) (two 20 mg tablets of the commercial presentation) and 100 and 200 mg of the slow-release (SR) formulation (Batch 03/756/1, 100 mg tablets). An interval of at least 2 days without medication was allowed between treatments. The substances were administered in the morning on an empty stomach. Two hours later, the subjects were given a light, standardized breakfast, and after a further 2 h a light, standardized lunch. At various times after administration, blood samples for platelet aggregation tests were withdrawn from an uncompressed vein. After each venepuncture, the curves for the determination of the systolic time-intervals were recorded. Platelet aggregation tests were made *in vitro* with the platelet-rich plasma (PRP) obtained in the first and third hours after placebo medication after the addition of phentolamine methane sulphonate in

Figure 1 Analysis of aggregation curves: (a) original curves showing effects of various concentrations of adrenaline (10, 2, 1, 0.6, 0.4, 0.3 μM). $\Delta OD_1/\text{min}$ = rate of decrease in optical density during first phase of aggregation (b) rate of aggregation in relation to concentration; (c) reciprocals of same values (Lineweaver-Burk diagram)

$$\text{tg } \alpha = \frac{\Delta \frac{1}{\Delta OD_1/\text{min}}}{\Delta \frac{1}{[\text{adrenaline}]}}$$

as index of first phase of aggregation

various concentrations (4.5×10^{-8} , 6.3×10^{-8} , 9.5×10^{-8} , 1.35×10^{-7} , 1.6×10^{-7} and 1.9×10^{-7} M). Aggregation was induced according to the method of Born & Cross (1963) with adrenaline (Stago, France) in concentrations of 10, 2, 1, 0.6, 0.4 and 0.3 μM. For this purpose, blood drawn from an uncompressed vein was immediately mixed in a ratio of 9 parts to 1 with 3.8% sodium citrate and centrifuged for 8 min at 150 g; the supernatant PRP was pipetted off. The residual blood was centrifuged for a further 10 min at 3000 g to produce platelet-poor plasma (PPP). The PRP was then diluted with PPP to give a platelet

count of 200,000/mm³. Platelet aggregation was determined nephelometrically with a Labintec aggregometer (Labintec, Montpellier, France). Samples of 0.2 ml PRP were brought to a temperature of 37°C in the aggregometer and stirred at 1100 rev/min. Aggregation was induced between 50 and 100 min after the withdrawal of blood. Optical density was calibrated at 0% for PPP and aggregation initiated by the addition of adrenaline at a setting of 100%.

Upon analysis of the results, the rate of the first phase of aggregation ($\Delta OD_1/\text{min}$) after the addition

of various concentrations of adrenaline was determined, as illustrated on the original curves shown in the uppermost panel of Figure 1. When the rate of aggregation is plotted against the concentration of adrenaline, curves are obtained that resemble those describing enzymatic reactions (Figure 1b). Plots of the reciprocals

$$\frac{1}{\Delta OD_1/\text{min}} \text{ v. } \frac{1}{[\text{adren.}]} \text{ (Lineweaver-Burk diagram)}$$

yield a rectilinear function, the slope of which, $\text{tg } \alpha$

$$\left(\frac{\Delta \frac{1}{\Delta OD_1/\text{min}}}{\Delta \frac{1}{[\text{adren.}]}} \right)$$

we take as an index of the first phase of adrenaline-induced aggregation (Figure 1c).

The results were analysed for each subject individually and means and s.e. means ($\bar{x} \pm s_{\bar{x}}$) were then calculated.

The systolic time-intervals were determined according to Weissler, Harris & Schoenfeld (1968) from simultaneous recordings of the electrocardiogram, carotid pulse and phonocardiogram. Our calculations are limited to the rate-corrected pre-ejection period (PEP_c), which is a reliable parameter of myocardial contractility. Heart rate was taken from the e.c.g. tracings. The recordings were made during the third minute of passive tilting to 70°. The statistical significance of the differences between any changes in the cardiovascular parameters observed after placebo and those produced by phentolamine was determined by the paired *t*-test and is indicated in the figures by * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

Results

Platelet aggregation

Only slight changes in the first phase of aggregation were noted after the administration of placebo. Both the addition of phentolamine to the samples *in vitro* (Table 1) and the oral administration of the drug led to a retardation of the first phase of aggregation, manifested in the Lineweaver-Burk diagram by an increase in the slope ($\text{tg } \alpha$). The greater the concentration of phentolamine added *in vitro*, the steeper was the slope (Table 1). From the slopes of the straight-line functions obtained with the various concentrations added *in vitro*, a standard curve was constructed for each of the six subjects. By transforming $\text{tg } \alpha$ into α , we obtained standard rectilinear functions (r of correlations for all subjects > 0.97) permitting more accurate interpolation. By comparing the values recorded after oral administration of phentolamine with these standard functions, we were able to ascertain the time-course of the effect of the substance in each subject (Figure 2). From these curves, it is evident that the duration of action and the maximum effect of the different treatments were highly consistent in all the subjects. There were nevertheless fairly marked differences in the intensity of the effects and hence presumably in the individual plasma concentrations. Calculations of the areas under the curves also revealed certain disparities in the activity ratios of the standard and slow-release formulations. Whereas the relative effects of the 100 mg and 200 mg doses of the SR formulation remained within roughly the same ratio of 1:3 to 1:5 in all subjects, the activity ratio of 40 mg SF to 200 mg SR phentolamine varied between 1:2.5 and 1:12.8. On the average (Figure 3), the maximum effect of a dose of 40 mg of phentolamine SF was reached after 30 min; 3 h after administration,

Table 1 Change in slope ($\text{tg } \alpha$) in Lineweaver-Burk diagram induced by addition of various concentrations of phentolamine *in vitro* to plasma from six subjects

Phentolamine concentration (M)	Subject					
	193 <i>tg</i> α	221 <i>tg</i> α	214 <i>tg</i> α	224 <i>tg</i> α	168 <i>tg</i> α	108 <i>tg</i> α
Blank value	0.017	0.023	0.014	0.021	0.014	0.024
4.5×10^{-8}	0.023	0.031	0.028	0.028	0.029	0.049
6.3×10^{-8}	0.029	0.055	0.037	0.032	—	0.071
9.5×10^{-8}	0.031	0.076	0.049	0.055	0.047	0.148
1.35×10^{-7}	0.051	0.084	0.068	0.071	0.059	0.287
1.6×10^{-7}	0.073	0.108	0.084	0.076	—	0.410
1.9×10^{-7}	0.079	0.127	0.137	0.091	—	0.795

Figure 2 Individual time-course of the effect of phentolamine (● 40 mg, SF), (○ 100 mg, SR), (■ 200 mg, SR) p.o. on platelet aggregation shown as calculated plasma concentrations.

about one-third of the maximum effect still persisted and after 6 h only a slight effect was demonstrable. The maximum effect of the oral dose of phentolamine SF corresponded to the effect of a concentration of approximately 12×10^{-8} M (≈ 34 ng/ml) added *in vitro*. In response to doses of 100 and 200 mg of the SR formulation, a distinct effect was apparent after 1 h, and the maximum was observed after 4 hours. In

Figure 3 Time-course of the effect of phentolamine (40 mg SF ●), (100 mg SR ○) and (200 mg SR ■) on platelet aggregation shown as calculated plasma concentrations. $\bar{x} \pm s_{\bar{x}}$; $n=6$ (100 mg SR, $n=5$).

this case the mean maximum effects corresponded to those of an *in vitro* concentration of roughly 4×10^{-8} and 15×10^{-8} M (≈ 11 ng/ml and 42 ng/ml), respectively. The effect of 200 mg of phentolamine SR was thus slightly greater than that of 40 mg of phentolamine SF. The effect subsided slowly and was still clearly in evidence after 12 h (1.5×10^{-8} and 8×10^{-8} M in relation to phentolamine *in vitro*). Twenty-four hours after administration, no further effect was demonstrable.

Cardiovascular studies

Heart rate (Table 2). The administration of phentolamine was followed by an increase in heart rate, compared with the placebo values. In the recumbent position, the changes were slight, the only statistically significant difference being recorded 4 h

Table 2 Mean increases in heart rate in recumbent (R) and upright (U) positions at various times after phentolamine 40 mg SF ($n=6$) and 100 mg ($n=5$) and 200 mg SR ($n=6$) in relation to changes after placebo.

Time after administration (h)	40 mg SF		100 mg SR		200 mg SR	
	R	U	R	U	R	U
Increase in heart rate (beats/min)						
0.5	2.8	14.3*				
1.0	4.7	8.3	4.0	2.2	5.2	4.2
1.5	2.0	12.6*				
2	(-1.0)	12.4	4.0	11.4	3.7	11.2***
3	(-0.7)	9.0				
4			6.0	15.8	10.0**	23.1**
6	0.8	1.5	1.2	7.8	8.0	9.5
8			0.8	8.8	9.2	18.2*
10			5.6	5.0	6.7	11.2*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

after a dose of 200 mg SR (+10 beats/min). During passive upright tilting, more marked increases were observed: 40 mg SF produced a maximum increase of 14.3 beats/min after 30 min and had a duration of action of slightly more than 3 h, but less than 6 h; after 100 mg and 200 mg SR, maximum increases of 15.8 and 23.1 beats/min, respectively were recorded and the effect persisted for more than 10 hours.

Rate-corrected pre-ejection period (PEP_c) (Table 3). Phentolamine shortened the duration of the PEP_c during passive upright tilting. A dose of 40 mg SF produced a decrease at all determination times, by comparison with the placebo value, with a maximum after 60 min (−23.9 msec). A dose of 100 mg SR led to a maximum decrease of 16.4 msec after 1 h and some effect was still detectable after 10 hours. After 200 mg SR, the duration of the PEP_c was shortened at all determinations, the maximum decrease of 22.2 msec being recorded after 4 hours.

Discussion

Phentolamine has a dose-related inhibitory effect on adrenaline-induced platelet aggregation. This effect is so pronounced and readily reproducible, especially during the initial phase of aggregation, that inferences concerning the plasma concentrations of the drug and their time-course can be drawn when the aggregation curves obtained *in vitro* and *ex vivo* are compared and

evaluated by a new analytical procedure. The method is based on the assumption that the inhibition of platelet aggregation *in vitro* is caused by unchanged phentolamine, which appears admissible since the main metabolite (2-[N-(*p*-carboxyphenyl)-N-(*m*-hydroxyphenyl)-aminomethyl]-2-imidazoline) has no effect on platelet aggregation. The calculated concentration of unchanged drug after the administration of 40 mg phentolamine SF also corresponds closely in its maximum and time-course with the values determined on other occasions by other methods (Imhof, Garnier, Brunner, Keller & Rohrer, 1975). The prolongation of effect achieved by the slow-release formulation is clearly evident upon comparison of the aggregation curves obtained after the administration of 40 mg phentolamine SF and 100 and 200 mg phentolamine SR. The two cardiovascular parameters determined in these studies, heart rate and the pre-ejection period in the upright position, had already proved in previous investigations to be reliable indicators of the pharmacodynamic action of phentolamine (Frei, Imhof & Dubach, 1974). The observed increases in heart rate and myocardial contractility are largely in keeping with the calculated plasma concentrations.

The foregoing results demonstrate that the slow-release formulation of phentolamine has a rapid onset of action (less than 1 h), induces its maximum effect after 4 h, and has a duration of action of about 12 h when given in a dose of 100 mg and distinctly more than 12 but less than 24 h when given in a dose of 200 mg.

Table 3 Mean decreases in rate-corrected pre-ejection period (ms) in upright position at various times after phentolamine 40 mg SF (*n* = 6), 100 mg SR (*n* = 5) and 200 mg SR (*n* = 6).

Time after administration (h)	40 mg SF	100 mg SR	200 mg SR
	Pre-ejection period (ms)		
0.5	20.5**		
1.0	23.9**	16.4*	17.2*
1.5	9.2		
2	13.0***	3.2	14.7*
3	11.8		
4		8.6	22.2*
6	11.0*	8.8*	7.3
8		3.2	9.5**
10		8.8	9.2

P* < 0.05; *P* < 0.01; ****P* < 0.001.

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(Received February 17, 1977)