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COMPARATIVE PHARMACOLOGICAL EVALUATION OF DIFFERENT SUBLINGUAL NITROGLYCERIN FORMULATIONS

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ABSTRACT

Using a double blind, randomized cross-over design, the heart rate and systolic blood pressure of five healthy human subjects were compared following sublingual administration of three different nitroglycerin tablets and a placebo. The nitroglycerin formulations included two commercially available tablets (one molded and one directly compressed) and an experimental tablet (directly compressed) which was developed in our laboratory. Significant increase in heart rate ($P < 0.05$) was demonstrated for two of the three nitroglycerin formulations

when compared to placebo, but no difference in heart rate elevation could be observed among the three active products. Decrease in systolic blood pressure was shown to be a less consistent circulatory response to sublingual nitroglycerin in this study, and no statistical significance ($P > 0.05$) in this parameter could be observed in the different treatments. It appeared that formulation factors such as addition of stabilizers and insoluble adjuvants to the dosage forms and change in tablet preparation methods did not significantly affect the pharmacological performance in circulatory effects of nitroglycerin.

INTRODUCTION

The use of sublingual nitroglycerin for the symptomatic relief in angina pectoris has been well established. Until recently, however, the formulation of a stable, non-volatile dosage form of nitroglycerin had been largely neglected. In the past few years, a number of attempts (1-5) had been made to prepare stabilized nitroglycerin tablets in which the volatility of the drug is substantially reduced through interaction with non-toxic macromolecules. Furthermore, there is now a trend to prepare nitroglycerin sublingual tablets by direct compression, replacing the classical molding technique which tends to give rise to more friable tablets with larger variations

in tablet weight and potency. Included in these efforts was our development of a stable, rapidly disintegrable, and directly compressed tablet containing a mixture of soluble and insoluble polyvinylpyrrolidone (4, 5).

The circulatory effects of nitroglycerin, i.e., blood pressure lowering and heart rate elevation, had been used to evaluate the bioavailability of nitroglycerin tablets in the dog (6) and of nitroglycerin inhalation aerosol in man (7). The same pharmacological effects were used in humans to demonstrate cross tolerance between nitroglycerin and pentaerythritol tetranitrate (8). Although preliminary data by Bogaert and Rosseel (9) could not demonstrate a direct relationship between heart rate and plasma nitroglycerin levels, it is generally believed that part of the beneficial effect of nitroglycerin may be due to its action in lowering systemic blood pressure, and hence, the oxygen requirements of the myocardium (10, 11). Using an exercise test in patients with angina, Goldstein et al (12) concluded that clinical improvement after nitroglycerin may be due to circulatory changes in blood pressure, heart rate and ejection time.

Although the papers cited above are of value to those investigating nitroglycerin products, there are rather considerable difficulties involved in comparative biological avail-

ability studies of products containing this drug. Its very short half life and lack of a really convenient chemical assay coupled with the difficulties in precisely evaluating clinical response, complicates biological availability determinations. In the present paper, data is presented which demonstrates the nature of this problem and points up the need for improved methodology in this area.

EXPERIMENTAL

Pharmacological measurements - Initially, six normal healthy human subjects, one female (subject SA) and five males, ranging in weight of 112-210 pounds and age 27-32 years, with no history of cardiovascular diseases were selected for the study. One subject (JL) had a precipitous drop in systolic blood pressure of more than 60 mm Hg during the first treatment, and he was subsequently dropped from the study. Each of the remaining five subjects were given sublingually a placebo tablet (P)[a], 0.6 mg nitroglycerin sublingual tablets A[b], B[c] and C[d] in a double-blind, randomized cross-over manner as shown in Table 1. The subjects were allowed to continue with their normal daily activities before and after each treatment. However, on treatment days prior to the administration of the different tablet formulations, they were asked to abstain from

TABLE I

EXPERIMENTAL DESIGN

	Treatment Group			
Subject	I	II	III	IV
SA	A	P	B	C
MB	B	C	A	P
DL	C	A	B	P
BF	C	B	P	A
CO	P	A	C	B
JL*	B			

* Dropped after Treatment #1

smoking, strenuous exercises, and caffeine. After the subject was seated in a comfortable position and well rested, as determined by stable baseline readings of both blood pressure and heart rate, the appropriate test formulation was administered sublingually. Heart rate was recorded by an electrocardiogram using LA, LL, RA, RL leads. A chart speed of 25 mm/sec was used during the study. The distance between two QRS cycles was measured and converted to beats per minute using a pre-calibrated ruler[e]. The mean of six consecutive measurements was recorded as the heart rate at a particular time. All blood

pressure measurements were recorded by one individual using a sphygmomanometer. Both of these parameters were monitored at -10, -5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 60 minutes after drug administration. The interval between each treatment was approximately one week on the average.

Nitroglycerin content in test formulations - Since it was essential to maintain uniformity in nitroglycerin content in all the test formulations during the whole course of the study, the following sampling procedure was adopted. Prior to the study, more than four bottles of the same production lot of commercial tablets A and B were collected. Tablet C was placed in a well filled glass container. Before each pharmacological experiment, a fresh bottle of A and B was opened and six tablets of each formulation were taken out for assay, using the kinetic method (13, 14). Individual unchipped tablets were selected and weighed before they were administered to the subjects.

RESULTS AND DISCUSSION

The nitroglycerin contents of the three test formulations during different treatment weeks are shown in Table II. All the nitroglycerin tablets tested lie within the official USP specification of 75 to 135% of labeled strength. Generally, tablet C, which was prepared in our laboratory, contained

TABLE II

CHEMICAL ASSAYS OF THE DIFFERENT NITROGLYCERIN SUBLINGUAL TABLET FORMULATIONS

Formulation	Labelled strength	Mean ± S. D.* Nitroglycerin Content (mg.) of Tablets Used in Treatment Group			
		I	II	III	IV
A	0.6 mg	0.604±0.079	0.582±0.057	0.606±0.057	0.667±0.087
B	0.6 mg	0.639±0.018	0.635±0.018	0.659±0.014	0.687±0.012
C	--	0.547±0.024	0.533±0.010	0.540±0.019	0.593±0.022

*Each figure represents mean and standard deviation of six different single nitroglycerin tablet assays.

slightly less active drug than the commercial products. The standard deviation was considerably higher in the molded tablet A, than the two directly compressed tablets, B and C. This finding is in agreement with that described by Dorsch and Shangraw (15) who also found better nitroglycerin content uniformity with directly compressed tablets.

The time-dependent changes in heart rate brought about by the test tablets were shown in Fig. 1. The placebo treatment was found to have little effect on the observed heart rate while all three nitroglycerin formulations produced noticeable elevation in this pharmacological parameter soon after nitroglycerin administration. Maximum increases were generally observed around four minutes post dosing. In all cases, the heart rate returned to its normal value about 20 minutes after administration of the drug.

The maximum increase in heart rate in each subject after each treatment is tabulated in Table III. Statistical treatment of the data using one-way analysis of variance showed a statistical significance between treatments at the 0.05 level. Pair analysis using the Tukey's method (16) showed that elevation of heart rate produced by treatment A and C were significantly different from that produced by placebo. However, no statistical difference could be demonstrated among the three

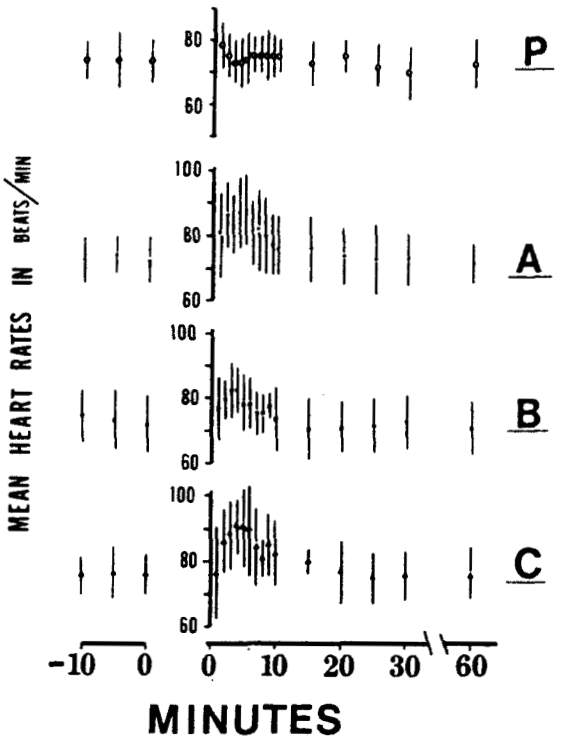

FIG. 1

Mean $\pm$ S.D. of heart rates in beats/min. of all subjects after administration of test formulations. Vertical lines represent 1 S.D.

active tablet formulations, or between tablet B and placebo. It is interesting to note that the average increase in the number of heart beats per minute produced by tablet A (19.6), B (12.0) and C (18.4) in this study was comparable to that produced (12.7) by a 0.52 mg dose of nitroglycerin administered through an aerosol (7).

TABLE III

STATISTICAL ANALYSIS OF THE MAXIMUM ELEVATION OF HEART RATE

Subject	Maximum increase in heart rate (beats/min.) produced by tablet			
	P	A	B	C
SA	8	24	6	21
MB	3	25	11	11
DL	7	19	22	28
BF	4	9	10	10
CO	3	21	11	22

ANOVA:

Source	d.f.	Sums of Squares (SS)	Mean SS	F	P*
Between Treatments	3	575.35	191.78	4.98	0.05
Error	16	616.40	38.53		
Total	19	1191.75			

Multiple Comparison Using the Tukey's Method:

Pair Compared	Mean differences**	P at <0.05 level
P Versus A	14.6	s
P Versus B	7.0	n.s.
P Versus C	13.4	s
A Versus B	7.6	n.s.
A Versus C	1.2	n.s.
B Versus C	6.4	n.s.

$P \leq 0.05$ when $F(3, 16) \geq 3.24$

Tukey allowable mean differences are 11.14 and 14.27 at P=0.05 and P=0.01 respectively.

s = Significant

n.s. = Not significant

Fig. 2 shows the changes in systolic blood pressure as a function of time after administration of the four test products. Again, the placebo tablet produced little changes in blood

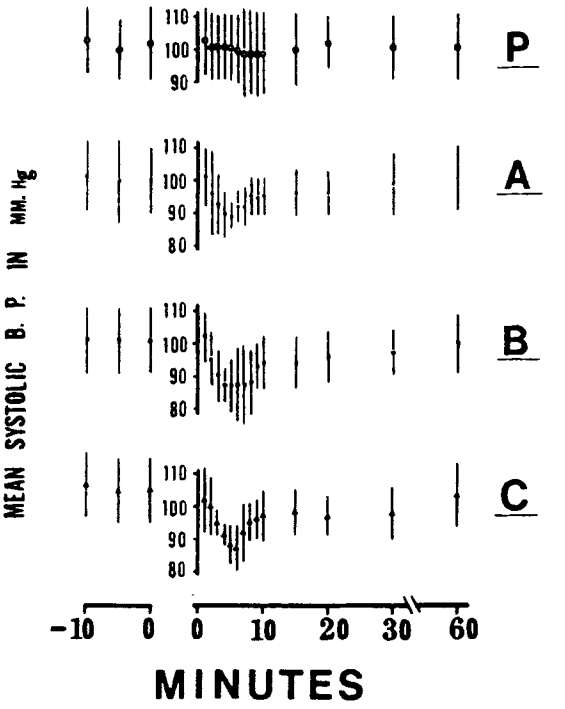

FIG. 2

Mean $\pm$ S. D. of systolic blood pressure in mm Hg of all subjects after administration of test formulations. Vertical lines represent 1 S. D.

Ordinate: Mean systolic blood pressure in mm Hg.

Abscissa: Minutes.

pressure whereas the three active products appeared to produce consistent decreases within the first ten minutes after drug dosing. Individual data are shown in Table IV. When a one-way analysis of variance was carried out, the F value barely missed out at the 0.05 level and statistical significance between treatments at this level, therefore, could not be demonstrated.

TABLE IV

STATISTICAL ANALYSIS OF THE MAXIMUM DEPRESSION OF SYSTOLIC BLOOD PRESSURE

Subject	Maximum decrease in systolic blood pressure (mm. Hg.) produced by tablet			
	P	A	B	C
SA	10	5	5	0
MB	0	25	42	20
DL	0	5	10	22
BF	0	15	15	20
CO	0	25	20	30

ANOVA

Source	d.f.	Sums of Squares (SS)	Mean SS	F	P*
Between Treatments	3	912.55	343.6	3.07	> 0.05
Error	16	179.24	112.1		
Total	19	2704.96			

$P \leq 0.05$ when $F(3, 16) \geq 3.24$

Inspection of the data revealed that one of the subjects, SA, gave a completely opposite blood pressure response to those observed for the others; in subject SA, the decrease in blood pressure was highest after placebo administration, and little or no effect was observed after dosing with the active tablets. The wide intersubject variability in the pertubation of systolic blood pressure observed here is consistent with another literature report (17). It should also be noted that estimation of blood pressure by means of a sphygmomanometer is rather subjective even though the same individual took all the measurements in the study.

The times at which maximum circulatory responses were observed for each formulation are of interest because they may be related to the *in vivo* release rate of nitroglycerin from the respective dosage form. Any gross interference by stabilizers or insoluble adjuvants in the dissolution rate of nitroglycerin from sublingual tablets may bring about concomitant changes in the onset of maximum circulatory responses. Table V and Vi show respectively the times at which maximum heart rate elevation (T_{hr}) and maximum hypotension (T_{bp}) occurred. It is evident that all three active products gave prompt circulatory responses. No statistical difference (P 0.05) could be observed between the test products in this respect.

TABLE V

TIME OF MAXIMUM HEART RATE RESPONSE

Subject	Time of maximum increase in heart rate (minutes) produced by tablet P	A	B	C
SA	9	5	3	6
MB	6	4	3	5
DL	20	4	3	5
BF	9	4	4	4
CO	2	2	2	5

TABLE VI

TIME OF MAXIMUM SYSTOLIC BLOOD PRESSURE RESPONSE

Subject	Time of maximum decrease in systolic blood pressure (minutes) produced by tablet P	A	B	C
SA	7	5	3	-
MB	-	4	7	5
DL	-	6	2	6
BF	-	2	5	6
CO	-	5	4	3

It is well known (8, 18) that repeated administration of organic nitrates may bring about tolerances toward circulatory responses. Existence of such a phenomenon in our study would

seriously complicate our interpretation on the relative pharmacological performance of the test products. Statistical analysis, however, revealed insignificant differences in the circulatory responses evoked by the different order of administration ($P > 0.05$). This indicated that the subjects apparently did not develop tolerance to the circulatory effects of nitroglycerin during the course of the study.

Results of this study showed that, at the dosage used, the circulatory responses elicited by the three different nitroglycerin formulations tested were essentially identical, both in terms of magnitude and rate of response. These three formulations differed in their method of preparation (molding vs. direct compression) as well as in the types and amounts of stabilizers and insoluble adjuvants contained therein. It appears, therefore, that these formulation factors did not significantly affect the pharmacological performance of nitroglycerin in these dosage forms, insofar as the circulatory responses were concerned. Caution must be exercised, however, when one wishes to extrapolate the apparent equivalence of these products in their pharmacological performance to mean equivalence in bioavailability. Any attempt in this regard must take into consideration the dose-response, or more vigorously, the biophase concentration-response, profiles of these pharmacological parameters.

FOOTNOTES

[a] Tablet P was prepared according to Formula II in reference 5 except that nitroglycerin was excluded.

[b] Table A: Eli Lilly & Co., 0.6 mg, lot #FAG5613.

[c] Table B: Warner/Chilcott, Div. Warner-Lambert Co., 0.6 mg, lot #4908064A.

[d] Tablet C was prepared according to Formula II in reference 5.

[e] Pfizer Laboratories Division, Pfizer, Inc., New York, NY 10017.

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