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EFFECT OF SESAME OIL EMULSION ON NITROGLYCERIN PHARMACOKINETICS

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SUMMARY

When nitroglycerin (3.5 mg/kg) was administered orally in a 20% sesame oil emulsion to rats, peak plasma concentrations were decreased but bioavailability was unaffected when compared to those observed using an aqueous solution vehicle. Pre-dosing with drug-free emulsion for 3 days, however, caused significant increases in nitroglycerin bioavailability (2.5X) and peak plasma concentrations (5X) compared to controls. This increase appeared to be reversible when emulsion dosing was withdrawn. The apparent increase in bioavailability was not due to a change in nitroglycerin distribution or elimination kinetics from drug-free emulsion administration nor could it be attributed to the surfactant used. There was no change in in vitro liver organic nitrate reductase activity or reduced glutathione concentration with emulsion pretreatment. The results suggested that, when using emulsion as a dosage form, repeated dosing of the vehicle could have an effect on the drug absorption process that was not predicted from single dose administration.

INTRODUCTION

The systemic clearance of nitroglycerin is very high: approximately 0.6 ℓ/kg/min in the rat and 0.4 ℓ/kg/min in man (Armstrong et al., 1979; Oh and Reid, 1979). Nitroglycerin also undergoes extensive first-pass metabolism after oral administration (Needleman et al., 1972); when rats were dosed orally at 7 mg/kg, the systemic bioavailability was only about 2% of an equivalent intravenous dose (Yap and Fung, 1978). Consequently, oral administration of nitroglycerin led to low plasma concentrations compared to those obtained after sublingual or intravenous administration (Blumenthal et al., 1977, Yap and Fung, 1978). Blumenthal et al. (1977) suggested that changes in heart rate and blood pressure after nitroglycerin dosing may be related to intact drug concentration. Wei and Reid (1979) recently reported that a correlation existed between plasma nitroglycerin concentrations and the maximal effect as well as the time course of changes in heart rate

and left ventricular diastolic dimensions in patients. If these beneficial hemodynamic effects are to be maximized at a given oral dose, enhancement in the oral bioavailability of nitroglycerin will be desirable.

Increased plasma concentrations of certain drugs can be achieved by using oil-in-water emulsions as vehicles. The absorption of such diverse molecules as insulin (Engel et al., 1969), heparin (Engel and Riggi, 1969), griseofulvin (Carrigan and Bates, 1973), indoxole (Wagner et al., 1966) and methyl orange (Ogata et al., 1975) was greatly facilitated when administered in emulsions. Increased plasma drug concentrations obtained with emulsion vehicles may result from a change in the physiologic conditions of the gastrointestinal tract (Carrigan and Bates, 1973, Wagner et al., 1966), but alterations in drug distribution and excretion (Kelleher et al., 1972; Patel and Jarowsky, 1976; Nitta et al., 1978; Kimura et al., 1978; Takada et al., 1978) may also occur as a result of emulsion dosing. Recently, Hashida et al. (1977) used parenteral emulsions for specific delivery of anticancer agents to regional lymphatics. These authors suggested the existence of a special transport mechanism by which drug and oil were delivered together when injected at the stomach wall. An increase in bioavailability (1.7 times) of iodohippuric acid was obtained with the water-in-oil emulsion compared to a control aqueous injection.

In addition to possible changes in systemic availability, use of emulsion vehicle for oral nitroglycerin dosing may also bring about sustained release characteristics (Kakemi et al., 1972 a,b). Prolonged absorption of nitroglycerin is desirable because its apparent elimination half-life is very short ($t_{1/2} \simeq 3$ min). In using an emulsion as a dosage form, however, the effect of continued oil intake on drug kinetics should be considered. Metabolites from esterified oils and fatty acids might alter drug pharmacokinetics because of possible interference with hepatic metabolism and/or tissue and protein binding. The effect of oil emulsion pretreatment on drug absorption and metabolism has not been well studied.

In the present report are described experiments to determine the effect of sesame oil emulsion co-administration and pre-treatment on the bioavailability and pharmacokinetics of orally administered nitroglycerin. The effect of oral administration of drug-free emulsion on the pharmacokinetics of intravenously administered nitroglycerin was also determined. Assessments of liver organic nitrate reductase activity and glutathione level following emulsion administration were made to explore the mechanism of the emulsion effect.

MATERIALS AND METHODS

Materials. Aqueous solutions of nitroglycerin and the internal standard, isosorbide dinitrate (Stuart Pharmaceuticals), for gas chromatographic assay were obtained and standardized by the procedure previously reported (Yap et al., 1978). Sesame oil (Fisher) and polysorbate 80 (J.T. Baker) were used as supplied.

Preparation of emulsion. 25 ml of a mixture containing sesame oil (40% v/v), polysorbate 80 (0.4% w/v) and water were sonicated (Branson Cell Disruptor 185) for 3 min at a fixed frequency setting (no. 5) in an ice bath. When nitroglycerin was to be administered in the emulsion dosage form, 25 ml of the emulsion was mixed with an equal volume of an aqueous nitroglycerin solution one hour before dosing.

Animal preparation and dosing. Male Sprague–Dawley rats (Blue Spruce Farms) weighing 280–360 g, were used. The right jugular vein was cannulated chronically with tubing, 0.020 in. (i.d.) × 0.037 in. (o.d.) (no. 602-135, Silastic Brand, Dow Corning), previously filled with physiological saline solution, secured and transferred to the back of the rat according to the procedure reported (Weeks and Davis, 1964). For intravenous studies, the rats were fasted overnight, and nitroglycerin aqueous solution was given as a bolus (0.35 mg/kg of rat weight) to each rat via the cannula, followed by flushing of the tubing with approximately 1 ml of saline solution. The volume of injection solution was approximately 0.2 ml. Blood samples (0.5 ml) were collected via the cannula before dosing and at appropriate intervals after intravenous dosing. The rat was allowed free movement within a metabolic cage during the experiment. For oral studies, rats were fasted overnight and nitroglycerin, 3.5 mg/kg, in a specified vehicle, was administered by gavage under slight ether anesthesia. The rat was then placed in a metabolic cage as described. Blood samples (0.5 ml) were obtained via the cannula at appropriate intervals.

Nitroglycerin pharmacokinetics. The intra-animal variability in nitroglycerin disposition kinetics was examined by comparing the nitroglycerin plasma concentrations obtained after repeated (2 or 3 times) intravenous doses, at 0.35 mg/kg, administered to the same animals. Dosing was repeated at 3-day intervals. The effect of emulsion as an oral vehicle for nitroglycerin was studied by administering nitroglycerin (3.5 mg/kg) to 6 rats in both the aqueous and emulsion vehicles in a randomized cross-over fashion. The two treatments were separated by an interval of 3 days. Plasma nitroglycerin concentrations were determined (Yap et al., 1978) after each treatment.

The effect of drug-free emulsion treatment on nitroglycerin pharmacokinetics after oral and intravenous administration was studied in a set of 4 experiments in which rats were treated according to the regimen described (Table 1). Group A received an oral dose of nitroglycerin in an aqueous vehicle, after overnight fasting, on the 1st, 5th and 9th days and plasma drug concentrations were determined at appropriate intervals after each dose. On the 2nd, 3rd and 4th days, these rats received once daily a dose of drug-free emulsion, 6.5 ml/kg, containing 40% sesame oil. On the 6th, 7th and 8th days of the regimen, saline was given in place of the emulsion. The saline treatment was incorporated in

TABLE 1

EXPERIMENTAL DESIGN

Animal group	Day								
	1	2	3	4	5	6	7	8	9
A	O-N	E	E	E	O-N	S	S	S	O-N
B	O-N	S	S	S	O-N	S	S	S	O-N
C	O-N	P	P	P	O-N				
D	I-N	E	E	E	I-N				

Key: O-N, oral nitroglycerin dose, 3.5 mg/kg; E, drug-free sesame oil emulsion; S, normal saline; P, polysorbate 80 solution; I-N, intravenous nitroglycerin dose, 0.35 mg/kg.

the experiment to determine whether any changes created by emulsion dosing were reversible.

Three control experiments (groups B, C and D) were also conducted: group B received saline instead of drug-free emulsion on the 2nd, 3rd and 4th days. The effect of polysorbate 80, the surfactant used in the emulsion, on nitroglycerin pharmacokinetics was examined in group C in which aqueous polysorbate 80, at a concentration used in the emulsion (0.667% w/v), was given in place of the emulsion. Finally, the effect of oral emulsion treatment on intravenous nitroglycerin kinetics was examined in animal group D (n = 7). An intravenous dose at 0.35 mg/kg was given before (day 1) and after (day 5) drug-free emulsion treatment. Except when fasted before nitroglycerin dosing, all animals were fed a normal diet (Charles River Diet RMH 1000) throughout the experiment.

Liver organic nitrate reductase activity. This enzyme assay was performed (Maier et al., 1980) in groups of animals which underwent one of the following treatments: (i) once daily dosing with saline (6.5 mg/kg) for 3 days; (ii) once daily dosing with sesame oil emulsion (6.5 ml/kg) for 3 days; and (iii) once daily dosing with sesame oil emulsion (6.5 ml/kg) for 3 days followed by a waiting period of 4 days. All animals were allowed free access to food and water throughout the experiment.

Determination of reduced glutathione levels in rat liver. The concentrations of liver reduced glutathione were determined (Ellman, 1959) in two groups of animals. One group (n = 14) received daily drug-free emulsion treatment for 3 days, as described, while the other group (n = 14) was given saline as control. After overnight fasting, the animals were decapitated and the liver was flushed with saline via the hepatic or hepatic portal vein until the lobes were pale brown. The entire liver was then removed, blotted dry with gauze pads, weighed, minced and then homogenized in 30 ml of cold distilled water in an ice bath. The volume of the homogenate was recorded and an aliquot of 5 ml was mixed with 20 ml of 5% trichloroacetic acid. After this mixture was centrifuged for 15 min at 17,000 rpm and 2°C, 1 ml aliquot of the supernatant was then added to 0.5 ml of a solution of 0.4% 5,5-dithiobis-(2-nitrobenzoic acid) and 1% sodium citrate in water and 4 ml of 0.3 M disodium hydrogen phosphate. The absorbance at 412 nm was measured and the reduced glutathione concentration calculated, after appropriate corrections for dilution, from a calibration curve constructed with solutions containing known concentrations of added reduced glutathione (A grade, Calbiochem) in aqueous solutions treated in the same manner. Assays were done in duplicate.

RESULTS AND DISCUSSION

Yap and Fung (1978) showed that the inter-animal variation of oral nitroglycerin bioavailability is large while that of intravenous kinetics is considerably less. Maier et al. (1980) confirmed this observation at a higher oral dose and showed also that much of the variability in the area under the plasma concentration–time curve (AUC) after oral administration could be explained by inter-animal differences in liver organic nitrate reductase activity. The degree of intra-animal variability, however, has not been reported. Since some of the experiments described in this report involved a cross-over design, it was deemed desirable to first assess the degree of intra-subject variability in nitroglycerin disposition kinetics. Fig. 1 shows the plasma concentrations of nitroglycerin in 3 rats which

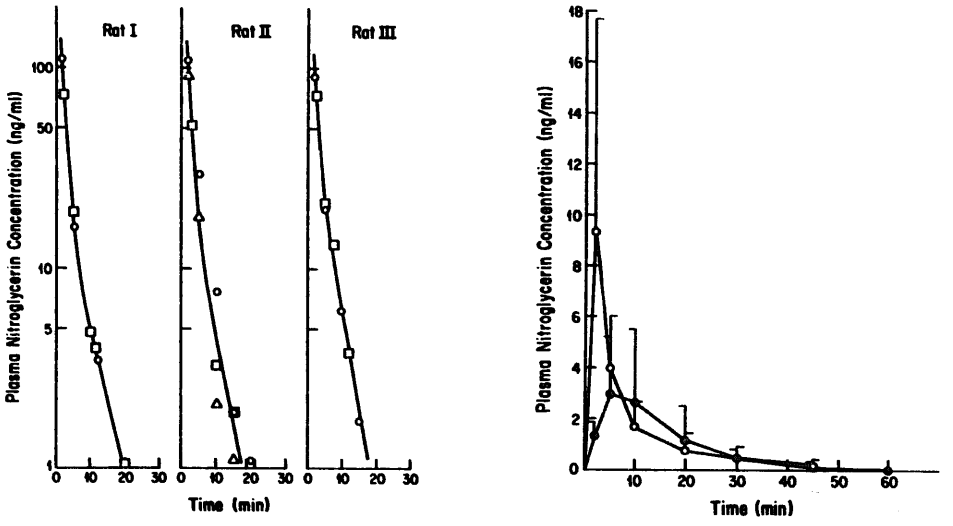


Fig. 1. Plasma nitroglycerin concentrations after repeated single intravenous doses (0.35 mg/kg) separated by intervals of 3 days. Symbols represent concentrations obtained after different doses.

Fig. 2. Plasma nitroglycerin concentrations after cross-over oral administration of nitroglycerin in aqueous solution (○) and sesame oil emulsion (●). Each bar represents 1 S.D.; n = 6.

received duplicate or triplicate intravenous dosing at 0.35 mg/kg. This dose level was chosen because it was anticipated that plasma nitroglycerin concentrations obtained with this intravenous dose would cover the range of concentrations observed after oral dosing at 3.5 mg/kg. The data showed that intravenous nitroglycerin kinetics were quite reproducible and that chronic implantation of a jugular cannula, repeated dosing and blood drawing did not apparently affect nitroglycerin disposition. The slope of the intravenous kinetic curves shown in Fig. 1 suggested the possibility of bi-exponential decline for nitroglycerin in the rat. A preliminary report (Yap and Fung, 1978) has shown that nitroglycerin kinetics could be described by a one-compartment model, but the blood collection schedule in that study might not have been frequent and long enough to reflect multi-exponential kinetics. Indeed, intravenous studies carried out at higher doses (Fung et al., unpublished data) confirmed this pharmacokinetic characteristic. Details of intravenous studies at various doses will be reported elsewhere.

Fig. 2 shows the plasma drug concentrations obtained when nitroglycerin was given orally in either an aqueous or emulsion vehicle. As expected, inter-animal variability in plasma nitroglycerin concentrations was quite large after both treatments. Peak plasma drug concentrations were in general higher after drug dosing with the aqueous vehicle (Table 2). In all dosing with the aqueous vehicle, drug concentration was invariably highest in the first blood sample (at 2 min), suggesting very rapid absorption of nitroglycerin under the experimental conditions. Nitroglycerin absorption appeared slower from the sesame oil emulsion vehicle. Since the partition coefficient of nitroglycerin between sesame oil and water is about 76 at 37°C, it is likely that the organic nitrate resides principally in the oily internal phase of the emulsion vehicle, thus possibly delaying its release into intestinal fluids. It is possible that gastric emptying may also be affected with emul-

TABLE 2

BIOAVAILABILITY OF NITROGLYCERIN FOLLOWING ORAL ADMINISTRATION WITH AQUEOUS AND EMULSION VEHICLES IN THE RAT

Rat no.	AUC (ng · min/ml)		Peak concentration (ng/ml)	
	Aqueous vehicle	Emulsion vehicle	Aqueous vehicle	Emulsion vehicle
1 [a]	39.4	23.4	6.6	2.0
2 [a]	112.8	155.2	8.5	8.0
3 [a]	123.9	39.2	26.0	2.5
4 [b]	44.8	27.0	2.0	1.3
5 [b]	68.3	96.2	6.5	5.0
6 [b]	32.7	10.9	7.1	1.3
Mean ± S.D.	70.3 ± 39.3	58.6 ± 55.9	9.3 ± 8.0	3.4 ± 2.7
P [c]	N.S.		<0.05	

[a] These animals received the aqueous vehicle first.
[b] These animals received the emulsion vehicle first.
[c] By paired signed-ranks test, two-tails. NS = not significant.

sion dosing. The extent of nitroglycerin absorption was not statistically different between the two vehicles, with both estimated to be about 1–2% of a comparable intravenous dose. This value was consistent with that reported previously (Yap and Fung, 1978). The composite results showed that a sesame oil emulsion vehicle, prepared as described, only provided some sustained release characteristics to oral nitroglycerin absorption, but the extent of absorption was unaffected.

Emulsion pre-treatment, however, produced a significant increase in apparent nitroglycerin bioavailability. Table 3 shows the AUC values obtained after nitroglycerin was administered to the emulsion (A) group and the saline control (B) group. There was no apparent difference in baseline nitroglycerin bioavailability in these two groups because their AUC values were not statistically different before emulsion or saline treatment (day 1). However, after emulsion treatment for 3 days (group A, day 5), apparent nitroglycerin bioavailability increased significantly when compared to that observed before emulsion treatment (group A, day 1) and that obtained in the control group treated with saline (group B, day 5). The plasma concentrations observed after nitroglycerin dosing on day 5 in groups A and B are shown in Fig. 3. Emulsion pre-treatment for 3 days, therefore, gave rise to mean increases of approximately 2.5-fold and 5-fold in nitroglycerin AUC and peak concentration, respectively, when compared to control.

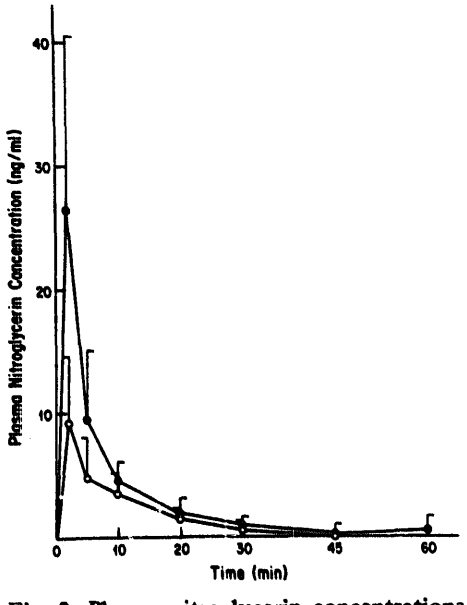
Because of technical difficulties in maintaining cannula patency for a long period, the number of animals studied with respect to nitroglycerin absorption and metabolism on day 9 was somewhat limited. A trend existed, however, which suggested that the apparent bioavailability enhancing effect from treatment with drug-free emulsion was reversible when that treatment was stopped. The mean AUC value in group A fell from 276 ng · min/ml on day 5 to 58 ng · min/ml on day 9. There was also an indication of a slight pro-

TABLE 3

EFFECT OF TREATMENTS WITH DRUG-FREE EMULSION AND SALINE ON AUC (ng · min/ml) OF NITROGLYCERIN FOLLOWING ORAL DOSING (3.5 mg/kg) IN THE RAT

Rat no.	Emulsion (group A)			Rat no.	Saline (group B)		
	Day 1	Day 5	Day 9		Day 1	Day 5	Day 9
7	136.4	152.0	88.9	13	360.2	136.4	a
8	74.6	184.1	23.7	14	51.0	58.1	36.8
9	115.1	568.9	a	15	100.7	106.8	71.3
10	265.8	387.1	62.5	16	218.9	109.8	a
11	23.7	179.6	57.4	17	100.9	35.3	58.1
12	36.8	185.0	a				
Mean ± S.D.	108.7 ± 88.4	276.1 ± 167.1	58.1 ± 26.8		166.4 ± 124.7	89.3 ± 41.3	55.4 ± 17.4
P	0.04 [b]				0.14 [b] (NS)		
	0.4 [c] (NS)						
	0.04 [c]						

[a] Not determined because of technical problems (e.g. blocked cannulae).
[b] By paired two-tail *t*-test, NS = not significant.
[c] By two-tail *t*-test.



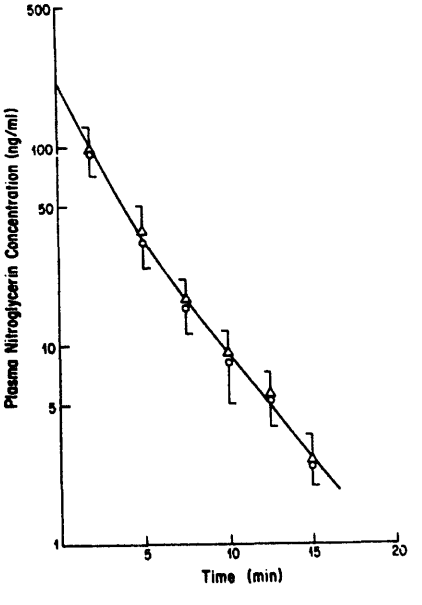


Fig. 3. Plasma nitroglycerin concentrations after oral drug dosing in an aqueous vehicle on day 5 in animal groups A (•) and B (○). Each bar represents 1 S.D.; n = 6.

Fig. 4. Plasma nitroglycerin concentrations following a 0.35 mg/kg intravenous dose before (○) and after (△) 3 days of oral emulsion treatment. Values are mean ± 1 S.D. (represented by bars); n = 7.

gressive decrease in nitroglycerin AUC upon repeated dosing. It is not known whether this apparent decrease was due to repeated fasting or handling. The hematocrit values found on days 1, 5 and 9 were, however, normal, suggesting that blood regeneration in the animals was apparently achieved between the study days.

There are several possible explanations why emulsion pretreatment apparently enhanced nitroglycerin bioavailability while concomitant emulsion dosing did not. (1) The effect may have been artifactually derived from continued dosing with polysorbate 80 rather than from sesame oil. (2) Emulsion pretreatment may produce fatty acid metabolites which alter: (a) nitroglycerin distribution in the body; (b) liver organic nitrate reductase activity; and/or (c) depletes liver glutathione which is a necessary co-enzyme for the metabolism of nitroglycerin. Experiments were carried out to explore these possibilities.

The effect of polysorbate 80 treatment on oral nitroglycerin kinetics was examined in animal group C. AUC values (mean ± S.D.) were 163 ± 55 and 157 ± 66 ng/ml/min and peak plasma nitroglycerin concentrations (mean ± S.D.) were 24 ± 16 and 22 ± 16 ng/ml in 4 rats examined before and after surfactant treatment, respectively. Apparently, the effect provided by the emulsion was not mediated by the surfactant.

Kelleher et al. (1972) indicated that tocopherol distribution to liver and kidney tissues was highly dependent on the vehicle of administration (polysorbate 80 emulsion or arachis oil). It is thus possible that the apparent increase in plasma nitroglycerin concentrations resulting from emulsion dosing might be derived from changes in tissue binding characteristics of the drug leading to a smaller volume of nitroglycerin distribution in the presence of fatty acid metabolites. If this were the case, intravenous nitroglycerin kinetics would be similarly affected by emulsion pretreatment. Plasma nitroglycerin concentrations were measured following identical intravenous doses before and after daily emulsion treatment for 3 days. Fig. 4 shows that intravenous nitroglycerin kinetics were identical on these two occasions. Thus, it is unlikely that the bioavailability enhancing effect of sesame oil

TABLE 4

EFFECT OF SESAME OIL TREATMENT ON LIVER ORGANIC NITRATE REDUCTASE ACTIVITY AND REDUCED CONTENT IN RATS

Treatment [a]	Liver organic nitrate reductase activity [b] (μmol/min)	Liver-reduced glutathione content [b] (μmol/g liver)
E	23.8 ± 6.1 (6) [c]	7.3 ± 1.8 (14)
F	21.0 ± 3.2 (6)	6.6 ± 1.9 (14)
G	21.6 ± 8.8 (5)	N.D. [d]

[a] Treatment E = once daily dosing with saline (6.5 ml/kg) for 3 days; treatment F = once daily dosing with sesame oil emulsion (6.5 ml/kg) for 3 days; and treatment G = once daily dosing with sesame oil emulsion (6.5 ml/kg) followed by a waiting period of 4 days.
[b] Mean ± S.D.
[c] Number in parenthesis indicates number of animals studied.
[d] N.D. = not determined.

emulsion resulted from alteration of distribution and elimination kinetics of nitroglycerin.

Several studies (Needleman et al., 1972; Maier et al., 1980) have shown that the liver possessed a high metabolic capacity for nitroglycerin metabolism. The enzyme responsible, organic nitrate reductase, requires glutathione as a co-factor for its activity. In comparison, the rat intestine was shown (Maier et al., 1980) to be essentially devoid of this glutathione-dependent enzyme. Thus, decreased first-pass metabolism of nitroglycerin from emulsion dosing might be suggested if decreases in liver enzyme activity or glutathione levels were found. Results in Table 4 show, however, that emulsion dosing did not significantly alter these parameters when compared to controls. Thus, the apparent increase in bioavailability after emulsion pretreatment was not reflected by an impaired in vitro capacity of the liver to metabolize nitroglycerin.

Although the mechanism of bioavailability enhancement after sesame oil emulsion pretreatment is yet to be determined, the implications of our findings are quite clear: (i) oral nitroglycerin bioavailability may be potentially affected by dietary habbits; and (ii) in using emulsion as a dosage form, repeated oil administration sometimes can have an effect on the drug absorption process that is not predicted from single dose administration. Further studies are being conducted to determine the mechanism of the emulsion effect on the oral nitroglycerin bioavailability in animals.

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