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IN VIVO SCREENING MODEL FOR EXCIPIENTS AND VEHICLES USED IN SUBCUTANEOUS INJECTIONS

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ABSTRACT

Incorporation of an insoluble drug in Subcutaneous (SC) dosage form requires addition of a cosolvent. The use of a cosolvent may result in burning sensation and/or severe skin inflammation response after the injection. In this investigation the inflammation response of rat skin after SC administration of excipients and vehicles was demonstrated by a simple and effective *in vivo* technique. Four hours after SC injection, of each vehicle with or without excipients, the skin fold thickness of rat skin was measured. Among the oils tested, Planters' peanut oil showed a higher increase in skin fold thickness (25%). In the mean time, the SC injection of MCT Estasan GT 80 oil resulted in an insignificant increase (<15%). A group of cosolvents prepared in sesame oil was tested in rats after SC injections. The magnitudes of the percentage increase in skin fold thickness of 10% benzyl alcohol, 10% ethyl oleate, 15 % phospholipon 100, 4% ethyl alcohol, and 0.2% triethanolamine were 172, 45, 38, 31, and 31, respectively. The rest of excipients evaluated showed minor inflammation responses. Different concentrations of benzyl alcohol (1, 2, 5 and 10% v/v) in sesame oil were injected. Only the 1% benzyl alcohol produced an insignificant increase in skin fold thickness.

INTRODUCTION

The design of a subcutaneous (SC) dosage form depends upon the drug properties (physical, chemical, pharmacological and toxicological) as well as the excipients used in the formulation. Cosolvent systems have been used quite effectively to achieve solubility for poorly soluble drugs (1).

There has been an increasing interest in evaluating subcutaneous formulations for pharmacokinetics, toxicity and efficacy (2). However, there has been few reports (3, 4) which have dealt with the selection of vehicles or excipients with respect to their irritation potential after SC administration.

This study was undertaken to demonstrate an *in vivo* method to screen the excipients and vehicles intended for use in SC formulations. The inflammatory response was evaluated by measuring the skin fold thickness.

EXPERIMENTAL

Materials: Table 1 represents the excipients and vehicles used in this study with their lot numbers and sources. All the materials were used without further purification. A summary of the concentration of the vehicles and excipients evaluated as well as their number of replicates in rats is given in Table 2.

Animals: Male Charles River rats, 600 to 800 grams were used. Rats were anesthetized with 0.5 mL intramuscular injection of ketamine : acepromazine (9 : 1) and 0.5 mL intraperitoneal injection of urethane solution (40% w/v in normal saline). The abdominal hair was shaved and eight injection sites were selected and marked on the abdominal skin. A micrometer was used to measure the skin fold thickness.

The time course of inflammation response was followed for the material that caused the severest inflammation up to five hours.

Effect of Normal Saline Injection on Skin Fold thickness: Subcutaneous injection of 0.5 mL of normal saline was given to 4 rats, two areas were tested in each rat. The skin fold thickness was measured at 0, 20, 30, 40, 50, 60, 100, and 240 minutes after administration.

TABLE 1

Excipients and vehicles tested

Adjuvant	Suppliers	Lot Number
Normal Saline	Abbott Laboratories	06-116-DM-07
Benzyl Alcohol	Givadon	1654-87
Peanut Oil	Ruger Chem. Co.	P33579II6
Refined Sesame Oil	Ruger Chem. Co.	P3452J19
Planters' Peanut Oil	Planters Co.	A8097
Dimethylacetamide (DMA)	Sigma Chem. Co.	26F-3405
Triethanolamine (TEA)	Chemical Dynamics	122662
Oleic Acid	Emery Chem. Co.	29178
Ethyl Alcohol	U.S. Industrial	JTN87L18
Phospholipon 100	American Lecithin Co.	5333987A
MCT Estasan GT 80*	Arteck Inc.	3009
Benzyl Benzoate	Sigma Chem. Co.	106F-0568
Ethyl Oleate	Jonas Chem. Corp.	-

* A grade of medium chain triglyceride.

TABLE 2

The number of replicates tried for each vehicle with or without excipients

Vehicle	No. of Replicates
Normal saline	8
10% Benzyl alcohol in sesame oil	6
5% Benzyl alcohol in sesame oil	4
2% Benzyl alcohol in sesame oil	4
1% Benzyl alcohol in sesame oil	5
Peanut oil	6
Planters Peanut oil	4
Refined sesame oil	10
MCT Estasan GT 80	4
15% Phospholipon 100 in sesame oil	4
10% Benzyl benzoate in sesame oil	4
4% Ethyl alcohol in sesame oil	4
10% Ethyl oleate in sesame oil	4
2% DMA* in sesame oil	6
1% DMA in sesame oil	6
0.2% TEA ** in sesame oil	4
2% Oleic acid in peanut oil	6

* Dimethylacetamide
** Triethanolamine

Calculations: The thickness of skin fold was measured at each selected area prior to drug administration. This value was taken as the zero time measurement (SFT_0). Subcutaneous injection of 0.5 mL of vehicle, with or without excipients, was given at each area. The skin fold thickness was measured at the specified time (SFT_A). The percentage increase in skin fold thickness (SFT_I) at each area was calculated according to equation 1.

$$SFT_I\,\% = \frac{(SFT_A - SFT_0)}{SFT_0} * 100 \qquad (1)$$

Where,

SFT_0 = Skin fold thickness at zero time

SFT_A = Skin fold thickness after a specified time

SFT_I = Increase in Skin fold thickness

RESULTS AND DISCUSSION

The skin fold thickness measured after SC injections of 0.5 mL normal saline is shown in Figure 1. After SC administrations of normal saline to rats, skin fold thickness was sharply increased by 30% followed by a rapid decrease to about 10% within 50 minutes. From 50 minutes up to 100 minutes the change in skin fold thickness was minimal, after which time it was followed by a gradual decrease to its zero time value after 4 hours. Since normal saline is tolerated by the subcutaneous tissue, the increase in the skin fold thickness after SC administration is believed to be due to a mechanical effect of the injection volume. This increase in skin fold thickness was considered to be the irritation threshold. It took four hours for the skin fold thickness to return to zero time value after SC injection of normal saline as presented in Figure 1. Accordingly, the skin fold thickness was measured, for the remainder of the study, 4 hours after the SC injection.

Figure 2 depicts the effect of various vehicles on skin fold thickness. Planters' peanut oil showed the highest response ($SFT_I\%$ = 25%). In the mean time, Estasan GT 80 (a grade of medium chain triglyceride) caused

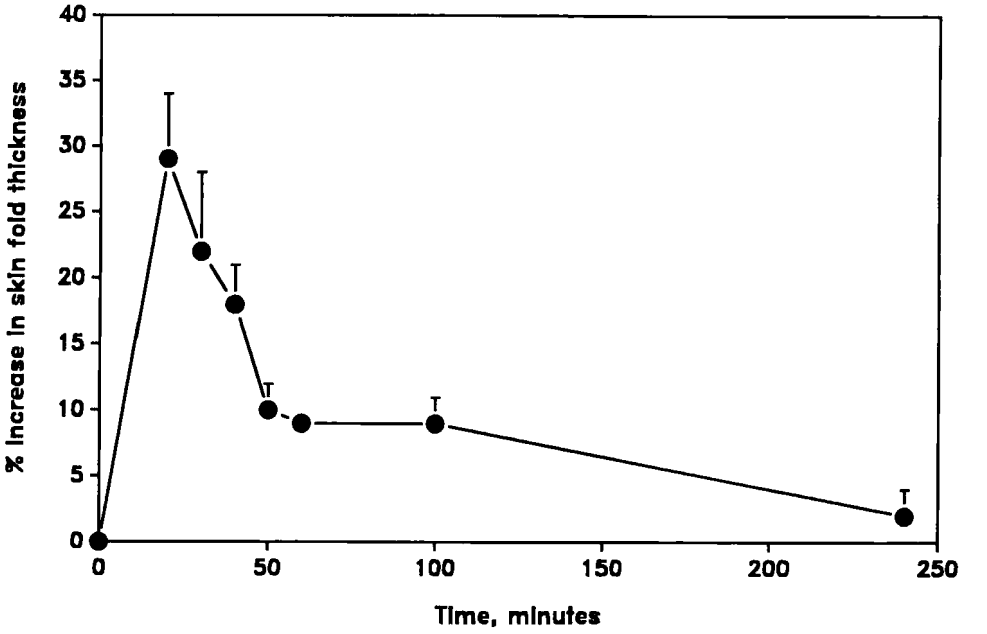

FIGURE 1

Time course of inflammation response of SC injection of normal saline in the rat (mean + SD)

the least effect to rat skin ($SFT_I\% < 10\%$). Therefore, a vehicle with excipients that caused ≤ 25% increase in skin fold thickness, was considered to have an insignificant irritation effect.

Subcutaneous formulations of insoluble drugs require the use of cosolvent to incorporate these drugs in liquid dosage forms. A group of cosolvents was tested for their effect on skin inflammation after SC injection. The inflammatory response of rat skin to various cosolvents is demonstrated in Figure 3. All the studied materials were dissolved in sesame oil. Increasing the concentration of dimethylacetamide DMA from 1 to 2 % resulted in minimal increase in skin fold thickness ($SFT_I\% < 15\%$). Oleic acid (2%) as well as benzylbenzoate (10%) showed a non significant

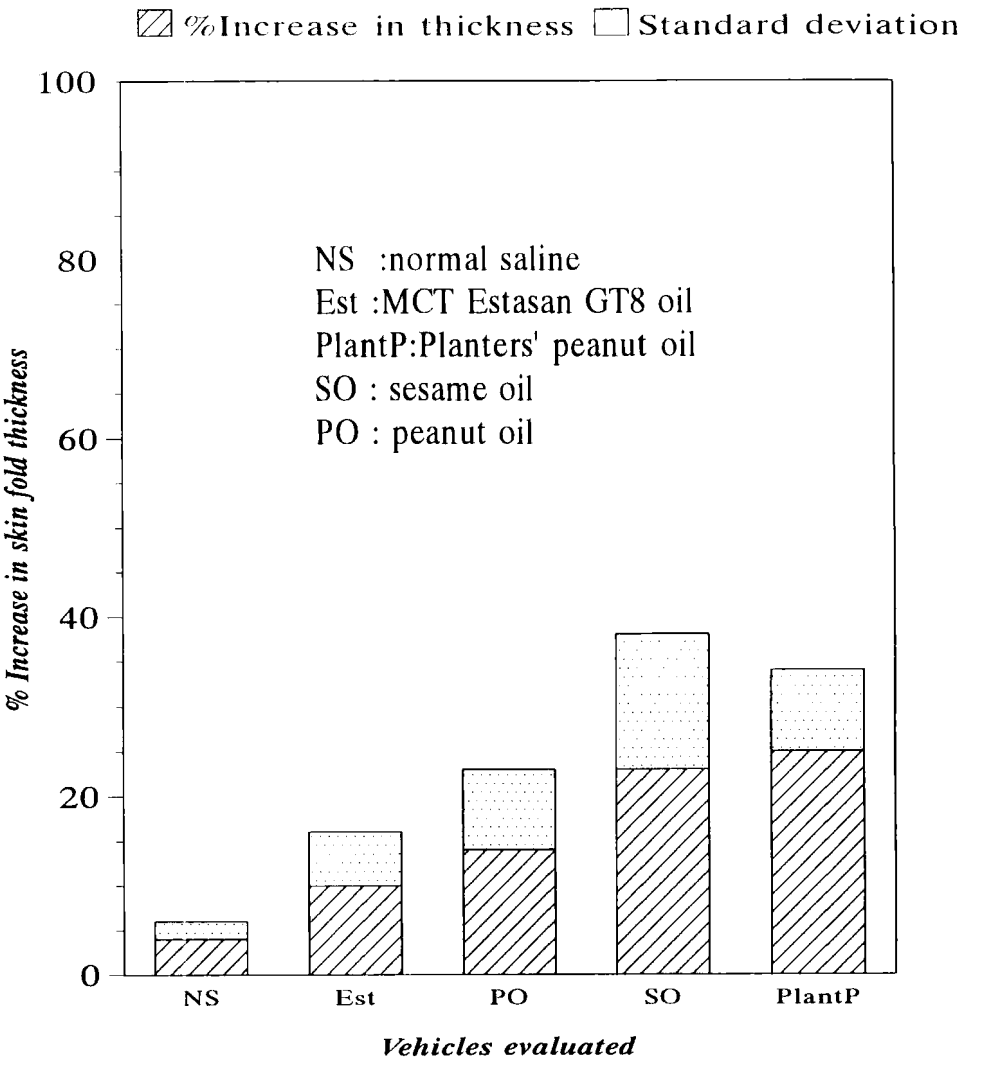

FIGURE 2

Inflammatory response (mean + SD) of rat skin to SC injections of vehicles

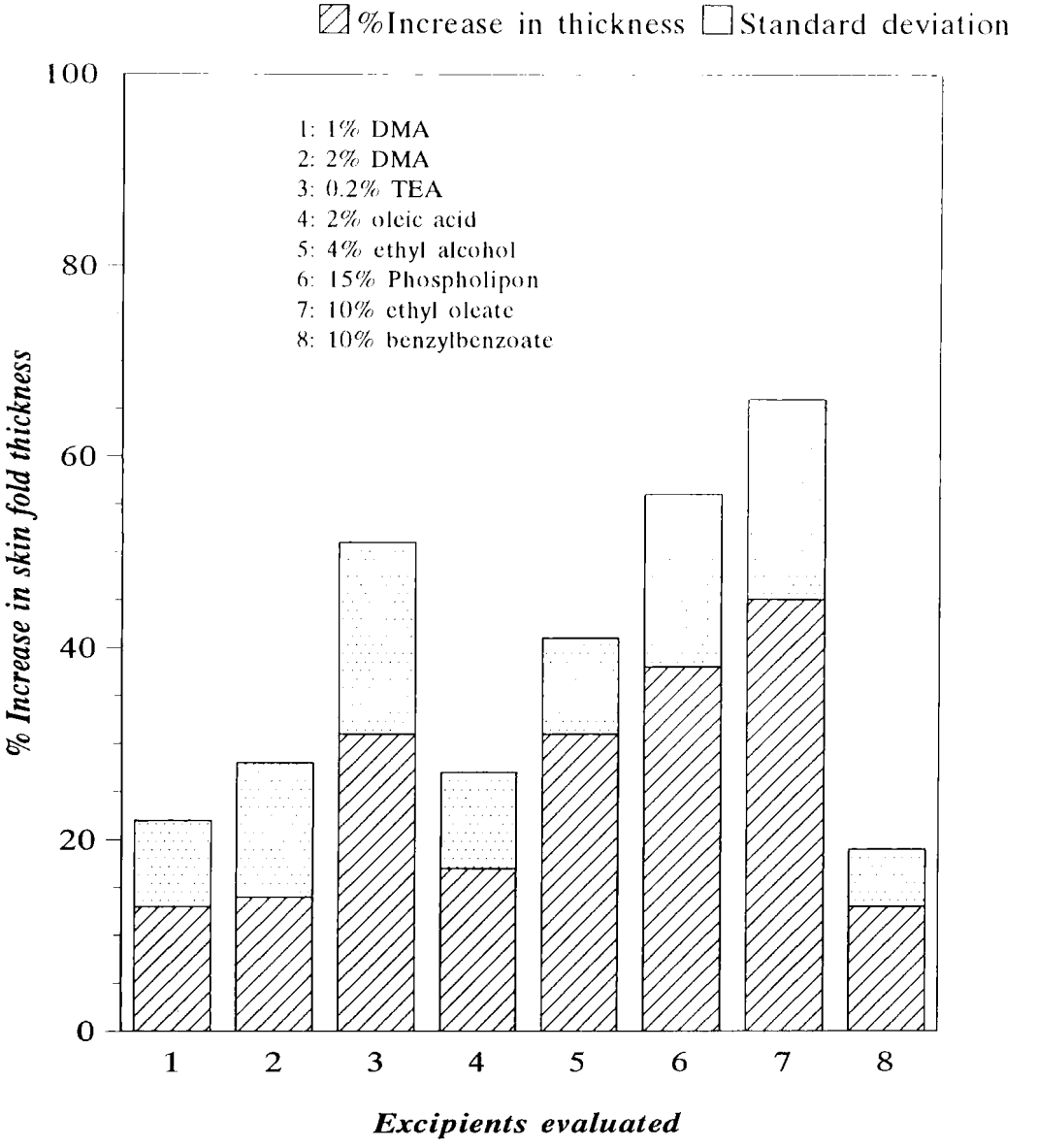

FIGURE 3

Inflammatory response (mean + SD) of rat skin to SC injections of excipients in sesame oil

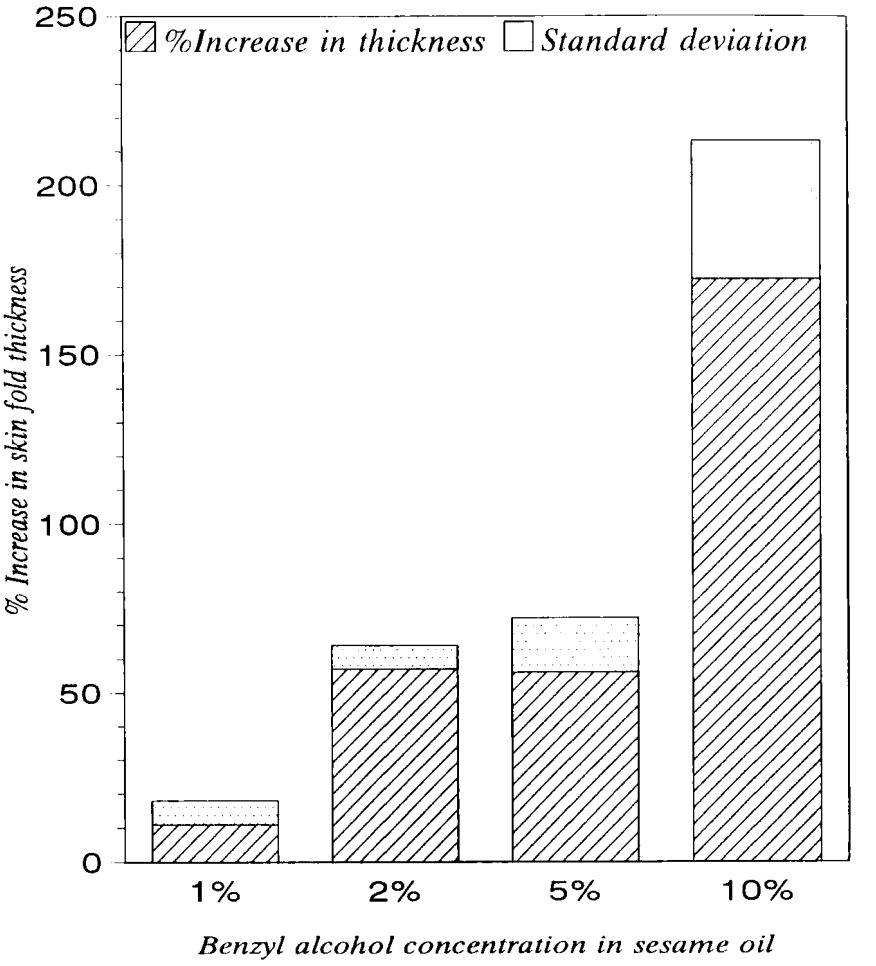

FIGURE 4

Inflammatory response (mean + SD) of rat skin to SC injections of benzyl alcohol in sesame oil

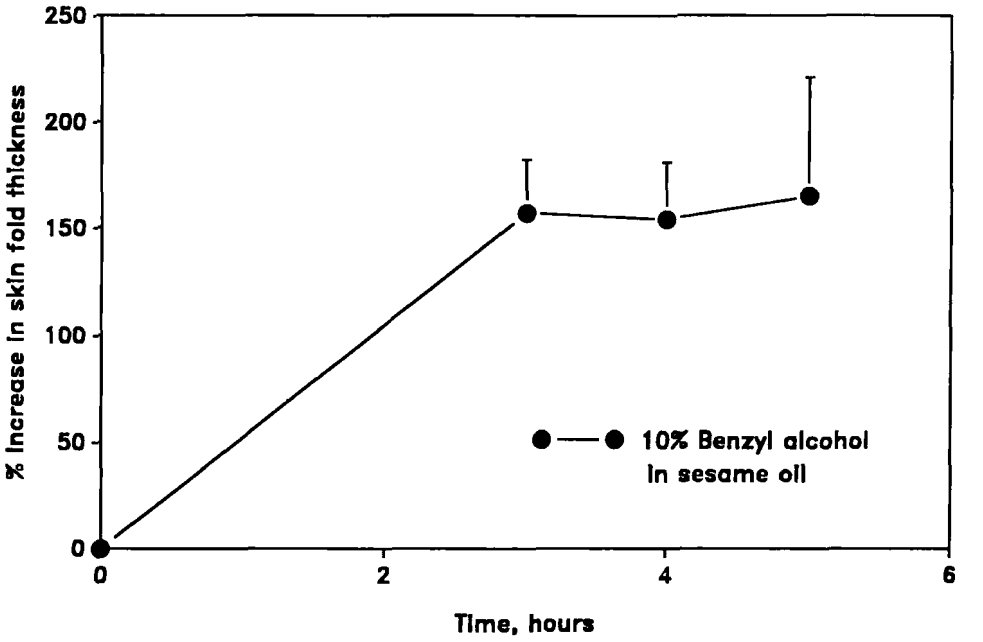

FIGURE 5

Time course of inflammation response of 10 % benzyl alcohol after SC injection in the rat (mean + SD)

change in the skin. For the other tested materials, a significant increase in skin fold thickness was observed, and was in this order 10% ethyl oleate > 15 % phospholipon 100 > 4% ethyl alcohol > 0.2% triethanolamine.

Benzyl alcohol is used as a 4% solution for topical block anesthesia (1). It is incorporated as a cosolvent in this study. The inflammatory response of rat skin to benzyl alcohol was investigated for 1, 2, 5, and 10% v/v (Figure 4). The lowest effect on rat skin was observed with 1% benzyl alcohol ($SFT_I\% < 15\%$). On the other hand, increasing benzyl alcohol concentration from 1 to 2%, produced 5 folds increase in skin fold thickness. However, when 2% and 5% of benzyl alcohol were injected, there was no significant change in the skin fold between those

concentrations. The highest skin response to excipients was measured after SC injection of 10% benzyl alcohol ($SFT_I\%$ = 172%). The severity of irritation response to 10% benzyl alcohol suggests its limitation to concentrations <5% in SC formulations.

The time course of inflammation response was followed for 10% benzyl alcohol as shown in Figure 5. The inflammation response rapidly developed after injection and was found to persist for more than 5 hours. Agreeably, selecting to measure the increase in skin fold thickness for all tested vehicles after 4 hours was adequate.

CONCLUSIONS

A simple and effective screening *in vivo* technique is introduced. The method is verified when applied to different vehicles and excipients. Therefore, this technique could be applied in preformulation stage for the selection of the least irritant materials to be used in SC formulations. Benzyl alcohol's concentration greater than 1% is not recommended in SC vehicles to avoid any patient discomfort. Also, the concentration of phospholipon 100 and ethyl oleate, if incorporated in SC injections, should be less than the tested ones. The oils evaluated in this study were found to be non-irritating in the rat animal model.

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