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Local Muscle Damage and Oily Vehicles: A Study on Local Reactions in Rabbits after Intramuscular Injection of Neuroleptic Drugs in Aqueous or Oily Vehicles

By

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Abstract: The local muscle damaging effect of a series of neuroleptics formulated in water or in oily vehicles (Viscoleo®, sesame oil, methyl oleate or squalane) has been investigated after intramuscular injection in rabbits. Each drug preparation (2 ml) was administered intramuscularly in the central part of the longissimus dorsi muscle of four rabbits. The rabbits were killed three days after the injection, and the injection site was examined macroscopically. Areas of necrotic muscle tissue were isolated by dissection and weighed. All preparations in aqueous solution caused local muscle damage (large necrotic area) at the injection site. This effect was almost completely neutralized by the Viscoleo® vehicle. The sesame oil, methyl oleate and squalane vehicles also neutralized the local damaging effect, but not to the same extent as that of the Viscoleo® vehicle.

Key-words: Vegetable oil - sesame oil - injection - intramuscular - neuroleptics - rabbit.

Vegetable oils have been used for decades as vehicles for intramuscular drug preparations. Vegetable oils are tolerated well at the injection site (Brown *et al.* 1944; Svendsen & Aaes-Jørgensen 1979). Oily vehicles are immiscible with the interstitial body fluid at the injection site. This means that a drug dissolved in oil, in contrast to an aqueous formulation, is largely prevented from coming into contact with the muscle tissue immediately on injection. As a consequence drugs in oily vehicles should be more gentle to the local tissue than their counterparts in aqueous solution.

A number of neuroleptic drugs are marketed as aqueous formulations for intramuscular administration. It is known from the literature (Bree *et al.* 1971; O'Connor 1980; Brumback *et al.* 1982) and investigations in this laboratory that several neuroleptic drugs cause local muscle injury at the injection site. Neuroleptic drugs therefore seem to be useful as a tool for studies on local damaging effect of different formulations.

In the present study in rabbits the local muscle damaging effect of aqueous formulations of neuroleptic drugs was compared to that of oily formulations of the same drugs.

Materials and Methods

New Zeeland White rabbits (2.2–3.9 kg) of both sexes were divided into groups of four animals. Each animal received one single intramuscular injection. Four rabbits were used for each preparation. The preparations used for the injections are presented in tables 1 and 2.

The following preparations, all commercially available in Denmark were used: Klorpromazin® (chlorpromazine, HCl, 25 mg/ml), Truxal® (chlorprothixene HCl, 10 mg/ml), Serenase® (haloperidol base, 5 mg/ml), Nozinan® (levomepromazine HCl, 25 mg/ml), Buronil® (melperone, HCl, 25 mg/ml), Trilafon® (perphenazine base, 5 mg/ml) and Torecan® (thiethylperazine maleate, 6.5

mg/ml). Formulations prepared in this laboratory in 0.9% sodium chloride were also used: cis(Z)-clopenthixol, 2HCl - 10 mg/ml, cis(Z)-flupentixol, 2HCl - 10 mg/ml, and fluphenazine, HCl - 10 mg/ml.

The salt of neuroleptics is not soluble in oily vehicles but the bases are highly soluble. The neuroleptic bases were prepared by Dr. Niels Lassen from our Department of Synthetic Chemistry as follows: From the aqueous solution of a salt, the neuroleptic base was precipitated with sodium carbonate and extracted with diethyl ether. The extract was dried with potassium carbonate, filtered and evaporated in vacuum at 40°. The evaporation residue was weighed and dissolved in the oily vehicles at concentrations as given in tables 1 and 2. All drugs were dissolved in Viscoleo®. Haloperidol, chlorpromazine and cis(Z)-clopenthixol were also dissolved in sesame oil, methyl oleate and squalane.

The injections were given via a needle (B-D®, 20 g 1½, 40-9) inserted centrally into the right longissimus dorsi muscle. The fix point for introduction of the needle was a transversal plane through the lumbar region halfway between the last rib and the crest of ilium and a sagittal plane in the middle of the muscle. This corresponds to about 4 cm ahead of the crest of ilium and 2 cm laterally from the spinous process of lumbar vertebrae. The needle was inserted about halfway (1.5 cm) into the vertical plane of the muscle. The thickness of the muscle at the injection site was evaluated from immediately preceding palpation.

Three days after the injection the rabbits were anaesthetized (50 mg/kg of pentobarbital intravenously) and killed by exsanguination. The muscle tissue at the injection sites was examined for macroscopic changes by serial sectioning. Areas of necrotic muscle tissue were isolated by careful trimming from the surrounding tissue and weighed. This procedure for demonstrating muscle damaging effect of injectable formulations has been described before (Svendsen *et al.* 1979).

Results

All aqueous preparations caused damage at the injection site as shown by presence of a large and well-defined necrotic area in all rabbits (table 1). The mean weight of the necrotic muscle tissue varied from 3.8 (Buronil®) to 8.8 g (cis(Z)-clopenthixol). The individual necrotic area varied considerably after injection of cis(Z)-clopenthixol (4.4–15.9 g), Klorpromazin® (3.2–11.1 g) and fluphenazine (1.3–8.3 g).

Little or no necrotic muscle tissue was found at the injection site after injection of the same drugs dissolved in Viscoleo® (table 1). The most consistent findings were focal haemorrhages, stripes of grey muscle fibres between normal muscle fibres and occasionally very small areas of necrotic muscle tissue.

Sesame oil, methyl oleate and squalane also reduced the local damaging effect of chlorpromazine and cis(Z)-clopenthixol but not to the same extent as it was reduced by Viscoleo® (table 2). Methyl oleate was less effective than sesame oil and squalane was less effective than methyl oleate. No necrotic muscle tissue was found at the injection site after injection of haloperidol in sesame oil, methyl oleate or squalane.

Discussion

The rabbits in the present study were killed for autopsy three days after the injection. Previous studies have indicated that a muscle lesion could then be expected to be at its maximum and also easy to dissect (Shintani *et al.* 1967; Löw *et al.* 1971; Kienel 1973; Fukawa *et al.* 1975; Steiness *et al.* 1978).

Previous studies have shown that chlorpromazine causes local muscle damage in animals (Benitz & Dambach 1966; Warnock & Ellman 1969; Meltzer *et al.* 1970; Bree *et al.* 1971; Klein *et al.* 1973; Küster 1973) and man (Meltzer *et al.* 1970; O'Connor 1980; Brumback *et al.* 1982). All aqueous formulations included in the present study caused local muscle damage at the injection site as indicated by the presence of a substantial amount of necrotic muscle tissue.

After injection of the same drugs dissolved in Viscoleo®, the most consistent findings were focal haemorrhages and grey stripes of degenerated muscle fibres between normal muscle fibres or occasional small areas of muscle necrosis. Consequently the present study has shown that neuroleptic drug preparations in the oily vehicle Viscoleo® are gentler than their counterparts in aqueous solution.

Microscopic examination of the muscle tissue was not included in the present study. All aqueous preparations caused massive muscle necrosis which was easily identified macroscopically by its abnormal colour and a greyish demarcating zone. Quantification of the size of the lesion by weighing of the dissected area of necrotic muscle tissue was therefore a reliable measure of damaging effect. The histopathology of similar muscle lesions in

Table I.

Post mortem findings at the injection site three days after intramuscular injection of 2 ml of different neuroleptic drug preparations into rabbits.

Preparation	Aqueous Solution		Viscoleo® solution	
	Weight (g) of isolated necrotic muscle tissue. Mean and range	Macroscopic findings	Weight (g) of isolated necrotic muscle tissue. Mean and range	Macroscopic findings
Cis(Z)-clopenthixol, 10 mg/ml	8.8 (4.4–15.9)	Well-defined necrosis	0.3 (0–0.7)	Focal haemorrhages and grey stripes
Cis(Z)-flupentixol, 10 mg/ml	4.0 (3.1–4.8)	Well-defined necrosis	0	No changes
Chlorpromazine 25 mg/ml, Klorpromazin®	7.9 (3.2–11.1)	Well-defined necrosis	0.3 (0–0.7)	Focal haemorrhages and grey stripes
Chlorprothixene, 10 mg/ml, Truxal®	4.5 (3.6–5.3)	Well-defined necrosis	0	Focal haemorrhages
Fluphenazine, 10 mg/ml	4.9 (1.3–8.3)	Well-defined necrosis	0	Focal haemorrhages and grey stripes
Haloperidol, 5 mg/ml, Serenase®	5.2 (3.5–7.6)	Well-defined necrosis	0.2 (0–0.8)	Focal haemorrhages and grey stripes
Levomepromazine, 25 mg/ml, Nozinan®	4.9 (4.7–5.2)	Well-defined necrosis	0	Focal haemorrhages and grey stripes
Melperone, 25 mg/ml, Buronil®	3.8 (3.2–4.4)	Well-defined necrosis	0	Focal haemorrhages and grey stripes
Perphenazine, 5 mg/ml, Trilafon®	4.3 (3.3–5.4)	Well-defined necrosis	0	Focal haemorrhages and grey stripes
Thiethylperazine maleate, 6.5 mg/ml, Torecan®	5.8 (4.6–7.0)	Well-defined necrosis	0	Focal haemorrhages and grey stripes

experimental animals has been described in several reports (Paget & Scott 1957; Hanson 1961; Benitz & Dambach 1966; Steiness *et al.* 1974 & 1978; Rasmussen & Svendsen 1976; Brumback *et al.* 1982). The histological changes were similar to those observed in massive infarction. In brief a large area of eosinophilic and hyalin fibres with pyknotic nuclei was described. The periphery of the necrosis was infiltrated with polymorphonuclear leucocytes and macrophages phagocyting the dead fibres. In addition there was evidence of beginning proliferation of fibroblasts and regeneration of muscle fibres. Previous studies have indicated that minimal histological changes are seen if no macroscopic changes are present (Paget & Scott 1957; Rasmussen & Svendsen 1976; Steiness *et al.* 1978). It has also been reported that vegetable oils such as Viscoleo® and sesame oil do not cause muscle damage (Svendsen & Aaes-Jørgensen 1979). For these reasons it was not considered necessary to include microscopic examination in the present study.

Table 2.

Post mortem findings at the injection site three days after intramuscular injection into rabbits of 2 ml of aqueous or oily solutions of chlorproma clopenthixol or haloperidol. The concentration of the aqueous solutions are given as concentration of the salt whereas the concentrations of the c refer to the base.

	Vehicles									
	Aqueous solution		Viscoleo®		Sesame oil		Methyl oleate		Squal	
Preparation	Necrotic area* (g)	Macroscopic findings	Necrotic area (g)	Macroscopic findings	Necrotic area (g)	Macroscopic findings	Necrotic area (g)	Macroscopic findings	Necrotic area (g)	M
Chlorproma-zine, 25 mg/ml (Klorproma-zine®)	7.9 (3.2–11.1)	Well-defined necrosis	0.28 (0–0.74)	Focal haemorrhages and grey stripes	0.49 (0–1.95)	Irregular necrosis. Focal haemorrhages and grey stripes	0.53 (0–1.75)	Irregular or well-defined ne-crosis. Focal haemorrhages and grey stripes	0.35 (0–5.42)	ha
cis(Z)-clopen-thixol, 10 mg/ml	8.8 (4.4–15.9)	Well-defined necrosis	0.26 (0–0.72)	Focal haemorrhages and grey stripes	1.15 (0.21–2.56)	Irregular necrosis. Focal haemorrhages and grey stripes	1.61 (0.64–2.83)	Well-defined necrosis	3.46 (2.98–4.38)	W
Haloperidol, 5 mg/ml (Serenase®)	5.3 (3.5–7.6)	Well-defined necrosis	0.23 (0–0.80)	Focal haemorrhages and grey stripes	0	Focal haemorrhages and grey stripes	0	Focal haemorrhages and grey stripes	0	ha

* Weight of isolated necrotic muscle tissue. Mean and range.

The present study has shown that Viscoleo® more efficiently neutralized the local damaging effect of cis(Z)-clopenthixol than sesame oil, methyl oleate and squalane. Squalane was in this respect least effective. A similar but less obvious trend was seen for the oily solutions of chlorpromazine. The concentrations of cis(Z)-clopenthixol (10 mg/ml) and chlorpromazine (25 mg/ml) in the oily vehicles were the same as those of the commercially available aqueous preparations. The aqueous solution of these two drugs caused identical local damage, indicating that cis(Z)-clopenthixol has more muscle damaging properties than chlorpromazine. This is also seen for the oily solutions.

The local muscle damaging effect of a drug preparation injected intramuscularly may be elicited by damage of the sarcolemma membrane of the muscle fibres or by toxic effects upon myofibrils or the sarcoplasmic organelles. The damage may be caused by concentration, pH or chemical properties of the drug or the vehicle. It is evident that the aqueous solutions must come into contact with a large number of muscle fibres immediately on injection. These muscle fibres are exposed to the drug preparation at the full concentration administered. The oily vehicles, on the other hand, are not miscible with the interstitial body fluids. Delay in drug release from the oily phase to the aqueous phase would mean that the initial concentration of the drug, to which the cells are exposed, is low. In addition, the high viscosity of the oily vehicle would tend to prevent the oil from reaching quite as many muscle cells initially. The net result, when drugs are presented in oily vehicles, would be fewer cells exposed to a lower initial drug concentration, than when the same drugs are presented in aqueous solution. It is suggested that these postulated differences in the number of cells exposed and in effective concentrations, would account for the observed difference in local toxicity of the two types of preparations.

Viscoleo® and sesame oil are triglyceride vegetable oils. The triglycerides in Viscoleo® contain short chain and saturated fatty acids, caprylic acid, capric acid and lauric acid, whereas the triglycerides in sesame oil contain mainly long chain saturated as well as unsaturated fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid and arachidic acid. The triglycerides are tolerated well at the injection site (Svendsen & Aaes-Jørgensen 1979). In comparison with sesame oil, Viscoleo® disappears relatively fast from the injection site (loc cit.).

Methyl oleate is an ester which to some extent is chemically related to sesame oil since oleic acid is the major fatty acid component of sesame oil.

Squalane is an oil obtained by complete hydrogenation of squalene. Commercial grades are obtained by direct hydrogenation of shark liver oil. According to Merck Index (1978) it is practically non-toxic in humans.

Recent studies have demonstrated that vehicles of vegetable oils injected intramuscularly are absorbed in part at the regional lymph nodes as liquid oil. Oil microembolisation may occur in the lungs if the oil-retaining capacity of the lymph nodes is saturated so that the excess oil leaves the lymph nodes and enters the thoracic duct (Svendsen & Aaes-Jørgensen 1979; Svendsen *et al.* 1980). Since very high dosages and frequent injections are needed to saturate regional lymph nodes, the implications of lymphatic absorption do not seem to be a problem for the use of oily vehicles.

From a local tolerance point of view there would be no reason for hesitation in recommending the use of vegetable oils as vehicles in drug preparations for intramuscular use. There are, however, several important aspects which have to be taken into consideration.

One fundamental prerequisite is that the drug is soluble in an oily vehicle. Salts of the neuroleptics used in the present study are hardly soluble in oil. The base of these drugs is on the other hand easily dissolved. Oil suspensions of sparingly soluble drugs were not included in this study but such a preparation may probably also be less irritating than an aqueous suspension. From a pharmaceutical point of view, however, suspensions are more troublesome than solutions.

The serum level profile of an oily drug preparation may differ from that of an aqueous preparation since the systemic absorption is delayed by the oil. Most aqueous drug preparations administered intramuscularly give an early and high plasma concentration followed by a rather steep decline. The serum concentration profile of cis(Z)-clopenthixol or cis(Z)-flupentixol in a Vis-

coleo® solution is in dogs characterized by a slower absorption and a plateau level lasting for more than 12 hours (Jørgensen and Aaes-Jørgensen, personal communication). This permits longer dosage intervals than the use of an aqueous solution but a minor delay in onset of action has to be expected. These two examples do not allow generalization, so similar studies would be necessary for each oily preparation.

A third aspect is stability of the drug in an oily vehicle and stability of the oily vehicle itself. Drug molecules with a hydroxygroup have been shown to be esterified to some extent with fatty acids form the triglycerides (Christensen, personal communication). Unsaturated fatty acids in the triglycerides may probably also be less stable than saturated fatty acids.

In conclusion the present study has shown that a number of neuroleptic drugs in aqueous solution cause local muscle damage after intramuscular injection. Oily solutions of the same drugs caused considerably less or even no local muscle damage. The muscle damaging effect of the drugs has consequently been diminished or even prevented by the oily vehicles. However, further studies are required as to whether this finding can be applied to other therapeutic agents known to cause local muscle damage in aqueous solution. Since the exchange of an aqueous vehicle with an oily vehicle may change the pharmacokinetic profile of the drug and probably also affect the stability of the preparation, these aspects need to be studied for each individual preparation.

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