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Research Paper

Acute Changes in Muscle Blood Flow and Concomitant Muscle Damage after an Intramuscular Administration

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Purpose. The intramuscular route (IM) is widely used but commonly induces injection site muscle damage. This study investigates the hemodynamic changes in an acute lesion induced by the IM administration of propylene glycol (PG) in rabbits.
Methods. Control groups received 1, 2, or 3 ml of PG (IM). Others were pretreated with pancuronium, dantrolene, indomethacin, or SR140333 and then received 2 ml of PG. The muscle blood flow (MBF) was assessed using fluorescent microspheres before and at 15, 45, 60, 90 min, 3 and 6 h after IM administration. Different areas within the muscle damage were quantified.
Results. Muscle contractions as well as a transient but major MBF increase were observed at the injection site. All treatments reduced hyperemia by up to 81% (dantrolene, 15 min) at 15, 45, and 90 min ($p < 0.05$). MBF had returned to basal values in all groups at 6 h. The central necrotic area was not modified, but peripheral damage (8.0 ± 1.3 g) was reduced by dantrolene, indomethacin, and SR140333 ($p < 0.05$), but not by pancuronium.
Conclusions. Muscle contraction and hyperemia are not responsible for muscle damage at the injection site, which is the multifactorial phenomenon, involving intracellular calcium and inflammation.

KEY WORDS: fluorscent microspheres; intramuscular administration; muscle blood flow; propylene glycol; skeletal muscle damage.

INTRODUCTION

The intramuscular (IM) route is widely used for drug administration. It was estimated in a study from the Boston Collaborative Drug Surveillance Program that 46% of patients received at least one IM administration during hospitalization (1). The IM route has many advantages over other routes of administration and often allows the use of drugs that are unsuitable for oral or intravenous administration. However, IM administrations commonly induce skeletal muscle damage and patient discomfort or pain. The muscle damage occurring during IM administration or soon after is mainly associated with pain, swelling, redness, and movement discomfort. There are also chronic consequences with local fibrosis of the muscles leading to muscle contractures (2), and abscesses (3) or nerve injury (4) have also been described.

Several studies focusing on different factors that could influence the extent of muscle damage are available. The influence of the nature of the actual drug, the vehicle, the pH of the solution, injected volume, concentration, viscosity, and speed of injection in the tissue have been documented (5). Paradoxically, very few studies are available on the pathophysiology of such muscle damage. Vasoactive events may be apparent during the acute phase of damage development after IM administration. Gross pathologic examination of the drug-induced lesions, usually reveals signs of inflammation and pathologic modifications in the microvasculature (hemorrhage, thrombosis, sludge, etc.). The skin temperature above the injection site was shown to change during the hours following IM administration in an ovine model (6). The temperature decreased during the first hour after the IM administration of 5 ml of a long-acting formulation of oxytetracycline, and this transient hypothermia was followed by local hyperthermia. This suggested that changes in skin and/or skeletal muscle blood flow (MBF) occurred and that the local muscle damage induced by an IM drug administration could involve a syndrome of ischemia-reperfusion (IR). In the current study, we used propylene glycol, an organic cosolvent that is used in many drug formulations and known for its poor local tolerability (5). We performed IM administrations in the rabbit to more precisely document the distribution and evolution of the actual blood flow in the injected muscle and to assess the effect of selected pharmacological modulators on these changes and concomitantly the extent of muscle damage at the injection site.

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ABBREVIATIONS: DAN, dantrolene; IM, intramuscular; IND, indomethacin; LD, *lumbar longissimus dorsi* muscle; MBF, muscle blood flow; MS, microspheres; PAN, pancuronium bromide; PG, propylene glycol; SPA, substance-P receptor antagonist.

MATERIALS AND METHODS

Experimental Design

Two experiments were performed: First, the distribution and the evolution of skeletal MBF were assessed in rabbits after the IM administration of increasing volumes of PG (1, 2, and 3 ml). In a second experiment, the skeletal MBF was similarly analyzed in rabbits pretreated with selected pharmacological agents.

The IM administration was performed into the left *lumbar longissimus dorsi* muscle (LD) of the anesthetized rabbit. The contralateral noninjected muscle was used as a reference. Fluorescent microspheres (MS) were administered in the left atrium of the heart after catheterization of the carotid artery and were used to assess blood flow in several organs and in the investigated muscles. Six MS solutions with different fluorescent dyes were used, once before the IM administrations, and at 5 critical times (i.e., 10, 30 min, 1, 3, and 6 h postinjection). At each administration of MS, a reference blood sample was collected from a carotid artery to allow calculation of the cardiac output and absolute quantification of the local blood flow. The rabbits were euthanized immediately after the final administration of MS.

Animals and Husbandry

New Zealand male rabbits (3 to 5 kg body weight) were purchased from Harlan France (ZI Le Marcoulet, Gannat, France) and acclimated to the experimental conditions for several weeks.

The animals were kept at the National Veterinary School of Toulouse, in facilities that are approved by the French Ministry of Agriculture in accordance with the current guidelines for animal care and use, and the research adhered to the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985). They were housed individually in stainless steel, wire-bottom cages, and provided with commercial rabbit chow and tap water *ad libitum*. They were subjected to daily observation throughout the experimental phases.

Chemicals and Drugs

Propylene glycol (PG) or 1,2-propanediol of analytical grade was purchased from Sigma-Aldrich Chimie (Lyon, France). The anesthetized rabbits were positioned in sternal recumbency. The lumbar area was thoroughly shaved. PG was injected in a standardized manner using a 15-mm-long 23-gauge needle inserted perpendicularly to the lumbar area into the middle of the left LD muscle. The contralateral noninjected muscle was also shaved and used as a control.

Pancuronium bromide (0.25 mg kg^{-1} h^{-1}) was purchased from Organon S.A. (Puteaux, France) as a commercial solution for intravenous use (Pavulon: 4 mg of pancuronium/2 ml).

Dantrolene sodium (IV bolus of 1.5 mg/kg and infusion of 1.5 mg kg^{-1} h^{-1}) was purchased from Merck Lipha Santé (Lyon, France) as a commercial solution for intravenous use (Dantrium: 20 mg of lyophilizate to be dissolved extemporaneously in 60 ml of sterile water).

Indomethacin (IV bolus of 3.2 mg/kg and infusion of 450 μg kg^{-1} h^{-1}) was purchased as a powder of analytical grade from Sigma-Aldrich Chimie (Lyon, France) and was prepared in solution (2 mg/ml) as described previously (7).

SR140333 (IV bolus of 100 μg/kg and infusion of 100 μg kg^{-1} h^{-1}), a potent nonpeptidic antagonist of NK-1 receptors, was generously provided by Dr. X. Emonds-Alt, Sanofi Research (Montpellier, France). It was prepared extemporaneously by dilution in saline.

Treatment Groups

Seven groups of four rabbits were used: The control groups CON1, CON2, and CON3 were not pretreated and received increasing doses of PG (1, 2, and 3 ml for groups CON1, CON2, and CON3, respectively) by intramuscular administration. The PAN group was intravenously infused with pancuronium (PAN). The DAN group received an intravenous infusion of dantrolene. The IND group was treated with indomethacin. The SPA group received an intravenous infusion of SR140333, an antagonist of the receptors for substance P. The selected dosage for SR140333 had been previously demonstrated (data not shown) to completely inhibit substance-P–induced hypotension in the rabbit (8). All treated rabbits received an intramuscular administration of 2 ml PG, similarly to group CON2.

Surgical Preparation

Anesthesia was induced by isoflurane delivered to the rabbit through a face mask. After tracheal intubation, the animals were anesthetized with 2.6% isoflurane supplied in 100% oxygen. Artificial ventilation was provided when needed. Body temperature was maintained by a heating blanket programmed to a set value of 39.5°C. The rabbit's eyes were lubricated with ophthalmic gel (Ocrygel, TVM, Lempdes, France) to prevent corneal drying. Anesthetic parameters were monitored continuously during anesthesia using an integrated system (Omnicare HP M1166A, Hewlett-Packard GmbH, Boeblingen, Germany): The inspired and end-tidal concentrations of the anesthetic gas were measured and continuously displayed using a gas analyzer (HP M1026A, Hewlett-Packard GmbH) connected to the endotracheal tube (HP 13905A, Hewlett-Packard GmbH). A lead-II electrocardiogram was continuously monitored (HP M1001A, Hewlett-Packard GmbH).

After reaching stable anesthesia, the animals were placed in a supine position. The right and left common carotid arteries were gently isolated from the connective tissues and nerves. A 20-gauge polyurethane catheter (Ref. 681037/681038, Becton Dickinson, Le Pont de Claix, France) was connected to a transducer and a pressure monitoring system, and was introduced through the left carotid artery and advanced into the left atrium for injection of the MS. The correct position of the catheter in the left atrium was identified by the typical pressure patterns of the left atrium and ventricle displayed by the monitoring system. For the collection of arterial blood, an 18-gauge catheter (Ref. 681037/681038, Becton Dickinson, Le Pont de Claix, France) was introduced through the right carotid artery and connected to a pressure transducer.

The myoelectrical activity of the injected LD muscle was registered in a separate group of anesthetized rabbits with an electroencephalogram machine (mini VIII; Alvar, Paris, France).

Measurement of Skeletal Muscle Blood Flow

Polystyrene MS 15 μm in diameter with 6 different fluorescent labelings (i.e., blue, blue-green, yellow-green, orange,

red, crimson and scarlet) were chosen (Fluospheres, diameter: 15 μm, Molecular Probes, Eugene, OR, USA). The MS were vortexed for 3 min, sonicated for 5 min, vortexed again for 3 min, and diluted in 0.9% saline to a total volume of 5.5 ml. An aliquot of the diluted solution was stored for determination of the total fluorescence administered. The rabbits received six administrations of MS: Once before the IM administration (control value) and at 5 successive time-points (10, 30, and 90 min, and 3 and 6 h) after the IM administration. The MS were injected within 1 min through the carotid catheter into the left atrium, to permit uniform mixing of the MS in the circulation. Simultaneously, a reference blood sample was withdrawn from the right carotid on citrate anticoagulation stabilizer at a known rate (3 ml/min) using silicon tubing (Masterflex L/S 13, model no. EL-96400-13, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) connected to a peristaltic pump (Masterflex L/S, model no. 7523-27, Cole-Parmer Instrument Co.) to allow calculation of the absolute blood flow in the organs.

Organ Sampling

After termination of the experiment by IV administration of an overdose of pentobarbital (pentobarbital sodium, CEVA, Libourne, France), samples from the *quadriceps femoris* muscles and LD muscles from both sides were excised after overnight storage of the carcass at 4°C to facilitate cutting of the muscles in a standardized way. The injected and control sites from the LD muscles were cut transversally into approximately 5-mm-thick slices starting from the center of the injection site. The slices were laid in order on a tray, and three muscular zones could be distinguished on a visual basis: a central zone with white necrotic tissue, red-colored reactive muscle tissue isolated in close periphery to the central zone, and normal surrounding tissue (Fig. 1). The smallest samples from each zone were pooled to obtain global samples of approximately equal weight. The mean weight was 4.5 ± 1.25 g. Each sample was immediately immersed in buffered 4% formaldehyde solution. After fixation, the samples were transferred to specific polyamide woven filtration units (Angelika Gaiser, Kunststoff- und Metallprodukte GmbH, Kappel-Grafenhausen, Germany), and analyzed for fluorescence.

Fluorescence Measurement

Immediately after sampling, the blood samples were filtered through the filtration units using a vacuum tap station. The withdrawal tube was washed with excess isotonic saline.

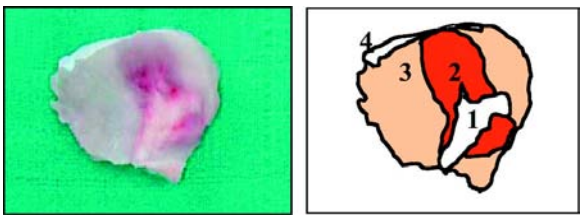

Fig. 1. Representative transversal slice of rabbit *longissimus dorsi* muscle 6 h after the intramuscular administration of propylene glycol (left panel) and the corresponding scheme (right panel) showing 3 main zones (1, center; 2, periphery; 3, normal) and the muscle aponeurosis (4).

The filter tube was placed in the filter holder and closed with a screw-cap.

Filters containing 10 μl of the administered MS diluted solution (3 per each MS administration), blood, or muscle samples, were processed and analyzed for fluorescence (9). Briefly, the organ sample was digested in 4N KOH solution for 6 h at a constant temperature of 60°C. The filter was then rinsed, washed and dried by centrifugation. The fluorescent dyes were released after dissolution of MS by adding 2 ml of cellosolve organic solvent to the filter. The fluorescence intensity was measured using a Perkin-Elmer luminescence spectrometer (LS50B) as described previously (10).

Calculation of Blood Flow

The cardiac output was calculated from the relation:

$$CO = R \times \frac{fl_{total}}{fl_{ref}}$$

where CO is the cardiac output (ml/min), R the withdrawal rate of the reference blood sample, fixed by the peristaltic pump (ml/min), fl_{total} is the total fluorescence intensity of the MS administered, and fl_{ref} is the fluorescence intensity in the reference blood sample.

The absolute blood flow to each sample was calculated using the following equation:

$$Q_i = \left(\frac{fl_i}{fl_{ref}}\right) \times R/W_i$$

where Q_i is the blood flow to the sample i (ml g^{-1} min^{-1}), fl_i its fluorescence intensity, fl_{ref} is the fluorescence intensity of the reference blood sample, R is the withdrawal rate of the reference blood sample (ml/min), and W_i is the weight of the *i*th sample (g).

The whole blood flow ($Q_{control}$) for the noninjected contralateral muscle was calculated as the mean of the absolute blood flow of each sample. This value was used to calculate the blood flow variation ($Q_{var\text{-}i}$) in each sample from the injected muscle relative to the noninjected contralateral muscle, used as a control:

$$Q_{var-i}(\%) = \left(\frac{Q_i - Q_{Control}}{Q_{Control}}\right) \times 100$$

Muscle Pathology

A separate group of rabbits was used for histologic analysis of the injured muscle. IM administrations of 2 ml of PG were performed in anesthetized rabbits as previously described to obtain muscular lesions at 10, 30, and 90 min, and 3 and 6 h after the administrations. Injected and control muscles were sampled and immersed in buffered 4% formaldehyde solution. After fixation, they were dehydrated and embedded in paraffin; 3-μm slices were obtained and stained with H&E. Several slices of each lesion were observed by a trained pathologist.

Statistics

A predictive interval including 95% of the individual values was constructed for each rabbit from the mean MBF values obtained from the muscle samples of the contralateral non-injected muscle. The upper limit of the 95% predictive

interval was defined as the mean MBF value for each time post-administration + 1.96 × SD, the lower limit of the predictive interval Mean MBF – 1.96 × SD. Thereafter, the samples for which the MBF value exceeded the upper limit of the predictive interval or was below the lower limit of the predictive interval were considered as hyperperfused or hypoperfused, respectively ($p < 0.05$).

A coefficient of variation was calculated for the muscle blood flow to estimate the heterogeneity of the measurements in the contralateral non-injected muscles. It was obtained from the arithmetic mean of the muscle blood flows divided by the standard deviation, and multiplied by 100 to be expressed as a percentage.

Data were analyzed with a general linear model (ANOVA). When a p value lower than 0.05 was observed, ANOVA was followed by adequate post-hoc tests: Dunett's test was used to compare values to a control group, and Fisher's least significant difference test was selected to compare each group to all the others. Data are presented as mean ± SD unless stated otherwise.

RESULTS

In Vivo Findings

A series of myotonic contractions or intense tremulations were observed in the untreated rabbits, for 10 min after the IM administration of PG, ipsilateral to the IM administration, mainly in the area rear to the injection site. Series of contractions were also seen in all rabbits pretreated with the pharmacological modulators but not in the PAN group.

Electromyographic examinations showed a spatial recruitment of the muscle contractions, which for the first few minutes were clonic and continuous (Fig. 2), then showed a slightly decreased frequency. This pattern was completely abolished by pretreating the rabbits with pancuronium bromide.

Assessment of the Skeletal Muscle Damage

The extent of necrotic damage to the muscle, in the control groups, was not different between the 3 volumes of PG administered. However, the weight of the peripheral red reactive tissue was significantly increased ($p = 0.013$) between the 2 and 3 ml PG IM administrations (8.0 ± 1.3 g and 14.5 ± 4.5 g respectively; Fig. 3). The extent of necrotic damage did not differ significantly between the control group administered with 2 ml of PG (CON2: 2.8 ± 1.1 g) and the groups treated with the pharmacological modulators (PAN, DAN, INDO, and SPA groups: 1.9 ± 0.8 g, 1.8 ± 0.3 g, 1.7 ± 0.8 g, and 1.7 ± 0.2 g, respectively). The extent of the peripheral red reactive tissue in the rabbits treated with DAN (4.9 ± 1.7 g), INDO (4.6 ± 0.6 g), and SPA (3.7 ± 0.5 g) but not with PAN (6.8 ± 2.2 g) was significantly reduced in comparison with CON2 (8.0 ± 1.3 g) ($p = 0.036$, 0.035 and 0.09, respectively) (Fig. 4).

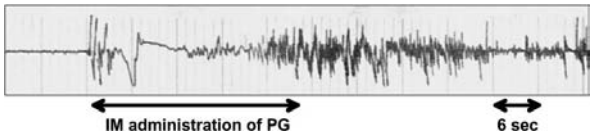

Fig. 2. Representative electromyographic pattern of skeletal muscle contractions observed after the IM administration of 2 ml of propylene glycol in the *longissimus dorsi* muscle of rabbit. This pattern was abolished by pretreatment of the rabbits with pancuronium bromide.

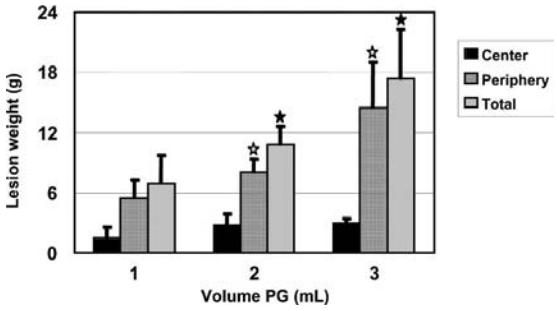

Fig. 3. Weight of the different areas (center, periphery, and total) of damaged muscle 6 h after the IM administration of propylene glycol in the lumbar muscles of the rabbit: effect of the administered volume (1, 2, and 3 ml) (mean ± SD, bars with stars are statistically different, $n = 12$).

Microscopic examinations showed a central zone exhibiting dissociation of the muscular cells at 15 min after the IM administration, without extensive degeneration and vascular events, except some sludge or microthrombosis. In the periphery, slight arterial vasodilation, congestion with hyaline plasma, mechanical extravasations, and interstitial edema were observed. At 45 min after the IM administration, larger, rounded myocytes, some of which were hyaline, were clearly dissociated. An arterial sludge and an accumulation of fibrin, red blood cells and leukocytes could be seen in the venules. Vascular congestion and hemorrhages were apparent in the peripheral zone.

At 90 min after the IM administration, the myocytes showed extensive degeneration and necrosis, with focal loss of myofibrillar structure but with intact cell membranes and nuclei. Some venules were dilated and empty. At 3 h after the IM administration, extensive, multifocal fragmentation and hypercontraction of some myocytes were observed, with several hyaline deposits. In the capillaries of the peripheral zone, the heterophilic blood cells were marginated and a diapedesis pattern sometimes observed. At 6 h after the IM administration, muscle fibers were dissociated by edema, and exudation was apparent. The muscular tissue was infiltrated by heterophilic blood cells, and thrombosis was observed in large vessels.

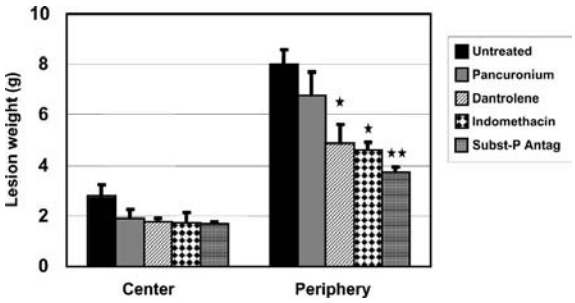

Fig. 4. Effect of different pharmacological modulators on the weights of the different areas (center and periphery) of the damaged muscle 6 h after the IM administration of 2 ml of propylene glycol (mean ± SEM, significant variation vs. untreated group is $*p < 0.05$ and $**p < 0.01$, $n = 20$).

Changes in the Whole Skeletal Muscle Blood Flow

The total cardiac output at the beginning of the study did not differ between treated and untreated rabbits (102 ± 8.6 ml kg^{-1} min^{-1}), and was comparable to the previously published values for pentobarbital-anesthetized rabbits [i.e., 92 ml kg^{-1} min^{-1} in resting conditions (11)], thus validating the absolute measurements of the organ blood flows. A slight decrease in the total cardiac output was observed throughout the duration of anesthesia, which was comparable for all groups of treated and untreated rabbits.

Under basal conditions, the mean control MBF assessed before the IM administrations in the LD muscle samples of rabbits from the CON1, CON2, and CON3 groups was 39 ± 6.2 μl min^{-1} g^{-1}, and the control MBF in the quadriceps femoris muscle samples was 44 ± 6.8 μl min^{-1} g^{-1}. No difference between left and right side for the MBF in the LD muscle samples (left: 40 ± 5.1 μl min^{-1} g^{-1}, and right: 37 ± 7.1 μl min^{-1} g^{-1}) and the quadriceps femoris muscle samples (left: 41 ± 3.3 μl min^{-1} g^{-1}, and right: 46 ± 8.1 μl min^{-1} g^{-1}) was evidenced. Moreover, the quadriceps femoris MBF remained stable for the total duration of the experiment, with final values of 49 ± 27.0 μl min^{-1} g^{-1} (left side) and 46 ± 18.6 μl min^{-1} g^{-1} (right side) 6 h after the IM administration.

In the contralateral noninjected muscle, the overall coefficient of variation for the MBF was 34% regardless of the time postinjection (from 0 to 6 h) under anesthesia and regardless of the status of the rabbit (pretreated or not with the pharmacological agents).

A significant increase in the MBF was observed in the injected muscle, compared to the contralateral noninjected muscle, of the CON1, CON2, and CON3 groups for all the volumes of PG at 15 min ($p < 0.05$). The mean increase was 351% (or 98 μl min^{-1} g^{-1}) of the contralateral value in CON3. The maximal value for the whole injected muscle (140 μl min^{-1} g^{-1}, i.e., a 623% increase) was observed in one rabbit in the CON3 group. The increase in MBF reached significance ($p = 0.013$) at 45 min postadministration, in CON1 and at 90 min in the CON1 and CON2 (Fig. 5). The whole MBF had returned to basal level at 6 h after the IM administration (CON1, CON2, and CON3).

The treated rabbits were compared to CON2, in which the observed increase in MBF for the whole injected muscle compared to the noninjected contralateral muscle was +175% at 15 min, +113% at 45 min, and +97% at 90 min. All treatments (PAN, DAN, IND, and SPA) significantly reduced hyperemia at 15, 45, and 90 min ($p < 0.05$), by up to 81% for the DAN group at 15 min (Fig. 6). The whole MBF had returned to basal values in all the treated groups at 6 h after the IM administration,

Blood Flow Distribution Within the Skeletal Muscle Lesion

The maximal MBF observed in the individual samples from the injected muscles was 242, 193, and 321 μl min^{-1} g^{-1} at 15 min in CON1, CON2, and CON3, respectively.

The mean weights of hypo- or hyperperfused muscle were not significantly modified by any of the pretreatments with pharmacological agents.

None of the slices of muscle from the red peripheric area was found to be hypoperfused, in any of the untreated rabbits, regardless of the volume of PG administered, whereas 81% (in weight proportion to the total weight of the area) of slices from this zone of the damage were found to be hyperperfused between 15 min and 3 h after the IM administration. In the central area, in the first 90 min, 0% of the area was found to be hypoperfused in CON1, 27% in CON2, and 48% in CON3. The evolution of blood flow in each of the samples from the injected muscle, compared to the predictive interval determined from the contralateral non-injected muscle values, is shown for a representative rabbit in Fig. 7.

DISCUSSION

This study is the first to report the evolution and distribution of skeletal MBF in a model of local iatrogenic muscle damage. The major findings are that:

1. A very rapid and dramatic increase in the whole blood flow occurs in the injected muscle.
2. The increase in MBF is transient, as all the values had returned to normal levels by 6 h after the IM administration.
3. The central area is mainly hypoperfused and is not modified by any of the pharmacological modulators. The red peripheral area close to the center, which is hyperperfused, is reduced by treatment of the animals with DAN, IND, or SPA, but not with PAN, whereas all four pharmacological modulators reduce the extent of hyperperfusion.

The rabbit model was chosen because of the availability of a large and homogeneous muscle mass in the paravertebral area, allowing adequate muscle samples. Moreover, the rabbit is a key species for testing muscle tolerance of injectable formulations (12) and has been used to document MBF during exercise (11). Propylene glycol, the test article selected here, is known to be poorly tolerated by the skeletal muscle (13,14).

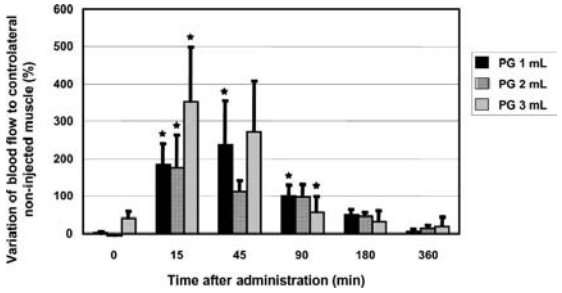

Fig. 5. Whole muscle blood flow evolution after the IM administration of propylene glycol in untreated rabbits: effect of the injected volume of propylene glycol (mean ± SEM, *significant variation vs. contralateral, n = 12).

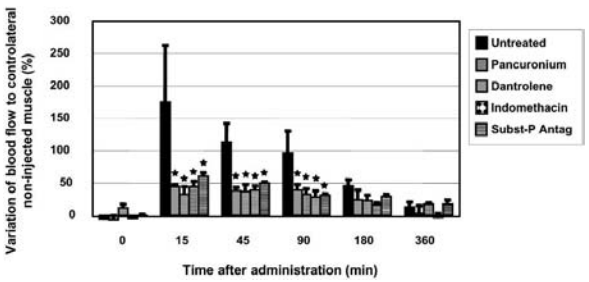

Fig. 6. Whole muscle blood flow evolution after IM administration of 2 ml of propylene glycol: effect of different pharmacological agents (mean ± SEM, *significant variation vs. untreated group, n = 20).

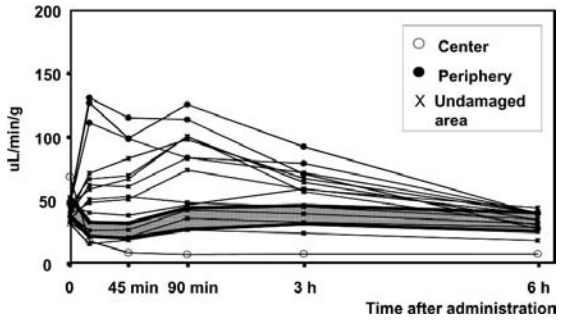

Fig. 7. Representative time course and heterogeneity of the absolute blood flow in muscle pieces from the center, periphery, and normal undamaged area of a rabbit muscle damaged by the IM administration of 2 ml of propylene glycol. The hatched area corresponds to the 95% predictive interval (mean ± 2 SD) of the blood flow values measured in the noninjected contralateral muscle.

It is used as an organic cosolvent at concentrations up to 100% for an increasing number of pharmaceutical formulations, as in recent years high-throughput biological screening and combinatorial chemistry has produced new, more lipophilic and less water-soluble chemicals (15). By inducing the lesion with a vehicle rather than with a pharmaceutical formulation containing vehicle plus active ingredient, any effects of the active ingredient on the observed mechanism of lesion development could be circumvented.

One limit of our study is that we have not documented the long-term reaction of the muscle tissue. This would require the use of a conscious model with chronic catheterism. Another issue is that an isoflurane-induced hemodynamic effect may interfere with changes in muscle blood flow. Even though the actual absolute values of blood flow may not be representative of those in the awake animal, the blood flow values from the injected muscle were intra-individually compared with the values obtained from the controlateral noninjected muscle at each time of measurement. Another potential limit is also the absence of a control group with saline instead of PG, or a sham-injected group (i.e., a needle inserted in the muscle with no injection of any drug) in order to discriminate precisely between the effects induced by the needle insertion and by the chemical properties of the drug itself. However, as well-tolerated nondamaging formulations such as normal saline are not a concern in clinical settings, the aim of this study was to document only the effects of damaging IM administrations on MBF. Groups receiving different volumes of PG (1, 2, and 3 ml) were included in order to obtain a large range of muscle lesions and to document the possible relationship between extent of muscle damage and change in MBF.

One of the major observations of this study was the spatial coexistence of muscle hypoperfusion and hyperperfusion in the acute phase after an IM administration. In myocardial infarcts or tourniquet-induced muscle ischemia, it is usually the temporal succession of ischemia and reperfusion that is reported and that is responsible for most of the damage (16).

In a study of hind limb muscles in the rabbit, MBF heterogeneity was evaluated at rest and during contractions-induced hyperemia (11). This heterogeneity remained unchanged (coefficient of variation of 31% to 41%) both during the different periods for which blood flow was averaged (from 10 s to 10 min duration) and during the periods of exercise, despite a major increase during the exercise. Moreover, the microspheres technique was found to be in good agreement with other methods used for the assessment of MBF. In our study, the coefficient of variation calculated for the contralateral non-injected muscles, regardless of the treatment and regardless of the time of assessment, was similar to the values reported by Iversen *et al.* (11) (i.e., between 31% and 41%). The spatial distribution of local blood flow within the rabbit myocardium is also heterogeneous, up to 200% (17). The effect of the injected volume was tested using 1, 2, and 3 ml of PG, but no clear relationship was seen between the volume and the increase in MBF, although the extent of the observed damage was volume-related.

No previous study has investigated the evolution of MBF within postinjection muscle damage. Therefore, several different hypotheses were taken into consideration in the selection of suitable pharmacological modulators for our experiments. The dosages of the different modulators were selected according to previously published data [e.g., pancuronium (18–20), dantrolene (21)] or pharmacokinetic calculation [e.g., indomethacin (22,23)]. The dosage of SR-140333 was determined in a separate pilot study (data not shown) and corresponds to the dose required to antagonize substance-P–induced hypotension.

As reflex muscle contractions were observed after PG administration, we first used a neuromuscular blocking agent, pancuronium bromide, to discriminate between a direct effect of the test article and an indirect effect, due to the contractions. Indeed, contractions result in high intramuscular tensions that can mechanically disrupt the myofibers, or permit phospholipase A_2 to directly lyse the components of the sarcolemma, together with high local temperatures in the muscle that can delete the protein structures (24). It was hypothesized that such contractions were responsible for the observed increase in blood flow due to an increased energy demand during muscular contraction. Indeed, contractions were abolished in the PAN group, and the increase in MBF was reduced but with no significant reduction of muscle damage. This suggests that the contractions were partly responsible for the increase in MBF, but that the increase in blood flow was not related to development of the muscle damage. Regarding the absolute values for blood flow, although some individual values in the areas close to the center of the damage were very high (up to 10-fold higher than the non-injected contralateral muscle or the basal values), the whole MBF had increased only 4.5-fold at 15 min after the IM administration of 3 ml of PG. In comparison, a 16-fold increase in whole blood flow (vs. rest value: 34 ± 3.3 μl min^{-1} g^{-1}) was observed in muscles of rabbits during exercise-induced contraction hyperemia.

The role of intracellular calcium has been studied in strenuous exercise, myocardial ischemia, muscular dystrophies, and injection-induced muscle damage. Muscle damage and creatine kinase release, after PG administration in isolated muscles, were associated with an intracellular mechanism involving the mobilization of calcium rather than direct damage to the sarcolemma (14). The loss of calcium homeostasis in myofibers and more specifically the increase in cytosolic free calcium is a major step in the cascade of events that results in cellular damage after exercise (24). Intracellular calcium has been shown to be an activator of phospholipase A_2 and also of the calcium-dependent proteases (25). The

release of calcium ions from the sarcoplasmic reticulum to the myoplasm is inhibited by dantrolene, which interacts with the ryanodine receptor, a major calcium release channel (26). It reduces creatine kinase release during exercise in the rat (27) and after repetitive blood sampling in the rabbit (28). It also prevents muscle damage in ischemic-reperfused hindlimb in the rabbit (21). In our study, the increase in blood flow to the PG-injected muscle was reduced in the DAN group.

The role of the prostanoïds in the evolution of blood flow and in the development of muscle damage was evaluated in our study by using IND, a cyclooxygenase inhibitor for both COX-1 and COX-2 at the selected dosage. COX-2 is induced in the subcutaneous skeletal muscle and local inflammatory cells at the RNA, protein and prostanoïd (PGE_2) levels, 3 h after carrageenan-induced inflammation in the rat (29). *In vitro*, well differentiated skeletal muscle cells produce COX activity and PGF_α when they are stretched (30). Prostanoïds are known to induce vasodilation of the muscle microcirculation. They are produced during episodes of local hypoxia or increases in blood velocity, which both occur during muscle contractions (31). Moreover, the administration of IND reduces contraction-induced hyperemia by 25% to 50% (32). In our study, inflammation was clearly observed by microscopic examination, and IND was shown to be effective in reducing the PG-induced increase in blood flow and the extent of the muscle damage.

Finally, the IM administration of drugs is most often painful (33) and we hypothesize that neurogenic inflammation might be involved in damage development. When the afferent axons are stimulated, a response by the tachykinins is induced, and substance-P (SP) is transported to the peripheral sensory endings, and released. Intense arterial vasodilation occurs in the tissue, with plasma extravasation and afflux of inflammatory cells. Moreover an axonal reflex produces a temporal and a spatial amplification of the reaction to neighboring endings (34). Although neurogenic inflammation has been described mainly for the eye, skin and intestinal tract, it seems that a neurogenic inflammation can be triggered from the skeletal muscle. Indeed, a colocalized expression of C-fos proteins (an index of pain) and NK_1 receptors in central neurons was evidenced after the IM administration of mustard oil in rat muscle (35). The activity of SP is mediated by the NK_1 tachykinin receptors, for which SR140333 has proven to be a potent nonpeptide antagonist (36). In our study, SR140333 reduced the increase in blood flow after the IM administration of PG in the muscle, in a similar way to the SP induction of arterial vasodilation in the tissues. Another action of SR140333 is to reduce the weight of the muscle damage, probably by limiting the spatial extension of inflammation by the axonal reflex, characteristic of neurogenic inflammation (34). Thus, the neurogenic inflammation process could be one of the pathways involved in the muscle reaction to iatrogenic damage, mainly by increasing the area of the inflamed tissue.

In conclusion, we have demonstrated that a major but transient increase in blood flow occurs within the skeletal muscle during the hours following IM administration of PG. Hyperperfused and hypoperfused areas coexist spatially within the lesion and its surroundings. Transient muscle contractions after the administration are partially responsible for the observed hyperemia but are not involved in the acute development of muscle damage. This seems to be induced by the loss of intracellular calcium homeostasis within the myofibers with a subsequent involvement of the inflammatory syndrome. None of the investigated pathways abolished the muscle damage or reduced the necrotic area, suggesting that direct toxicity of the test article and mechanical disruption of the myofibers are also major factors involved in muscle damage.

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