Effects of Pharmacological Intervention on Infarct Size following Induced Myocardial Infarction in Rats

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Abstract □ Several therapeutic agents that alter infarct size were administered to rats with myocardial infarcts induced by electrocauterization. The myocardial tissue damage and infarct size correlated well with the creatine phosphokinase myocardial band activity, and these markers were utilized to assess the action of the therapeutic agents on myocardial tissue damage. Hyaluronidase, insulin, potassium chloride, and isoproterenol increased myocardial tissue damage whereas heparin and prednisolone administration resulted in decreased myocardial tissue damage after induced myocardial infarction in rats.

Keyphrases □ Infarct size—effect of various therapeutic agents, rats with induced myocardial infarctions □ Myocardial tissue damage—effect of various therapeutic agents, rats with induced myocardial infarctions □ Creatine phosphokinase myocardial band—correlated with infarct size and myocardial tissue damage, rats with induced myocardial infarctions

Currently available methods for assessing myocardial ischemia include measurements for coronary blood flow, serum lactate levels, angiography, radioactive scanning, and ECG mapping (1–3). However, these methods study the qualitative aspects of myocardial ischemia without quantitatively ascertaining the infarct size.

BACKGROUND

Correlations between infarct size and myocardial enzyme levels have been recognized for many years. Myocardial infarct size, measured by excision and weighing of the damaged tissue, correlated well with the extent of the release of myocardial creatine phosphokinase (4, 5). This enzyme offered many advantages over the other myocardial enzymes since it rose first following an infarction and returned to normal levels in approximately 3 days (6). Sequential serum changes in creatine phosphokinase monitored myocardial infarction in experimentally infarcted dogs (7).

The presence of the cardiac specific creatine phosphokinase fraction, creatine phosphokinase myocardial band, in elevated amounts is a much better index of necrotic damage since this enzyme is exclusively of myocardial origin (8). Evidence supports the contention that enzyme release from the ischemic myocardium occurs once necrosis is either inevitable or complete (9). One could prevent cellular mortality by preventing necrosis in endangered, but not yet necrotic or irreversibly damaged, myocardium.

Serial determinations of creatine phosphokinase have been performed to predict the infarct size by utilizing data from only the first 7 hr postinfarction in patients and after 5 hr postinfarction in animals (10). With clinical experimentation, the variation between observed infarction and the enzymatically predicted infarction was only 5%. It appeared that the jeopardized zone was a reflection of the creatine phosphokinase predicted infarct size and that this zone would ultimately become necrotic without a beneficial intervention (10).

Experimental models show that brief coronary occlusions do not alter creatine phosphokinase activity and do not produce histological necrosis. An occlusion of sufficient duration, however, results in creatine phosphokinase release (9, 11). The myocardial depletion of creatine phosphokinase reflects the infarct size in experimental animals (12, 13) and is indicative of necrosis as determined with radioactive microspheres.

In this study, the activity of the creatine phosphokinase cardiac specific myocardial band fraction was correlated to the grams of damaged necrotic myocardium produced after induced infarction. These biochemical and histological markers are used to illustrate the degree of myocardial tissue necrosis following pharmacological interventions in rats with induced myocardial infarction. This study demonstrates a model for evaluating various therapeutic agents capable of altering infarct size. The pharmacological agents studied were prednisolone, heparin, hyaluronidase, insulin, potassium chloride, and isoproterenol.

EXPERIMENTAL

Animals—One hundred and five Sprague–Dawley[1] male rats, 300–400 g, were divided into seven groups. Group I (45 rats) was used as a control to establish a correlation between infarct size and creatine phosphokinase myocardial band activity. The remaining rats were divided into six groups of 10 rats each.

Group II was treated with 100 units of heparin/kg iv by the penile vein at 15 min postinfarction. Group III was treated with 1.5 units of insulin sc 15 min following infarction. Group IV animals were treated with 0.025 mEq of potassium chloride subcutaneously at 15 min postinfarction. Group V was treated at the same time interval following infarction with prednisolone butylacetate at 11.4 mg/kg im. Group VI received 100 U iv of hyaluronidase.

Group VII was used as a positive control since many reports on isoproterenol suggest that the increased chronotropic and inotropic activity of this drug would increase the expected infarct size. This final group received a dosage of isoproterenol of 0.02 mg/kg.

All animals were given food[2] and water *ad libitum*.

Procedure for Inducing Infarction—The animals were anesthetized with pentobarbital sodium[3] (35 mg/kg ip), and the ECG was monitored throughout the surgical procedure. Following depilatation and sterilization with povidone-iodine[4], a 2.54-cm long incision was made through the skin in the left thoracic area to the skeletal muscle. The muscle layers were separated with minimal damage, using blunt dissection, and a purse string suture was prepared around the incision site.

After the ribs had been spread, the thorax opened, and the heart exposed, a branch of the left anterior descending artery was cauterized[5] carefully. Artificial respiration was not employed since the procedure involved an open thorax for only 15 sec. The chest was slightly compressed to create a negative intrathoracic pressure, and the purse string suture was closed.

For the measurement of the creatine phosphokinase levels, blood was taken from the tail at 0, 6, 18, 30, 42, and 54 hr since creatine phosphokinase activity previously returned to baseline by this time. After each experiment, the surviving animals were sacrificed, their hearts were removed, and the infarcted tissues were excised carefully. The necrotic tissue was weighed for future correlation with enzyme release data.

Assay of Creatine Phosphokinase Myocardial Band—A 0.1-ml volume of serum was used for the assay of creatine phosphokinase levels in a commercial spectrophotometric kit[6].

RESULTS

Group II (heparin) was treated with a dose expected to increase clotting time and result in a diminished clot surrounding the occluded vasculature. Clotting at the infarction site has been implicated with the extension of ischemia in the immediate area of the occlusion as well as in the distal vasculature. The current finding of a diminished necrosis and released enzyme is in total agreement with previous work (14–19) demonstrating

[1] Taconic Farms, Germantown, N.Y.
[2] Purina Rat Chow, Ralston-Purina, St. Louis, Mo.
[3] Sedasol, EVSCO Pharmaceutical Corp., Oceanside, N.Y.
[4] Betadine, Purdue-Frederick, Yonkers, N.Y.
[5] Lawton Co., New York, N.Y.
[6] Creatine phosphokinase 45-UV, Sigma Chemical Co., St. Louis, Mo.

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Table I—Mean Necrosis *versus* Mean Enzyme Released

Group	Necrotic Mass[a]	Enzymes[b]	Treatment[c]	Correlation Coefficient[d]
I	0.175	47.52	Control	0.930
II	0.173	32.65	Heparin	0.924
III	0.193	65.50	Insulin	0.836
IV	0.197	74.80	Potassium chloride	0.984
V	0.151	24.10	Prednisolone	0.672
VI	0.184	52.40	Hyaluronidase	0.963
VII	0.277	153.90	Isoproterenol	0.819

[a] Necrotic mass = necrosis in grams. [b] Enzymes = mean creatine phosphokinase myocardial band released in 54 hr. [c] Treatment = agent administered to the group. [d] Correlation coefficient between creatine phosphokinase myocardial band and necrotic mass.

that decreased infarct size is associated with an inhibition of platelet aggregation. Although not statistically less than the experimental group, there was a mean decrease in infarct size. A similar minimal decrease in the enzymes demonstrated a definite trend.

Insulin treatment was carried out as a positive control because Smith and Bird (20) showed that hypoglycemia increases infarct size by eliminating anaerobic metabolism of glucose as an energy source. The inhibition of glucose from access to the cell enhances the damage to the ischemic myocardium. This agent is often given with glucose to overcome hypoglycemia and with potassium to replenish the ion lost to the interstitial space during infarction. In this study, a nonsignificant increase in infarct size was shown by the mean increase in the necrotic mass at postmortem and also by the mean increase in the released enzyme.

Potassium was administered to the myocardially infarcted rats to determine if an increase in intracellular potassium in the deficient myocardial cell would affect myocardial salvage. The dosage was administered subcutaneously in an attempt to release the ion slowly into the circulation. A small dosage was chosen to eliminate the chance of toxicity associated with larger doses. The cells of the body store this ion, once administered, if it is administered at a rate exceeding the body's ability to utilize it or to excrete it *via* the kidney (21). An increase in necrosis was associated with this drug, which can probably be attributed to the toxic effect of the ion on the myocardium.

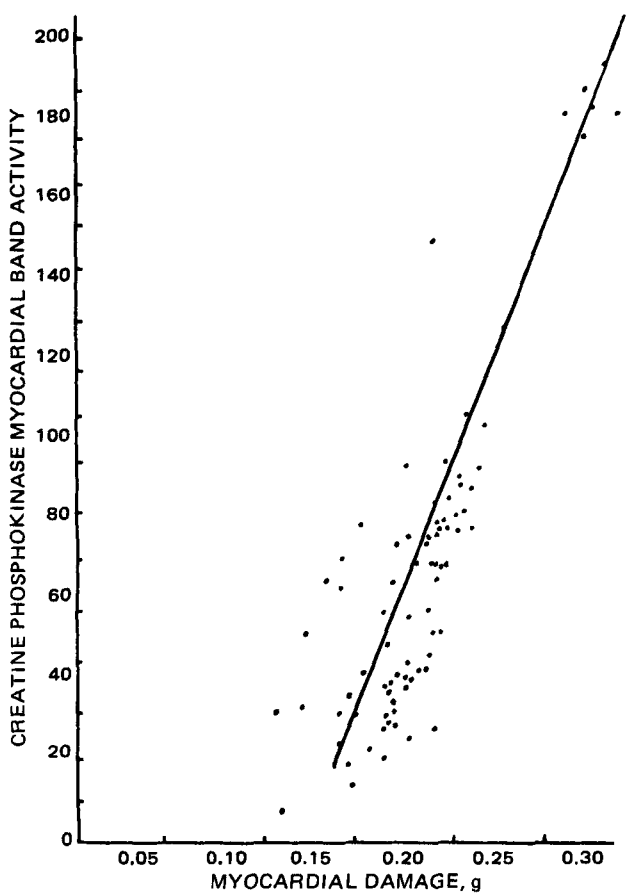

Figure 1—*Linear regression of all animals.*

Although the use of steroids to protect the ischemic myocardium has been studied extensively, controversy still surrounds this application. In the present study, there was a definite reduction in necrotic tissue as well as a reduction in the observed serum creatine phosphokinase attributed to this drug.

Steroids are thought to stabilize the lysosomal membrane and the cellular membrane itself. These effects limit the spread of lysosomal hydrolases capable of further damaging the myocardium (22). The efficacy of the glucocorticoids in decreasing infarct size was undoubtedly demonstrated in this study. This work supports the clinical finding of Morrison *et al.* (23) when a single dose of methylprednisolone decreased infarct size.

The effect of hyaluronidase on the extent of myocardial necrosis following infarction was studied to determine if the spreading factor associated with this agent would efficiently allow the transport of nutrients and oxygen to the ischemic portion of the myocardium and thus salvage the tissue. Hyaluronidase increased the level of necrosis and also the enzyme release but not significantly.

Isoproterenol was administered to assess the effect of the positive inotropic and chronotropic activities of this drug and its oxygen-wasting activity on the ischemic myocardium. Studies (24) utilizing such an intervention on the surgically occluded left ventricle of the dog demonstrated an increase in infarct size. The present study agrees with this work since the increased workload and oxygen requirements associated with the drug significantly increased infarct size in this group. The serial determination of the creatine phosphokinase myocardial band also correlated with the increased necrotic mass observed at postmortem.

The proof of the sensitivity of this model is depicted in Table I where it is demonstrated that each group correspondingly increases, *i.e.*, potassium chloride is the sixth in both total enzymes and necrotic mass. The two groups in which statistically significant alterations were observed were at the extreme ends of the experimental animal groups.

Although several drugs significantly reduced infarct size and enzyme release, Table I displays an important insight into the sensitivity of the model; in each case, the group with the lowest mean released enzyme had the lowest mean necrotic mass and the group with the highest release of enzymes had the highest necrotic mass as well. The middle groups responded sequentially.

Figure 1, a scatter diagram, shows the linear regression of all animals in which the analysis with creatine phosphokinase myocardial band was carried out.

The procedure offers a simple, easily performed method for the induction of myocardial infarction in the rat. The model offers enzyme sensitivity, reliability, and speed with a minimum of mortality following the intervention. Therefore, this procedure can be part of a routine screening for the pharmacological efficacy of agents that limit the extent of infarction in the rat.

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Effect of Surfactants on Percutaneous Absorption of Naproxen II: *In Vivo* and *In Vitro* Correlations in Rats

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Abstract □ The effect of surfactants on percutaneous absorption of naproxen was studied using rat *in vivo* models. The *in vivo* normalized relative absorption rates were in good agreement with the *in vitro* relative flux. An antipyretic model in the rat could not show relatively small increases in percutaneous absorption caused by the surfactants. Based on these results, it is apparent that reliance on the rat, using either *in vitro* or *in vivo* models, may lead to erroneous conclusions when considering the effect of surfactants on human percutaneous absorption of drugs such as naproxen.

Keyphrases □ Surfactants, various—effect on percutaneous absorption of naproxen, *in vivo* and *in vitro* correlations in rats □ Naproxen—percutaneous absorption, effect of various surfactants, *in vivo* and *in vitro* correlations in rats □ Absorption, percutaneous—naproxen, effect of various surfactants, *in vivo* and *in vitro* correlations in rats □ Anti-inflammatory agents—naproxen, percutaneous absorption, effect of various surfactants, *in vivo* and *in vitro* correlations in rats

Percutaneous absorption generally is studied by two types of model systems: those employing excised skin in some type of a diffusion cell and those using living animals or humans *in situ*. Many studies suggested that these two model systems may differ in their mode of percutaneous absorption, namely, the *in vitro* measurement of steady-state flux *versus* the pharmacological activity mainly induced by the small amount of drug penetrating through the appendageal route in the early stages of diffusion.

BACKGROUND

Penetration through sweat ducts under a potential gradient occurred within 1–5 min, with no comparable transport through the stratum corneum within this period (1). Histological studies (2) also demonstrated follicular diffusion occurring within 5 min. Similarly, perifollicular wheals were observed 5 min after the application of 10% histamine free base (3). Some studies also related the *in vitro* steady-state flux to the *in vivo* steady-state flux. For example, the *in vitro* and *in vivo* transport of alkylmethyl sulfoxide across rabbit skin was studied, and the steady-state urinary elimination rate was about one-half of the *in vitro* steady-state flux (4).

Because of the question of the absorption mechanism, *i.e.*, intra-appendageal or transepidermal, in *in vivo versus* model systems and further complications due to variations in skin permeability from site to site, human to human, and species to species, comparable quantitative data between *in vivo* and *in vitro* model systems are sparse. A notable exception may be data (5) showing a similarity between the *in vitro* skin penetration and *in vivo* vasoconstriction response of fluocinonide and fluocinolone acetonide.

Previously (6), an *in vitro* model using excised skin from laboratory animals and humans was used to study the effect of surfactants on percutaneous naproxen absorption. The results suggested that excised skin of laboratory animals may lead to erroneous conclusions about the effect of surfactants on excised human skin.

This paper reports the effect of surfactants on percutaneous naproxen absorption in the rat *in vivo*. Comparisons between the *in vivo* normalized relative absorption rates and the *in vitro* relative flux in the rat indicate good correlation between the two model systems. Since surfactants showed only a small increase in percutaneous absorption in the rat, an antipyretic bioassay model in the rat could not show a similar correlation.

EXPERIMENTAL

Materials—The surfactants sodium lauryl sulfate[1], sodium laurate[2], and methyldecyl sulfoxide[3] were used as received.

Nonlabeled naproxen[4] was at least 99% pure. The tritiated naproxen was purified by radiochromatography and was at least 98% pure. The two solvent systems used were hexane–ethyl acetate (85:15) and benzene–tetrahydrofuran–acetic acid (90:9:3). All other chemicals were analytical reagent grade unless otherwise indicated.

Preparation of Creams—The method for the preparation of creams was as described previously (6).

***In Vivo* Percutaneous Absorption in Rats**—Male Sprague–Dawley rats[5], 250–300 g, were employed. Hair was removed[6] from the skin of the dorsal area between the forelegs and hindlegs on both sides of the spine, 18 hr prior to the application of cream. Throughout the shaving and dosing operations, the animals were kept under light anesthesia with anesthetic grade ether[7].

A cardboard template with an outside measurement of 4 × 5 cm and an inside measurement of 2 × 3 cm was devised to restrict and control the contact area between the cream and skin of the rat. The cardboard was covered on both sides with waterproof plastic tape[8] and was held in position by medical adhesive tape[9]. An accurately measured dose (0.5 ml) was delivered with a 1-ml tuberculin syringe to a 2 × 3-cm area. A

[1] E. I. du Pont de Nemours & Co., Menlo Park, Calif.
[2] Eastman Kodak Co., Rochester, N.Y.
[3] Institute of Organic Chemistry, Syntex Research, Palo Alto, CA 94304.
[4] *d*-2-(6′-Methoxy-2′-naphthyl)propionic acid, Syntex Research, Palo Alto, CA 94304.
[5] Simonsen Laboratories, Gilroy, Calif.
[6] Model-A2 animal electric clipper with size 40 blade, John Oster Manufacturing Co., Milwaukee, WI 53200.
[7] Mallinckrodt Chemical Works, St. Louis, MO 63100.
[8] Scotch No. 471, 3M Co., St. Paul, Minn.
[9] Zonar Porous Tape, Johnson & Johnson.

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