Burn Edema Reduction by Methysergide Is Not Due to Control of Regional Vasodilation

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To determine the extent to which edema modulation by methysergide is due to a blunting of the regional vasodilator response to scald and/or local reduction of transvascular fluid flux, a canine hind limb lymphatic was cannulated. Femoral blood flow (Q; ml/min), lymph flow (QL; l/min/100 g), and lymph-to-plasma protein ratios (CjCp) were monitored in groups of five dogs before and 4 hr after 5-sec, 100°C foot paw scald; high (1.0 mg/kg) or low (.5 mg/kg) dose of methysergide 30 min before scald. The compression on a clamp placed around the femoral artery in other dogs was adjusted after scald to simulate the blunting effect on Qs observed in methysergide treated dogs. Hind leg venous pressure was elevated to ~40 mm Hg before experimentation until steady state Qs and (CjCp)min were reached. Protein reflection coefficient (rj; 1 - CjCp) and fluid filtration coefficient (Kr) were calculated. Compared to preburn values, all groups showed significant (P < 0.002, analysis of variance) increases in CjCp and Kr. Contrasted with the burn only group, methysergide blunted increases in Qs, Kr and paw weight gain in a dose-dependent fashion, with no effect on the reflection coefficient. Compression clamp control of femoral Qs caused no effects on permeability. Methysergide limits burn edema in a dose-related fashion, though not due to a blunting of the regional vasodilator response. Local, not regional, mechanisms likely mediate this response.

INTRODUCTION

Beyond producing a marked increase in blood flow directed to the wound, a burn dramatically increases capillary permeability at the site of injury. In a patient with a large body surface area burn, this so-called “capillary leak” causes rapid depletion of intravascular volume which, left untreated, will lead to hypovolemic shock. Clearly, aggressive intravenous fluid resuscitation is warranted to maintain stable hemodynamic parameters during this acute phase of injury. However, among the consequences of this chain of events is the accumulation of massive amounts of edema beneath the burn wound, with its known deleterious effects, including respiratory insufficiency [1], compression of the vascular supply of the extremities [2], and inhibition of cell-mediated immune responsiveness [3].

In an effort to blunt the accretion of edema, investigators have long sought to devise burn resuscitation regimens that would either mitigate the marked regional vasodilator response to the burn or “seal” the leak in those capillary beds that, as a result of the burn, are more permeable to the transvascular flux of fluid and protein into the interstitium at the (local) site of injury. In this regard, we have demonstrated that continuous activation of serotonin receptors is to a large extent responsible for the burn-induced regional vasodilator response in dogs, and that this effect can be markedly inhibited by the serotoninergic receptor antagonist, methysergide [4]. In a subsequent series of experiments, we found that pre- or postburn administration of methysergide also limited the otherwise marked burn-induced increase in capillary hydrostatic pressure and in transvascular flux of fluid, and significantly reduced edema formation at the (local) site of injury [5]. Since we were unable to determine the extent to which the reduction of edema by methysergide was due to modulation of the otherwise marked regional vasodilator response and/or the reductions in burn-induced increases in capillary hydrostatic pressure and in transvascular fluid flux known to occur at the (local) site of injury, the following series of experiments was designed.

MATERIALS AND METHODS

All experimental procedures were completed in accordance with the guidelines and approval of the Tulane University School of Medicine Advisory Committee for Animal Resources.

Surgical protocol. Mongrel canines weighing 20–25 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg body weight). The animals were intubated and mechanically ventilated with a Harvard ventilator pre-set to deliver 15 cc/kg/min room air at a rate of 12 breaths/min. A foreleg vein was cannulated for the administration of maintenance fluid (1–2 ml/kg/hr Ringer’s lactate) and intermittent doses of sodium pentobarbital as needed to maintain an operative plane of anesthesia. An ipsilateral foreleg artery was cannulated for continuous recording of mean arterial pressure.
Through a groin incision, the femoral artery, vein, and nerve were exposed (Fig. 1). Side branches of the vessels were individually ligated and divided, beginning at the level of the inguinal ligament and continuing along its distal length for a distance of 10–15 cm. An umbilical tape was placed loosely around the thigh, 5–10 cm distal to the inguinal ligament, excluding the femoral artery, vein, and nerve. An ultrasonic flow probe positioned around the common femoral vein (Transonic Systems) was used to provide a source for the continual recording of femoral blood flow (Q0). In certain dogs (denoted later), the femoral artery was encircled distal to the ultrasonic blood flow probe with a non-crushing compression clamp; in these dogs, minute-to-minute control of Q0 could be achieved by simply tightening or loosening the clamp.

The animal was then positioned in the lateral decubitus position, and a small segment of skin over the lateral saphenous vein was removed to expose the lateral saphenous vein and the prenodal lymphatic trunk paralleling the vein. Through a small branch from the saphenous vein, a cannula was advanced to its point of confluence with the saphenous vein, so as not to impede saphenous outflow (and to measure capillary pressures) and to obtain blood samples. The lymphatic trunk on either side of the lateral saphenous vein was proximally ligated. The ankle was elevated on a stand to the level of the heart, and arterial and venous pressures were maintained at 0 mm Hg. This maneuver increased transcapillary fluid flux, thereby improving lymph flow (QL).

By tightening the previously placed umbilical tape around the thigh, venous pressure was elevated to 40 mm Hg. This maneuver increased "washing down" lymph total protein tension as adjusted by totally encircling the saphenous vein and the prenodal lymphatic trunk. Through a groin incision, the femoral artery, vein, and nerve were exposed (Fig. 1). Side branches of the vessels were individually ligated and divided, beginning at the level of the inguinal ligament and continuing along its distal length for a distance of 10–15 cm. An umbilical tape was placed loosely around the thigh, 5–10 cm distal to the inguinal ligament, excluding the femoral artery, vein, and nerve. An ultrasonic flow probe positioned around the common femoral vein (Transonic Systems) was used to provide a source for the continual recording of femoral blood flow (Q0). In certain dogs (denoted later), the femoral artery was encircled distal to the ultrasonic blood flow probe with a non-crushing compression clamp; in these dogs, minute-to-minute control of Q0 could be achieved by simply tightening or loosening the clamp.

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FIG. 2. Changes in mean arterial pressure over time in each animal group. There were no within or between group differences at any point during the experimental period.

FIG. 3. Time-course analysis of femoral blood flow alterations. Manipulation of the femoral artery compression clamp, and administration of both high and low dose methysergide, all blunted the dramatic and persistent increase in femoral blood flow following burn in equivalent fashion. *P < 0.0001 vs preburn; +P < 0.05 vs MET, HIMET/BN, LOMET/BN, BN/OCCL.

FIG. 4. Alterations in capillary hydrostatic pressure depicted as a function of time. The significant, albeit short-lived, increase in capillary pressure in the burn alone group was ameliorated to an equivalent extent by high and low dose methysergide. Femoral artery compression had no effect on this variable. *P < 0.007 vs preburn; +P < 0.05 vs BURN, BN/OCCL.

FIG. 5. Changes in lymph flow graphed as a function of time. Following burn, lymph flow in all groups increased immediately. When compared to the burn only group, both groups of animals given methysergide demonstrated a significant reduction in lymph flow towards the terminal stages of the experiments. Femoral artery compression had no effect on this variable. *P < 0.0001 vs preburn; +P < 0.0006 vs BURN: HIMET/BN, LOMET/BN, BN/OCCL.

RESULTS

In the absence of scald injury, peripheral intravenous infusion of methysergide produced no lasting alterations in mean arterial pressure, capillary pressure, QL, filtration or reflection coefficients. Methysergide administration did produce a mild decrease in Qa, consistent with its known vasoconstrictor properties.

As shown in Fig. 2, systemic mean arterial pressures were unchanged in all burn groups, suggesting that the scald injury was not of sufficient magnitude to produce appreciable alterations in systemic hemodynamics. In marked contrast, the regional effects of the scald injury were underscored by the dramatic and sustained increase in Qa in the burn alone animals when compared to all other groups (Fig. 3). Of note, while Qa:s did mildly increase immediately after scald in animals pretreated with high and low dose methysergide (data not shown), they uniformly returned to baseline values within 30 post-burn min. Finally, modulation of the post-burn regional vasodilator response with the compression clamp to the same extent as was achieved with high or low dose methysergide was clearly affirmed by the lack of statistically significant differences in Qa among these groups throughout the period of observation.

The local hemodynamic effects of the scald were similarly well demonstrated by the significant, albeit short-lived, increase in capillary hydrostatic pressure in the burn alone animals (Fig. 4). This phenomenon was unaltered by mechanical modulation of femoral artery blood flow, though it was significantly blunted by high and low dose methysergide pre-treatment.

When compared to their respective preburn levels, the dramatic increases in Qa observed in all burn groups persisted throughout the experiments (Fig. 5). However, Qa:s in both methysergide-treated groups were significantly lower than both untreated burn groups after the initial postburn hour. Despite increased Qa:s in both untreated burn groups, the paws gained significant weight when compared to those of the unburned animals (Fig. 6). Pretreatment with methysergide dramatically reduced edema formation to an amount statistically identical to that of unburned...
animals. Low dose methysergide also reduced paw weight gain, though not to the same extent as did high dose methysergide, suggesting a dose-response effect. Moreover, regional control of $Q_a$ by the compression clamp failed to produce a significant impact upon weight gain.

The filtration coefficient, $K_f$, increased immediately and remained elevated throughout the observation period in all burn groups (Fig. 7). However, by the 3rd post-burn hr, animals given high dose methysergide demonstrated a significant reduction in $K_f$ when compared to that of the burn only animals, an effect not seen with low dose methysergide. This implies that methysergide blunts the burn-induced increase in transmembrane fluid conductance in a dose dependent fashion. Again, modulation of the burn-induced femoral vasodilator response by the compression device had no effect upon the filtration coefficient.

As mentioned previously, the reflection coefficient, $U_d$, describes the plasma protein selectivity of the capillary membrane. A $U_d$ of “0” implies a membrane that is completely permeable, and a $U_d$ of “1” indicates a membrane totally impermeable, to protein. An immediate, marked, and persistent decrease in $U_d$ was observed in all burn groups (Fig. 8). This significant increase in capillary membrane permeability was unaltered by either dose of methysergide, signifying that this agent has no appreciable effect on the forces that control macromolecular permeability at the site of scald injury. The lack of a perceptible effect of the compression clamp on $U_d$ further reinforces the concept that at present, mechanical or pharmacological efforts to control the regional vasodilator response to scald injury have no impact on the transvascular flux of macromolecules (e.g., protein) at the injury site.

**DISCUSSION**

It has been well documented in experimental and human studies that blood flow to the site of injury greatly increases immediately after a burn and remains elevated for a prolonged period of time [4, 13–15]. Using ultrasonic flow probe techniques, we determined that the marked femoral vasodilator response to an ipsilateral canine hind paw scald was not dependent upon activation of adenosine$_A$, muscarinic, $\beta_2$-adrenergic, histaminergic$_C$ or histaminergic$_D$ receptors, on cyclooxygenase products, endothelium-derived relaxing factor, or $K_{ATP}$ channels. Rather, this response could be almost completely blocked by pre- or postburn intravenous administration of methysergide, suggesting that continual activation of serotoninergic-like receptors is primarily responsible for the effect [4]. Since the hind paws of animals given methysergide appeared appreciably less swollen than were those of untreated, similarly injured animals, it was suggested that this agent may play a role in minimizing edema formation.

To examine this issue, a canine model was subse-
quently employed that, in the hands of several investiga-
gators, has proven a reliable means of monitoring
changes in Starling forces across a skin/soft tissue mi-
crocirculatory bed that has been subjected to a small
scald [16]. We found that methysergide, whether given
before or after scald, dramatically reduced edema for-
to a marked blunting of the regional vasodilator re-
sponse to the injury, methysergide may provide a re-
gional infow brake that would limit exposure of the
intravascular blood volume to "leaky" capillary mem-
branes at the injury site. Methysergide treatment was
also found to reduce the total transvascular fluid flux
within the burned tissues. These data suggest that
methysergide may as well possess local effects, perhaps
limiting serotonin receptor activation that would other-
wise promote: (1) a local vasodilation and a redistribu-
tion of blood flow directly to the injury site [17]; (2)
direct microcirculatory vascular leakage, as has been
reported to occur in rat and rabbit cremaster muscle
exposed to serotonin [18].

The present series of experiments was conceived to
determine the extent to which the reduction in early
burn-induced edema formation by methysergide is ex-
erted through regional and local mechanisms. The magnitude of the scald (3% total body surface area)
was limited by design, to diminish the possibility that
alterations in systemic hemodynamics would influence
Starling forces monitored at the site of injury. In this
regard, it has previously been demonstrated in dogs
that this specific injury produces no changes in cardiac
index, pulmonary artery pressure, pulmonary capillary
wedge pressure, or mean systemic arterial pressure [4].
Likewise, no changes in systemic mean arterial pres-
sure were observed in the experimental groups re-
ported herein, enabling us to monitor the local and
regional impact of methysergide on burn-induced
changes in capillary permeability.

The dramatic alterations in femoral artery blood
flow, capillary pressure, \( Q_c \), reflection and filtration
coefficients, and paw weight gain in the burn only
group are in keeping with previously reported data [16,
19, 20]. This overall increase in microvascular filtration
is thought to be generated through several different
physiologic mechanisms. Immediately after the injury,
the increase in microvascular filtration is driven by
hydrostatic (e.g. increased capillary pressure) forces, a
factor that wanes within hours after injury. At that
point, the permeability alterations (e.g. reduced reflec-
tion coefficient and increased filtration coefficient) that
are present from the onset of injury assume the domi-
nant role in sustaining the increase in microvascular
filtration across the damaged capillary membranes.
These phenomena, combined with the marked, persis-
tent increase in regional blood flow, are considered re-
sponsible for the clinical endpoint of the injury—
the peak in the accumulation of edema in the early postinjury
period of observation. Additional, as of yet not clearly
defined, mechanisms likely account for each of these
effects. For example, we have recently demonstrated
that activation of postganglionic autonomic nerves is at
least partially responsible for mediation of the regional
vasodilator response to burn [21]. However, the
impact of this observation or, for that matter, the in-
fluence of other locally produced vasodilator, cytokine,
or neuropeptide substances on serotonin-independent edema formation is at present undetermined.

One explanation for this phenomenon might be found in the effect that methysergide exerted on the marked, albeit short term, burn-induced increase capillary pressure—considered to be a primary force promoting increased microvascular filtration early after burn. Low dose methysergide blunted this local increase in hydrostatic pressure to the same extent as did high dose treatment. These observations imply that this effect on capillary pressure could account for the mild but significant reduction in the clinical endpoint of an increase in microvascular filtration—wound edema—seen following low dose methysergide therapy. Again, regional arterial inflow compression failed to have any effect on locally measured capillary pressure or on edema formation after scald, reaffirming the impression that regional forces have minimal influence on the accumulation of edema at the injury site. It is unclear as to whether the effect of methysergide in this experimental model can be assigned solely to alterations in capillary pressure and/or in the transvascular flux of fluid into the soft tissue spaces. Moreover, the source(s) of serotonin that, in the absence of methysergide, are presumed to drive this edemogenic response are ill defined. In this regard, others have suggested that, in response to injury produced at the microcirculatory level, platelet-derived serotonin not only promotes arteriolar vasodilation and venoconstriction, but also increases capillary and venular permeability, leading to the formation of edema [17]. Additional experiments are presently underway to delineate the answers to these queries. Nonetheless, data reported herein lead us to the conclusion that, in this animal model, the known regional vasodilator response to burn injury, while marked and persistent, plays little role in the early post-burn accumulation of edema at the site of injury.

REFERENCES


