

Original Article

HDFx: a novel biologic immunomodulator is therapeutically-effective in hemorrhagic and intestinal-ischemic shock: importance of microcirculatory-immunological interactions and their potential implications for the warfighter and disaster victims

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Abstract: Recently, we have reported on the discovery of a new, conserved protein (35-40 kD), termed HDFx, that protects rats, guinea-pigs, mice and rabbits against lethal hemorrhage, endotoxins, and traumatic injury when given, systemically, as a pretreatment. HDFx was also found to stimulate several arms of the immune system. The present report demonstrates, for the first time, that HDFx, when administered post-hemorrhage and post-intestinal ischemia shock -trauma, yields increased survival rates, elevates falling arterial blood pressures, possesses unique actions in the microvasculature, stimulates depressed RES phagocytosis (normally observed in animals and humans during blood loss, sepsis and trauma), and preserves cytokine levels in lymphocytes obtained from animals subjected to hemorrhage and traumatic shock. We believe that HDFx presents a potential brand new therapeutic approach: 1) for the injured warfighter on the battlefield, 2) for victims of major disasters, 3) as an adjunct for patients undergoing high-risk surgical procedures commonly found in open-heart surgery, cancers, and in neurosurgeries. Use of HDFx could potentially allow oncologists to decrease chemotherapy dosing, while increasing patient survival chances.

Keywords: Macrophages, circulatory shock, cytokines, blood pressure, arterioles, venules, phagocytosis, reticuloendothelial system, vasoactive molecules

Introduction

Despite the vast body of experimental and clinical information that has accumulated over the past 40-50 years, in the study of circulatory shock, except for more rational, vigorous approaches to volume expansion, electrolyte balance, surgery and pH maintenance, relatively little of this data has been found suitable for precise therapeutic application to humans. This becomes crucial in battlefield casualties and in disaster victims who suffer severe blood loss and multiple body trauma. The identification of specific etiologic mechanisms or substances that generate and sustain lethal progression of the shock syndrome continues to remain elu-

sive and a matter of much uncertainty despite a history of more than 100 years of intensive research, and treatment continues to be empiric. Failure of peripheral exchange-vessel (microcirculatory) blood flow in the tissues remains the single valid common denominator in most and perhaps all forms of both experimental and clinical low-flow state syndromes [1-4]. A secondary concern is subsequent dysfunction of the immune system.

Almost any therapy that either directly or indirectly improves local exchange-vessel blood flow in critical vascular beds within a reasonable time interval is beneficial. But many of these studies do not emphasize drugs or techniques

that are suitable for immediate clinical use. General experience with vasoactive drugs as acute therapy in shock-trauma has not been satisfactory; their use is considered by many clinicians and investigators to be ineffective or even unwarranted [1, 2, 5, 6]. The ineffectiveness of vasoactive drugs as acute therapy in shock may be a result of at least four factors: 1) inability to be able to measure, quantitatively, at a tissue level, the severity or course of the shock syndrome; 2) unavailability of vasoactive drugs that exert selective microvascular actions; 3) since shock syndromes consist of at least two distinct phases (i.e., compensatory and decompensatory in nature) [7], two different types of vasoactive agents may be required for effective treatment. In regard to the latter, the exact type of vasoactive would be dependent on the phase of the low-flow state syndrome; the early phase may require a selective dilator or pharmacologic agent (antagonist) capable of attenuating an overcompensated (heavily constricted) microvasculature, whereas the late phase may require a vasopressor agent that can exert selective, but mild constrictor action on the muscular venules, thereby maintaining a vis a tergo and preventing pooling of blood in the capacitance side of the microcirculation, or may require pharmacologic antagonists that can reverse the widespread vasodilation (or loss of vasomotor tone seen later in shock. Lastly, or 4) a therapeutically-effective agent would have to sustain an effective immune system.

Recently, our group has reported the discovery of a naturally-occurring biologic in several species of rodents and rabbits which we have termed HDFx that stimulates several arms of the innate immune system and leads to protection against lethal hemorrhage, trauma, endotoxins, sarcoma -180 and combined injuries when administered as a prophylactic treatment [8]. Some of our studies suggested that HDFx might have vasoactive properties in addition to its immunomodulatory attributes. The current studies were designed to determine if HDFx: 1) is therapeutically-effective in two different forms of circulatory shock: hemorrhage and intestinal ischemia; 2) alters arterial blood pressure in these forms of experimental shock; 3) increases rates of survival when administered as a therapeutic agent; 4) sustains phagocytic function of the monocyte-phagocytic system in animals subjected to shock; and 5) administration will prevent depression of the immune system. Lastly,

we sought to determine if the therapeutic effectiveness of HDFx is associated with beneficial changes in the microcirculation of animals subjected to different forms of experimental shock.

The studies reported, herein, serve to demonstrate, for the first time, that: 1) administration of HDFx in animals subjected to either lethal hemorrhage or intestinal ischemia trauma leads to increased survival; 2) HDFx administration in shocked animals elevates arterial blood pressure; 3) HDFx administration prevents loss of phagocytic competence in macrophages of the RES-monocyte system in animals subjected to hemorrhage or intestinal ischemia; 4) HDFx administration ameliorates deterioration of the immune system; and 5) HDFx prevents loss of venular tone in the microcirculation of shocked animals.

Materials and methods

Animals, anesthesia and survival studies

Young, adult male inbred Wistar strain rats (165-200g) were used for all studies. All animals were anesthetized prior to surgery, hemorrhage and intestinal ischemia shock-trauma, and blood withdrawal with pentobarbital sodium (Nembutal, 4 mg/100g i.m.) to reduce genetic variability as a factor [9, 10], littermates from our breeding colony were used throughout these studies [8]. All animals were at least 70 days old and, thus, sexually mature [8]. Only male animals were included as the sex of animals has been demonstrated by a number of workers to influence the outcome of shock-trauma studies and the responses of macrophages and other cellular elements of the immune system to pathophysiologic stimuli [9, 11-13]. All animals were given sterilized distilled water to drink and Purina rat chow pellets ad libitum. Aseptic technique was used throughout the studies. Each study group (controls and experimental) were comprised of 10-16 rats. Each anesthetized animal, prior to surgery or animal model procedure, was placed on a temperature-controlled table and maintained under temperature-controlled conditions. A femoral arterial and vein were cannulated in each animal for monitoring of arterial blood pressure and i.v. infusion of either saline or HDFx. Different groups of animals subjected to hemorrhage (controlled arterial bleedings to maintain arterial blood pressure at 35-40 mm Hg) or intesti-

nal ischemia shock-trauma (temporary ligation of the superior mesenteric artery) were administered i.v. sterile physiologic saline or HDFx (in sterile physiologic saline) at a constant rate over 60 min. After removal of catheters, some animals were monitored for survival for seven days; others were utilized for removal of blood for RES phagocytic function and cytokine levels in lymphocytes [8]. All animals were autopsied at death or sacrificed. Rats dying within 15 min of the start of infusion were not included in the data. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised in 1996) and was approved by the Animal Use and Care Committee at SUNY Downstate Medical Center.

Hemorrhage model

The procedure for normal animals followed closely methods we have described previously [8]. Following initial control observations, over a period of 15-20 min, the animal was bled in a graded fashion over a 2 hr period to a mean blood pressure level of 35-40 mm Hg and maintained at this level by additional small bleedings as necessary [8]. No blood was reinfused during the hypotensive episode after which 2.0 ml of saline alone or containing HDFx were infused intravenously at a constant rate for 60 min. At the conclusion of the infusions, the shed blood was reinfused (intra-arterially) over a 20-30 min period. Observations were continued post-transfusion for 30-45 min. The animals were either sacrificed or studied for survival for seven days.

Intestinal ischemia trauma-shock model

Intestinal ischemia trauma-shock was instituted by temporary ligation of the superior mesenteric artery for 90 min after which the temporary ligation was released for a 60 min period of time [14], following which 2.0 ml of saline alone or containing HDFx were infused at a constant rate for 60 min. Serial hematocrits were determined. Observations were continued post-transfusion for 30-45 min. The animals were either sacrificed or studied for survival for seven days.

RES phagocytic function

The procedure in these experiments essentially consisted of determining RES phagocytic indi-

ces (K values) [15, 16] in the different groups of experimental animals identical to the models used above. The phagocytic indices were determined 45 min after either the infusions of saline or HDFx by measuring the rate of clearance of colloidal carbon (4 mg in calf skin gelatin/100 g body wt) [15, 16]. Precisely timed blood samples were obtained at 2, 4, 8, 12 and 15 min after the i.v. colloidal carbon injection, hemolyzed in 0.1 % sodium carbonate and the carbon concentration measured photometrically at 675 mu [15, 16]. The colloidal carbon utilized in these studies was Pelikan C11/1431 (Gunther-Wagner, Hanover, Germany). Phagocytic indices were calculated:

$$K = \log_{10} C_1 - \log_{10} C_2 / t_1 - t_2$$

Where K is the phagocytic index and C_1 and C_2 are the colloidal carbon concentrations in mg/100 ml of blood at t_1 and t_2 [15, 16].

Effects of hemorrhage and intestinal ischemia on cytokine levels in lymphocytes with/without treatment with HDFx

Lymphocytes were harvested from control animals, hemorrhaged survivors, and survivor animals subjected to intestinal ischemia shock-trauma according to previously-described routine density gradient methods utilizing a typical Metrizoate-Ficoll mixture [8, 17]. The lymphocytes were then analyzed for levels of IL-2, IL-6, and interferon γ (IFN- γ) using commercially available antibody-kits (Santa Cruz Labs, Santa Cruz, CA) as previously [8]. Different groups of animals received either infusions of isotonic saline or HDFx in isotonic saline, as above, prior to harvesting the lymphocytes.

In-vivo microcirculatory effects of isotonic saline and HDFx in animals subjected to hemorrhage and shock-trauma

Rats were prepared as described above. In addition, prior to hemorrhage or intestinal ischemia shock and infusions, the mesoappendix was exteriorized in-vivo for direct microscopic visualization using an image-splitting TV microscopic recording system devised in our laboratories [18]. This quantitative microscope recording system can make 45-60 measurements/min of lumen sizes of all types of microvessels (i.e., arterioles, venules, metarterioles, precapillary

sphincters, and capillaries [18]. Microscopic observations were made at magnifications of 60 to 1,000 x for changes in microvessel lumen sizes, rate of blood flow, and vasomotion (i.e., the spontaneous opening and closing of precapillary sphincters where they exist). Vascular reactivity to topically applied epinephrine was determined at frequent intervals and quantified by the characteristic equivalent response to varying concentrations (parts per million) of 0.1 ml aliquots of epinephrine [19]. Microcirculatory parameters were monitored continuously for the entire acute experiment extending for at least 45 min post-infusion.

Isolation, preparation and standardization of HDFx for infusions

Briefly, heparinized blood was obtained from control, naïve rats stimulated to produce HDFx by adaptation to whole-body LD 80- trauma, as shown previously [8]. Precautions were taken for blood collections as described [8]. All blood collection tubes and glassware were tested for endotoxins using a Limulus Amebocyte assay as described [8]. We set one unit of purified HDFx to be equivalent to raising the RES phagocytic index (K-value) 100% in naïve rats [8]. Two units of HDFx raised the RES phagocytic index over 200% in naïve rats [8]. This latter dose of HDFx (in 2.0 ml of sterile isotonic saline) was administered i.v. over 60min (via a constant Harvard infusion pump) into the animals subjected to hemorrhage and intestinal ischemic shock-trauma in our study.

Statistical analyses

Where appropriate, means and means \pm S.E. were calculated. Differences between means were assessed for statistical significance by Student's t-tests and ANOVA, followed by a Newman-Keuls test. Mortality/survival differences were tested for statistical significance by Chi-square. A P value <0.05 was considered significant.

Results

Microcirculatory effects of HDFx in hemorrhagic shock

Normal saline solution infusion (12 rats). During the initial period of hemorrhage (5-15 min), when the hypotensive effect of blood loss was

most marked, all components of the mesenteric microvascular bed became hyperactive and overall blood flow was more rapid. Increased shunting via larger A-V shunt vessels became quite apparent. The small arteries (80-120 μ m in diameter), arterioles (30-50 μ m in diameter) and muscular venules (40-70 μ m in diameter) were variably but markedly constricted. Vasomotion of precapillary sphincters and metarterioles (15-22 μ m in diameter) was augmented (i.e., the microvessels were more constricted than vasodilated). Capillary inflows were restricted. Reactivity of the arterioles and metarterioles to topical epinephrine was increased (5-25 times normal). In the succeeding period of saline infusion, the overall hyperactivity and rate of blood flow fluctuated irregularly and the microvascular bed remained hyperactive. During this time, arterial and arteriolar vasoconstriction was maintained as was the increased vascular reactivity to epinephrine; A-V shunting increased. Vasomotion eventually disappeared. Capillary ischemia in the entire bed became intense. Caliber of the small veins and muscular venules initially demonstrated enhanced vasoconstriction but eventually gave way to venular dilation followed by loss of venular tone, stasis of red and white blood cells in the venules, and leukocytic sticking on the venular walls. This was often followed by rupture of collecting venules with extravasation of red and white blood cells. These events using saline infusion are summarized in **Table 1**. Even though the infusion of saline resulted in some rise in blood pressure (**Figure 1**), it did not appreciably alter the state of the microcirculation which progressively exhibited decompensation. Reinfusion of blood resulted in some rise in arterial blood pressure concomitant with slight, transient improvement of flow in the larger channels; but the preexisting venular stasis, congestion, and rupture of venules were markedly exaggerated by the transfusion of blood.

HDFx infusion (12 rats)

For a brief period of time (5-15 min) following the administration of HDFx, the microvascular bed also became hyperactive. The microcirculation, however, exhibited some of the characteristics of the early compensatory responses to the onset of less severe hemorrhage in that precapillary shunting was less pronounced (compared to saline controls), capillary inflow was more readily noted and vasomotion was

HDFx for treatment of hemorrhagic and intestinal-ischemic shock

Table 1. Microcirculatory effects of saline versus HDFx infusion in rats subjected hemorrhagic shock

Parameter	Normal saline (controls)	HDFx
Arteriolar constriction	++++*	+
Capillary inflow	markedly reduced (4+)	improved (+)
Vascular reactivity**	markedly increased	near-normal
Vasomotion	dilated-absent	near-normal
Venular tone	absent	present
Stasis-petechiae***	++++	+

Microvessels of rat mesentery were observed by direct in-vivo microscopy during last 15-20 min of saline infusion. Mean blood pressure prior to infusion maintained at 35 mm Hg for 2 hr. *Indicates generalized vasoconstriction sufficient to result in intense ischemia. **Response to various concentrations ($\times 10^{-6}$ M) of epinephrine HCl applied topically. ***Indicative of severe permanent, irreversible microvascular injury.

quite evident unlike the saline controls (above). Moreover, epinephrine reactivity was not significantly increased and, surprisingly, often actually was decreased. During the succeeding period of HDFx infusion, epinephrine reactivity clearly was decreased (only 2-4 times normal) and vasomotion was maintained, quite unlike the saline infused controls. The arterial and arteriolar limbs resembled closely a normal mesenteric microvascular bed; A-V shunting was minimal. Venous and venular vessels maintained their tone (i.e., did not progressively widen) except in an occasional vein and venule. Venular outflow (return) was quite rapid and unidirectional. Most areas of the mesenteric microbed appeared entirely normal except for some axial streaming in the arterial limb (characteristic of blood loss). Interestingly, infusion of HDFx resulted in a rapid rise of arterial blood pressure (**Figure 1**).

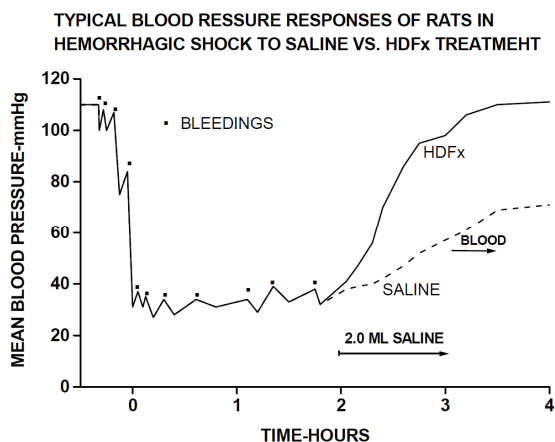


Figure 1. Typical blood pressure responses of rats during hemorrhagic shock with infusions of normal, isotonic saline solution versus HDFx. Dots indicate times at which blood was withdrawn to main arterial blood pressure at 35 mm Hg. N=12.

Transfusion of blood resulted in a further rise in blood pressure to normotensive levels (**Figure 1**); this was concomitant with a return of overall blood flow, near-normal vasomotion, near-normal reactivity to epinephrine, and a capillary flow close to the initial normal level. Some of these results are summarized in **Table 1**.

Survival rates in hemorrhagic shock treated with saline and HDFx

Table 2 summarizes the survival rates which were intended to establish a hemorrhagic episode yielding a mortality of approximately 50%. It is clear from our data that HDFx resulted in close to a 90% survival rate.

Table 2. Influence of normal, isotonic saline solution versus HDFx infusion on survival rates after hemorrhagic shock in rats*

Group	Survivors/Total	% Survival
Naïve controls	6/16	38
Saline controls	7/16	44
HDFx	14/16**	88**

*See methods for hemorrhage details.

**Significantly different from naïve and saline controls ($P < 0.01$, Chi-Square).

Microcirculatory effects of HDFx in intestinal ischemia shock-trauma

Normal saline solution infusion (10 rats)

During occlusion of the superior mesenteric artery (SMA), all mesenteric blood flow ceased. Upon release of the SMA occlusion, there was a resumption of flow which was very similar to

Table 3. Microcirculatory effects of saline versus HDFx infusion in rats subjected intestinal ischemia shock-trauma

Parameter	Normal saline (controls)	HDFx
Arteriolar constriction	++++*	+
Capillary inflow	markedly reduced (4+)	improved (+)
Vascular reactivity**	markedly increased	decreased
Vasomotion	absent	present
Venular tone	dilated	present
Stasis-petechiae***	+++	0-+

Microvessels of rat mesentery were observed by direct in-vivo microscopy during the last 15-20 min of infusion. *Indicates generalized vasoconstriction sufficient to result in intense ischemia. **Response to various concentrations (x 10⁻⁶ M) of epinephrine HCl applied topically. ***Indicative of severe permanent microvascular damage.

control levels, which was maintained for about 15 min. However, blood flow appeared most rapid in the arteriolar-venular shunts which bypassed the “true” capillary channels. The mesenteric bed demonstrated an overall vasodilation of venules and an augmentation (i.e., constriction) of vasomotion with a heightened increase in vascular reactivity to epinephrine (approx. 4-64x normal). During the next 15-45 min, blood flow in the collecting and muscular venules became more and more diminished, the larger resistance microvessels (arterioles, 40-70 μm) and smaller feeding arterioles (18-30 μm) were intensely constricted; no flow could be observed in either the feeding metarterioles or capillaries. Vasomotion gradually disappeared completely. Epinephrine reactivity increased 200-400-fold. Infusion of normal, isotonic saline did not appreciably alter the curtailment of blood flow or the heightened increase in vascular reactivity. But, within minutes of the start of the saline infusion, the status of the microvascular bed underwent profound deterioration characterized by pooling of blood in the post-capillary, collecting, and muscular venules followed by rupture of post-capillary and collecting venules with extravasation of red and white blood cells; numerous petechial formations were observed. Concomitant with these deleterious changes, we observed a rapid decline of arterial blood pressure (Figure 2). These microvascular alterations are summarized in Table 3.

HDFx infusion (12 rats)

Within 3-15 min, infusion of HDFx resulted in a microvascular bed with less A-V shunting of blood flow, increased “true” capillary blood flow, and a regeneration of vasomotion. Unlike that seen with saline, vascular reactivity to epineph-

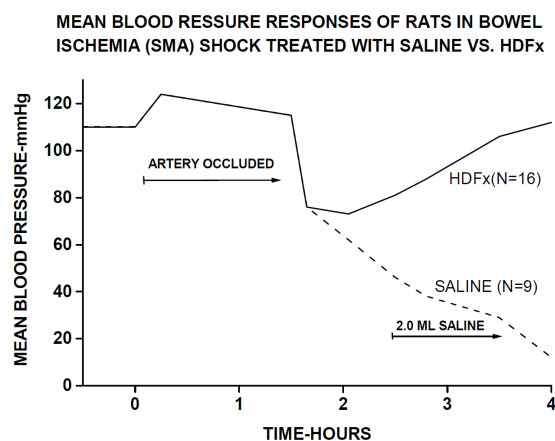


Figure 2. Typical mean blood pressure responses of rats during intestinal ischemia shock-trauma with infusions of normal, isotonic saline versus HDFx. N=16 for HDFx; N=9 for saline infusion.

rine demonstrated progressive decreases in responsiveness to the catecholamine (e.g., 3-6 x normal). Towards the end of the HDFx infusion vascular reactivity to epinephrine approached normal levels. Arteriolar and venular tone also approached normal levels. Good continuous arteriovenous shunt flow was maintained in contrast to that seen with saline infusion. Little or no stasis or petechial hemorrhages were observed in the venous side of the microvascular bed. Arterial blood pressure continued to rise towards normal levels, quite unlike the continuous decline seen with saline (Figure 2). A summary of these findings using HDFx is shown in Table 3.

Hematocrit patterns seen with saline vs. HDFx infusion in intestinal ischemic shock-trauma

Hematocrit patterns were consistent and typical

for hemoconcentration reported previously in this model of shock-trauma (i.e., a 15-22 % rise in hematocrit over control levels indicative of hemoconcentration) [20]. With infusion of saline, there was a fall in hematocrit (i.e., 5-8 % decrease), suggestive of hemodilution. In animals treated with HDFx, there was a greater fall in hematocrit (i.e., 8-14 %) which appeared to peak towards the end of HDFx infusion.

Survival rates in intestinal ischemic shock treated with saline and HDFx

Table 4 summarizes the survival rates which were intended to establish an episode yielding a mortality of approximately 25%. It is clear from our data that HDFx resulted in more than a 90%

Table 4. Influence of normal, isotonic saline versus HDFx infusion on survival rates after intestinal ischemia shock-trauma*

Group	Survivors/Total	% Survival
Naïve controls	3/16	19
Saline controls	4/16	25
HDFx	15/16**	94**

*See methods for details of animal model.

**Significantly different from naïve and saline controls (P<0.01, Chi-Square).

survival rate.

RES phagocytic indices early after hemorrhage and intestinal ischemia shock-trauma with and without saline or HDFx treatment

Table 5 indicates that irrespective of the model of untreated shock, the RES phagocytic indices are severely and significantly depressed similar to that reported previously [9, 12, 15, 16]. However, the results with treatment, using HDFx, indicate that within 3 hr of the end of the infusion, the RES phagocytic indices demonstrate a hyperactive phagocytic capability, quite unlike that seen early after saline infusion.

Effect of hemorrhage and intestinal ischemia shock-trauma with/without treatment with saline and HDFx on cytokine levels in lymphocytes

Recently, we have demonstrated that pretreatment of animals, subjected to various types of systemic injuries, with HDFx protected lympho-

Table 5. RES phagocytic indices 3 hr after saline versus HDFx infusions in rats subjected to hemorrhage and intestinal ischemia shock – trauma*

Group	K-value
Controls	0.048+/- 0.004
Hemorrhage alone	0.008+/-0.002**
Hemorrhage w/saline	0.010+/-0.002**
Hemorrhage w/HDFx	0.065+/-0.006***
Intest Isch alone	0.006+/-0.002**
Intest Isch w/saline	0.012+/-0.004**
Intest Isch w/HDFx	0.060+/-0.006***

*See methods for details of models. **Significantly different from controls (P<0.01, ANOVA).

***Significantly different from controls and shocked models without HDFx (P<0.01, ANOVA).

cytes obtained from these stressed animals against sustained losses of key cytokines (i.e., IL-2, IL-6, IFN-gamma) [8]. We hypothesized that therapy of shocked/traumatized animals with HDFx might protect lymphocytes, allowing them to continue to produce near –normal levels of these important cytokines. The results shown in **Table 6** indicate that, as expected, HDFx treatment of rats subjected to either hemorrhage or intestinal ischemia shock-trauma protects lymphocytes against the profound losses of cytokines/chemokines usually observed in systemic circulatory stresses [11, 21].

Discussion

Overall, we believe that our present experiments demonstrate the potential utility of our recently-discovered protein, HDFx, for the warfighter on the battlefield, for victims of major disasters (e.g., earthquakes, plane-train-automobile crashes, severe blood loss, etc.), in emergency rooms, etc. HDFx appears to exhibit a number of powerful, heretofore, unseen attributes, e.g., increases survival rates after hemorrhage and bowel ischemia shock; elevates arterial blood pressure from very low levels towards normal; restores the microcirculation towards normalcy; prevents formed elements from pooling in the venous side of the microcirculation in shocked animals; stimulates phagocytosis; and prevents loss of lymphocyte functions.

The fact that HDFx appears to restore vasomo-

HDFx for treatment of hemorrhagic and intestinal-ischemic shock

Table 6. Effects of hemorrhage and intestinal ischemia shock-trauma with and without saline or HDFx treatment on cytokine levels in lymphocytes harvested from rats*

Group	IL-2	IL-6	IFN-gamma
Controls-shams	418+/-66	1.9+/-0.3	8.2+/-0.4
Hemorrhage alone	122+/- 24**	0.32+/-0.08**	0.72+/-0.12**
Hemorrhage w/saline	142+/-28**	0.38+/-0.12**	0.66+/-0.10**
Hemorrhage w/HDFx	338+/-56***	1.6+/-0.24***	7.1+/-0.5****
Intest Isch alone	84+/-12**	0.20+/-0.06**	0.42+/-0.08**
Intest Isch w/saline	78+/-14**	0.16+/-0.06**	0.40+/-0.10**
Intest Isch w/HDFx	296+/-38***	1.5+/-0.26***	6.8+/-0.4****

Data are mean values +/- S.E.M. Values are given in U/ml. *See methods for details of animal models. **Significantly different controls-shams (P<0.01, ANOVA). ***Significantly different from controls and respective shocked model w/without saline (P<0.05, ANOVA). ****Significantly different from respective shock model w/without saline (P<0.05, ANOVA).

tion and vascular reactivity towards normal levels suggests, to us, that HDFx may possess unique vasoactive properties, which will have to await further studies. Vasoexcitor protein- drugs which unlike amines, lipids, and peptides, have suitably selective modulating actions on the various types of microvessels [18] as seen, herein, should allow sufficient capillary inflow for adequate tissue-blood exchange. Such unique vasomotor effects could attenuate or avoid the genesis of progressive microcirculatory degeneration, the final common pathway to irreversibility in most forms of clinical shock-trauma. This concept of selective pharmacologic manipulation of the microcirculation is compatible with and mimics the normal intrinsic microvasculature, being a focus of our laboratories for more than 50 years [14, 18, 22-24]. We have demonstrated that a number of synthetic analogues of vasopressin and oxytocin also possess some of the attributes of HDFx in shock-trauma [14, 18, 22-24]. However, none of these peptide analogues can be used as both a therapy and a prophylactic as appears to be the case with HDFx.

It should be pointed out, here, that acute occlusion of the SMA is all too frequently completely refractory to either surgical or supportive therapy, and demonstrates, clinically, mortalities varying between 80-95%. However, in our experiments, use of HDFx yields survival approaching in excess of 90%.

Specific molecular mechanisms which might account for the remarkable therapeutic, benefi-

cial effects observed in the present experiments, or which might account for the selective vasomotor and immunological actions of HDFx are beyond the scope of the present work. However, there is much "classic" evidence that almost any procedure which improves regional blood flow in shock, particularly in the splanchnic area, can increase survival [3, 5, 14, 22-24, 25-27]. In relation to microcirculatory mechanisms predisposing to increased survival in hemorrhagic and SMA shock treated with HDFx, the selective microvascular effects on precapillaries and metarterioles to sustain their vasomotion, the maintenance of relatively normal venular tone, and the absence of significant intravascular hemolysis and pooling of blood are no doubt contributory on a hemodynamic basis.

Modern combat conditions including blast injuries, terrorist attacks, and major disasters (e.g., earthquakes; mine cave-ins; vehicle and airplane crashes; etc.) have always resulted in a high number of casualties, often with major body trauma, blood loss, internal organ damage, severed limbs, etc. which require on-the-spot treatment by paramedics and ancillary health care personnel prior to transport to field stations and properly equipped hospitals and trauma centers. In these situations, time of emergency treatment usually means whether a person will recover or eventually die. During the first three decades of life, trauma is the leading cause of death in The Western World. Our discovery of HDFx [8] presents great promise as both a therapeutic measure and as a pre-

treatment to significantly enhance survival rates and, potentially, shorten the hospital stays of: 1) patients undergoing open-heart surgery, cancer surgeries, and neurosurgical procedures; and 2) Armed Forces personnel subjected to potential battlefield casualties and bioweapons. Further, by virtue of HDFx's macrophage and natural – killer(NK) cell -stimulating activities [8] and abilities to attenuate the virulence of pathogenic microorganisms [8], as well as its unique micro-circulatory attributes [present report], it could be pivotal in increasing the activities of infection-fighting cells (i.e., macrophages, monocytes, polymorphonuclear leukocytes, and lymphocytes) in cancer patients who have had bone marrow transplants, and allow oncologists to decrease chemotherapy dosing ,while increasing patient survival chances. HDFx should prove to be particularly of promise in the critical care arena and as an adjunct to many types of chemotherapies.

Lastly, we would be remiss if we did not point out that the current conflicts in Iraq and Afghanistan (as well as terrorist attacks around the globe) have resulted in complex body and head injuries which present physicians and surgeons often with almost impossible tasks for the wounded and warfighter to survive and preserve a high degree of body and brain integrity. Blast injuries (often caused by IEDs) can cause secondary injuries from flying debris and bomb fragments propelled by blast winds. Tertiary blast injury often results when an individual is thrown by the blast into stationary objects. Quaternary blast injury usually includes all other blast-related injuries and their complications, such as burns, crush injuries, and respiratory problems [28-31]. Since we have demonstrated that HDFx can increase survival of animals exposed to lethal burn injuries [unpublished findings], accelerate wound healing [unpublished findings], and we have shown that HDFx is a conserved molecule (as it is present in numerous species) [8], we believe HDFx has the potential to dramatically enhance survival of severely wounded personnel who have suffered diverse types of blast-burn injuries.

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