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PREKALLIKREIN AND PROTEASE INHIBITOR LEVELS IN PLASMA FROM CONSCIOUS, HEMORRHAGED AND EUVOLEMIC SWINE INFUSED WITH 7.5% NACl/6% DEXTRAN-70

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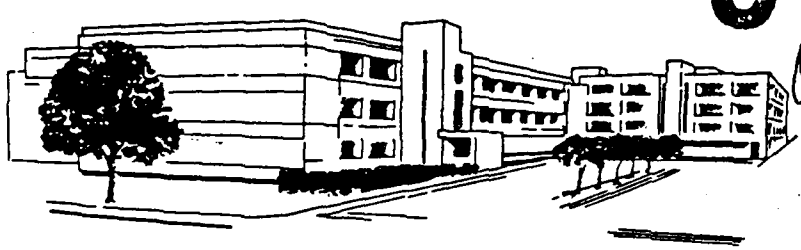

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Prekallikrein and Protease inhibitor levels in plasma from conscious, Hemorrhaged and Euvolemic swine infused with 7.5% NaC1/6% Dextran-70, M.A. Dubick and C.E. Wade

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The kallikrein-kinin system can initiate a number of effects capable of further aggravating the effects of hemorrhage. The present study examines plasma prekallikrein concentrations and the levels and functional activity of circulating protease inhibitors known to bind kallikrein, in 5 euvolemic and 6 hemorrhaged swine infused with 7.5% NaCl/6% Dextran-70 (HSD). Pigs were hemorrhaged progressively over a 45 min period at 27 ml/kg body weight. HSD was then infused over a one (1) minute period into both groups of pigs at a dose of 4 ml/kg. Blood samples were withdrawn before, immediately after the hemorrhage period and at 5, 60 and 120 min following HSD infusion. Neither group of pigs showed significant changes in plasma prekallikrein concentrations in response to either hemorrhage or HSD infusion. In addition, plasma concentrations of α_1-PI and α_2-M and the functional activity of α_1-PI were unaffected by hemorrhage or HSD. These data indicate that Dextran-70, when administered as HSD, is not an activator of the kallikrein-kinin system.

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Abstract

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Prekallikrein and protease inhibitor levels in plasma from conscious, hemorrhaged and euvolemic swine infused with 7.5% Nacl/6% dextran-70 -- Dubick et al.

INTRODUCTION

A 7.5% NaCl/6% Dextran-70 solution (HSD) has recently been introduced for the management and treatment of hemorrhagic shock (1,2). Of the safety issues concerning the use of dextran solutions in humans (3-7), one area of attention focuses on the effects of dextran on hemostasis (4-7). In this regard dextran sulfate, at least in vitro, is a well known and potent activator of kallikrein activity (8) and this activation occurs at lower concentrations of dextran than those observed in plasma following its use for the treatment of hemorrhagic shock (9,10). Kallikrein is a serine protease found in serum and tissues. Under normal circumstances, the enzyme exists in plasma as prekallikrein and any active enzyme is usually bound and inactivated by α_2-macroglobulin (α_2M) (11). Activation of this enzyme system, leading to activation of bradykinin and the onset of a number of physiological responses (12) has been associated with different types of traumatic injury (13-15). Activation of the kallikrein-kinin system results in hypotension, natriuresis, arterial vasodilation, increased renal blood flow and a fall in peripheral resistance (12), effects directly opposed to the clinical objectives of HSD therapy for the treatment of hemorrhage. In addition, trauma and injury have been reported to lower plasma concentrations of α_2-M and other protease inhibitors in both pigs and humans (16,17), and to activate prekallikrein (16). Thus, tramatic injury resulting in hemorrhage subsequently treated with HSD may result in potentiation of the actions of the kallikrein-kinin system.

Currently, little is known of dextran metabolism, when administered as HSD, for the treatment of hemorrhagic shock. Whether activation of the kallikrein cascade can occur with neutral dextrans or following their metabolism has not been addressed. The present study investigates plasma prekallikrein and protease inhibitor concentrations following HSD administration to evolemic and hemorrhaged pigs.

MATERIALS AND METHODS

Animals and Experimental Protocol

Eleven immature (21.6 $\pm$ 0.6 kg) Yorkshire pigs (J.G. Boswell Co., Corcoran, CA) were randomly assigned to either the euvolemic (n=5) or hemorrhage (n=6) group. Animals were individually

housed in a common indoor laboratory holding facility with a 12 h light/dark cycle maintained at constant temperature and humidity, and fed a commercial chow and water, ad libitum. After a 1 to 3 week adaptation period to the laboratory environment, each pig was splenectomized and chronically instrumented with arterial and renal vein catheters and an abdominal aortic sideport catheter to remove blood during hemorrhage (18). A pyelostomy was also performed. Other aspects of the catheterization protocol and conditioning to the Pavlov sling have been detailed previously (19). All surgeries were performed under aseptic conditions, and the pigs were allowed to recover 5 days before the start of each experiment.

Following an 18 h fast, pigs were placed in the Pavlov sling and allowed to rest quietly until stable values for O_2 consumption were obtained. Two baseline blood and urine samples (time 0) were collected. Immediately thereafter, animals were hemorrhaged progressively at 27 ml/kg body weight over a 45 min period. The 6% Dextran®70/7.5% NaCl (HSD) solution (Pharmacia AB, Uppsala, Sweden) was then administered intravenously into the pulmonary artery at 4 ml/kg body weight over a 1 min period. Blood specimens were collected at the end of the hemorrhage or mock-hemorrhage period in euvolemic pigs, and at 15, 60, and 120 min following HSD infusion. This experimental protocol was designed to simulate conditions in which an injured person is brought to a hospital 2 h after injury for full resuscitation and replacement of lost blood.

Biochemical Assays

Plasma kallikrein activity was determined using H-D-prolyl-L-phenylalanyl-L-arginine-p-nitroanilide (S-2302) as substrate. Plasma samples were incubated with substrate solutions with or without prekallikrein activator at 37° C for 5 min and the absorbance read against a blank at 405 nm (20). Results were compared to a standard curve of kallikrein activity and data expressed as units/ml plasma.

Plasma concentrations of α_2- macroglobulin (α_2-M) and α_1-protease inhibitor (α_1-PI) were assessed by rocket immunoelectrophoresis according to the method of Laurell and McKay (21). In addition, plasma protase inhibitory capacity of α_1-PI was determined as described by Dubick, et al. (22) using purified porcine pancreatic elastase and bovine trypsin as standards.

Statistical Considerations

Data were analyzed by 2-way ANOVA and analysis of covariance to evaluate the effects of HSD and time following infusion on

plasma kallikrein and protease inhibitor concentration and activities. A p value < 0.05 obtained by the Greenhouse-Geisser probability method was considered statistically significant.

RESULTS

The data indicate that neither hemorrhage nor HSD infusion significantly affected plasma prekallikrein concentrations in pigs (Table 1). In addition, essentially undetectable activated kallikrein activity was observed in both groups of pigs at all time points (data not shown).

Determination of the plasma protease inhibitory capacity of α_1-PI, assayed as both trypsin and elastase inhibitory capacity, revealed no significant effects induced by HSD or hemorrhage (Table 1). Consequently, the ratio of elastase-to-trypsin inhibitory capacity did not change, thereby verifying the functional integrity of α_1-PI. Determination of immunoreactive of α_1-PI concentrations in plasma were found to be within normal limits and were not affected by hemorrhage nor HSD infusion (Table 1). In contrast, α_2-M concentrations in plasma tended to be 10-20% lower in response to hemorrhage, but quickly returned to prehemorrhage levels following HSD infusion (Table 1).

DISCUSSION

Plasma prekallikrein is a key enzyme in activating bradykinin and regulating normal circulatory homeostasis (12). It has also been shown to regulate Factor XII-dependent activation of prorenin and inactivates the first component of complement (23). Endogenous substances such as Hageman Factor (Factor XII), in the intrinsic clotting cascade system, and enzymes such as trypsin and plasmin can activate prekallikrein to kallikrein (8,12,13). In addition, a number of exogenous substances can activate prekallikrein. Kaolin, ellagic acid and dextran-sulfate are among the more potent prekallikrein activators (8,24). Kluft (8) reported maximum activation with in vitro dextran-sulfate concentrations of 25 ug/ml, i.e., concentrations over 100-fold lower than peak dextran concentrations reported in plasma following HSD-infusion at doses of 4 ml/kg body weight (10). In the present study, the plasma concentrations of prekallikrein observed are in close agreement with those reported by others for normal individuals (25). In addition, the amidolytic assay employed has shown good correlation with previous assays and has been used sucessfully to monitor plasma prekallikrein activity in critically ill or multiple traumatized patients (14, 15). Since prekallikrein concentrations were not affected by HSD infusion in the present

study, and no significant activated kallikrein activity was detected, it appears that as a component of HSD, Dextran-70® is not an activator of the kallikrein-kinin system in euvolemic or hypovolemic swine. Althought prekallikrein can be activated following trauma (15), the mechanisms responsible are not well understood. Potent activators of this system have been employed in vitro, and it is unknown to what extent these activators may be involved in the pathophysiology of prekallikrein activation. Nevertheless, since low concentrations of sulfates exist as anions in plasma, even a small potential for formation of dextran sulfate during the metabolism of HSD, could have dire consequence.

In plasma, kallikrein activity is inhibited by circulating protease inhibitors, particularly α_2-M, C1-esterase inhibitor and α_1-PI (8,23,24). The effectiveness of these inhibitors is illustrated by higher kallikrein activity in plasma incubated at 0°C than at 37°C (8,24). Although it has been reported that plasma levels of α_1-PI and α_2-M may be reduced following injury and trauma (16,17), the results from the present study do not indicate any significant effect of hemorrhage or HSD-infusion on α_1-PI or α_2-M concentrations in plasma. As indicated by a lack of change in the EIC-to-TIC ratio, the functional inhibitory capacity of α_1-PI was also unaffected. It is known that the active site region of α_1-PI can be oxidized under a variety of circumstances, resulting in a lower elastase inhibitory capacity and lower EIC-to-TIC ratio (26). For example, α_1-PI can be oxidized by reactive oxygen compounds released by activated polymorphonuclear leukocytes (27). Although higher white blood cells have been observed in experimental animals infused with HSD (28), it does not appear that they have been activated, at least to a physiologically significant extent. Therefore, the data from the present study indicate that HSD administration to hemorrhaged or euvolemic pigs at the therapeutic dose of 4 ml/kg, does not activate prekallikrein or significantly affect plasma protease inhibitor concentrations or their function.

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Table 1

Prekallikrein and Protease Inhibitors in Plasma from Euvolemic (E) and Hemorrhaged (H) Swine Infused with HSD

		Time (min)			
		0	5	60	120
	Group				
Prekallikrein	E	130±43(4)	137±37(4)	148±34(4)	160±34(4)
(U/dl)	H	136±45(5)	125±47(4)	140±40(4)	161±46(4)
α_1 - PI[2]	E	295±19(5)	298±20(5)	297±17(5)	296±12(5)
(mg/dl)	H	285±12(6)	276±15(5)	287±18(5)	310±18(5)
α_2-M[3]	E	344±16(5)	352±11(5)	353±22(5)	320±20(5)
(mg/dl)	H	320±18(6)	336±27(5)	371± 8(5)	369±5(5)
TIC[4]	E	47.8±4.8(4)	51.0±3.4(4)	51.0±5.9(4)	48.8±8.4(3)
(% inhibition/ 20 µl)	H	57.8±4.0(6)	53.0±6.8(3)	59.7±2.7(6)	59.6±3.9(6)
EIC[5]	E	84.4±1.4(4)	86.0±1.1(4)	85.3±1.1(4)	87.0±2.1(3)
(% inhibition/ 20 µl)	H	85.1±1.1(6)	85.7±0.1(6)	86.3±0.5(6)	86.0±0.8(6)
EIC/TIC Ratio	E	1.82±0.18(4)	2.07±0.29(4)	2.06±0.21(4)	2.37±0.34(3)
	H	1.52±0.11(6)	1.68±0.23(3)	1.47±0.07(6)	1.47±0.10(6)

[1]Data expressed as mean ±S.E.(n)
[2]α_1-Protease inhibitor
[3]α_2-macroglobulin
[4]Trypsin inhibitory capacity
[5]Elastase inhibitory capacity

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