Comparative effects of trichothecene mycotoxins on bovine platelet function:
Acetyl T-2 toxin, a more potent inhibitor than T-2 toxin

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Abstract

The effects of the trichothecene mycotoxins (acetyl T-2 toxin, T-2 toxin, HT-2 toxin, palmityl T-2 toxin, diacetoxyisirpenol (DAS), deoxynivalenol (DON), and T-2 tetraol) on bovine platelet function were examined in homologous plasma stimulated with platelet activating factor (PAF). The mycotoxins inhibited platelet function with the following order of potency: acetyl T-2 toxin > palmityl T-2 toxin = DAS > HT-2 toxin = T-2 toxin. While T-2 tetraol was completely ineffective as an inhibitor, DON exhibited minimal inhibitory activity at concentrations above $10 \times 10^{-4} \text{M}$. The stability of the platelet aggregates formed was significantly reduced in all mycotoxin treated platelets compared to that of the untreated PAF controls. It is suggested that the increased sensitivity of PAF stimulated bovine platelets to the more lipophilic mycotoxins may be related to their more efficient partitioning into the platelet membrane compared to the more hydrophilic compounds.

Introduction

While a wide variety of trichothecene mycotoxins have been isolated from foods, the relative potency of these contaminants, and the underlying biochemical mechanism(s) responsible for their toxicity have not been fully elucidated (1 - 5). The results of in vitro studies with various cell types have indicated that T-2 toxin can interact with cell membranes (6 - 11). T-2 toxin-induced changes in the bovine platelet membrane phospholipid bilayer have recently been reported (11). In addition, the inhibition of platelet function by T-2 toxin has been well documented in porcine (12), ovine (13), bovine (14 - 16), guinea pig (17), and human platelets (18). Previous studies have indicated that T-2 toxin and possibly other trichothecenes are unique inhibitors of bovine platelet function (19).
In the present study, the comparative effects of the trichothecene mycotoxins; T-2 toxin, HT-2 toxin, acetyl T-2 toxin, palmityl T-2 toxin, DAS, DON, and T-2 tetraol on bovine platelet function were examined. Platelet activating factor (PAF; 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine), a potent natural lipid chemical mediator (20) was used as the platelet agonist in part because of the sensitivity of bovine platelets to stimulation by this agonist (19, 21). Furthermore, PAF induced aggregation is not dependent on thromboxane production in the stimulated bovine platelet but rather is associated with calcium mobilization (19, 22).

Materials and Methods

Platelet sample preparation

Blood was drawn from healthy mature Holstein cows by puncture of the jugular vein using an 18 gauge needle attached to a plastic syringe containing 0.15 M trisodium citrate. The blood and citrate, in the proportion 9:1, were thoroughly mixed and transferred to siliconized glass centrifuge tubes. Following centrifugation at 220 g for 15 min at 22°C, the upper phase containing the platelet rich plasma (PRP) was removed. To obtain platelet poor plasma (PPP), the lower phase was recentrifuged at 2,510 g for 15 min at 4°C. Platelet counts were adjusted to 2 x 10^5 platelets per μL with homologous PPP using Unopettes (Becton-Dickinson Co, Rutherford, NJ, USA).

Preparation of reagents

The toxins, T-2 toxin (Myco-Lab Co, Chesterfield, Mo, USA), HT-2 toxin, DAS, acetyl T-2 toxin, DON, and T-2 tetraol (Sigma Chemical Co, St Louis, Mo, USA) were dissolved in methanol: 0.85 % saline (1:1, v/v) to give a 20.0 x 10^-4 M final stock solution. The final stock solution for DON and T-2 tetraol was 50.0 and 80.0 x 10^-4 M, respectively. Palmityl T-2 toxin, a generous gift from Dr B Yagen, was dissolved in 100 % methanol to give 20.0 x 10^-4 M. The methanol:saline solvent was used for subsequent dilutions of each toxin.

Platelet activating factor (PAF, Calbiochem, San Diego, Ca, USA) was dissolved initially in 100 % ethanol and further diluted with modified Tyrodes buffer containing 0.1 % bovine serum albumin and 0.11 % CaCl₂ to give a stock solution of 5 x 10^-4 mg/mL. Subsequent dilutions were made with modified Tyrodes buffer giving a final PAF concentration of 3.6 x 10^-8 M in the platelet suspensions.

Evaluation of platelet aggregation

Platelet aggregation was monitored in a Payton Dual Channel aggregometer (Payton Associates Ltd, Scarborough, Ontario, Canada). The 100 % aggregation limit corresponding to 100 % transmittance of light through the plasma was calibrated with PPP and methanol: saline solvent. To establish the 0 % aggregation limit (0 % light transmittance), unstimulated PRP and methanol: saline solvent were used. To assess platelet aggregation, 215 μL aliquots of PRP, warmed to 37°C, were incubated with 10 μL of each toxin or methanol: saline solvent at 37°C for 1 min before the addition of 25 μL PAF. The platelet aggregation response was monitored for 5 min or, until deaggregation was observed.

Maximum percent aggregation was calculated by measuring the greatest difference in light transmission through the plasma before and after the addition of PAF. The time to disaggregation was determined by measuring the time elapsed between the addition of PAF and the start of the dissolution of the platelet aggregates. Homologous plasma obtained from one animal was used to determine the effects of all the toxins and duplicate samples were run on each sample for all toxins.