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Original Article

Characterization of inhibitory activity of camel urine on human platelet function

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الملخص

الأهداف: أظهرت دراسات سابقة أن كلا من بلازما الإبل وأبوالها تحمل خاصية مثبطة لوظائف الصفائح الدموية البشرية. لذا هدفت هذه الدراسة لمعرفة ما إذا كان وجود العنصر المثبط لوظائف الصفائح الدموية البشرية، تتم تصفيته من بلازما الإبل إلى أبوالها أو يتم إفرازه من الكلى، ودراسة ما إذا كانت ظروف الإبل الإنجابية لها أي تأثير على هذا النشاط المثبط.

طرق البحث: شملت الدراسة ٦٧ من إناث الإبل (حواملا وأبكارا ومرضعات) واستُعمل اختبار وظائف الصفائح الدموية بقياس أثر التكدس على نقل الضوء، وباستعمال جهاز تحليل وظائف الصفائح الدموية.

النتائج: أظهرت النتائج انخفاضا كبيرا في استجابة تراكم الصفائح الدموية للإبل للمواد المحفزة بالمقارنة بالصفائح الدموية البشرية. وعلاوة على ذلك أحدثت إضافة أبوال الإبل تثبيطا كبيرا لاستجابة تراكم الصفائح الدموية البشرية للتحفيز. ولقد وُجد عند بعض الإبل خاصية تثبيط تراكم الصفائح الدموية البشرية عند إضافة البلازما والبول، بينما في البعض الآخر وُجد لديهم خاصية تثبيط تراكم الصفائح الدموية البشرية إما في البلازما أو البول. وُجد أن أبوال الإبل التي لديها تثبيطا كبيرا للصفائح الدموية، تحدث تثبيطا كبيرا لصفائح الدم البشرية أيضا. وقد تأكد هذا التثبيط من خلال جهاز تحليل وظائف الصفائح الدموية. هذا وقد أظهرت الإبل المرضعة نشاطا مثبطا أقوى لأبوالها مقارنة بالفئات الأخرى من الإبل.

الاستنتاجات: تشير النتائج إلى أن عامل تثبيط الصفائح الدموية البشرية الذي ثبت وجوده في أبوال الإبل يصل إلى الأبوال من خلال تصفيته من بلازما الدم. لكن لا يمكن استبعاد الكلى كمصدر منتج لها. كما يبدو أن الإبل المرضعة تمتلك نشاطا مثبطا أقوى لأبوالها مقارنة بغيرها من الإبل. هذه النتائج تدعم حقيقة المقولات المعروفة للخصائص العلاجية المفيدة لأبوال الإبل التي قد تنشأ جزئيا في الكلى، كما يمكن أن تتم تصفيتها من البلازما.

الكلمات المفتاحية: أبوال الإبل؛ العمل المثبط للصفائح الدموية؛ الإبل المرضعة؛ بلازما الإبل؛ ثنائي فسفات الأدينوزين

Abstract

Objectives: Previous studies have shown that both camel plasma and urine display inhibitory action on human platelet function. This study aimed to determine whether the platelet-inhibiting activity in camel plasma is filtered into urine or if this activity is initiated by the kidney and to evaluate the impact of the camel's reproductive status on this inhibitory activity.

Methods: The study included 67 non-pregnant, pregnant and lactating female camels. Platelet function was tested in the camels by light transmission aggregometry and platelet function analyser (PFA-100®) studies.

Results: In comparison to the results in human beings, camel platelet aggregation responses to both adenosine diphosphate (ADP) and arachidonic acid (AA) agonists showed a significant reduction. Furthermore, human platelet aggregation responses were significantly inhibited by camel urine. Some camels displayed inhibitory activity in both plasma and urine, while others displayed this activity in either blood or urine. In camel categories with markedly inhibited platelet aggregation responses, urine caused marked inhibition of human platelets. In camels with antiplatelet urine effects, camel platelet inhibition was also confirmed by prolongation of platelet function analyser 100 (PFA-100®) closure times in all categories.

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Lactating camels showed stronger urine inhibitory activity compared to other groups.

Conclusions: These findings suggest that an inhibitory factor could be filtered from camel plasma; however, a renal source cannot be excluded. Lactating camels seem to possess more potent urine inhibitory activity compared to other camel groups. These findings support the fact that the claimed beneficial therapeutic properties of camel urine originate in part from the kidney and could be filtered from plasma.

Keywords: Adenosine diphosphate; Camel plasma; Camel urine; Lactating camel; Platelet inhibitory action

Introduction

Compared to human platelets, camel platelets display markedly inhibited platelet aggregation responses to common aggregation agents, and their platelet function analyser (PFA-100®) closure times[1,2] are prolonged. Camel plasma and camel urine have each been shown to have an inhibitory effect on human platelets, and lactating camel urine is the most potent inhibitor of human platelet aggregation.[3] Interest in these findings stems from the fact that these inhibitory actions resemble those of the widely used (dual) anti-platelet drugs clopidogrel and aspirin. Neither of the two studies of[1,3] platelet function measured blood and urine simultaneously from the same camel, and thus, this study aims to determine whether the platelet inhibitory activity displayed in camel urine originates from plasma and whether the reproductive state of the camel has any influence on this inhibitory activity, bearing in mind the belief in our local community that the urine of virgin camels has more potent therapeutic efficacy than that of either pregnant or lactating camels.

Materials and Methods

Sixty-seven female camels were studied (virgins = 21, pregnant = 22, lactating = 24). The camels' ages ranged from 2 to 10 years. The animals were sampled from a private farm near Riyadh. The camels were healthy, kept under intensive management and veterinary care and had free access to food and water. The healthy human samples were collected from blood donors.

Blood collection and processing

Camel blood collection for platelet aggregation and platelet function analyser (PFA-100®) studies

Venous camel blood samples (50–60 ml) were collected from a large vein in the neck directly into vacutainer tubes containing sodium citrate (0.129 M) to give a blood/citrate ratio of 9:1 (Terumu Co. Japan). Blood samples were transported without delay (within 2 h of collection) to the Coagulation Research Laboratory, College of Medicine. This study was approved by the Institutional Review Board (IRB) of The College of Medicine, King Saud University.

Camel urine collection

The collection of urine from the same camels from which blood samples were collected was undertaken during the feeding time by experienced camel attendants, as detailed recently.[3]

Laboratory procedures

Preparation of platelet-rich and platelet-poor plasma for aggregation studies

Platelet-rich plasma (PRP) was prepared by centrifugation of citrated whole blood at 1000 rpm for 5 min at room temperature. The PRP was removed, and the remaining sample was centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP), which was used as a standard for the aggregation studies. The platelet count in the PRP was adjusted to fall in the range of $200-300 \times 10^9$/L.

Platelet aggregation studies were undertaken using a Platelet Aggregation Profile® Model PAP-4 (BioData, 155 Gibraltar Road Horsham, PA 19044-0347, Corp., USA) using 20 μmol/l adenosine diphosphate (ADP, BioData, USA) and 5 mg/ml arachidonic acid (AA, BioData, USA), as detailed previously.[3,4]

Camel platelet aggregometry

The aggregometer's macro-cuvette (8.75 × 50 mm) was used in the entire study. Using plastic tips, 0.5 ml of PRP was pipetted into the cuvette, followed by 0.05 ml of the aggregating agent, and the recording started. The machine automatically registers the aggregation result as maximum aggregation (%) against the control platelet-poor plasma (PPP) and the slope of the aggregation curve.[4]

Effect of camel urine on human platelets

Using plastic tips, 0.45 ml of human PRP was pipetted into the cuvette, followed by 0.05 ml raw camel urine, and a plastic coated magnetic stirrer stirred the mixture for two min. Then, 0.05 ml of the aggregating agent was added and the recording started.

Camel blood platelet function analyzer 100 (PFA-100®) studies

The platelet function analyser (PFA-100®) was employed according to the instructions provided by the manufacturer (Dade Behring Inc., Miami, FL, USA) using either replaceable disposable collagen-ADP (CADP) or collagen-epinephrine (CEPI) cartridges, as detailed previously.[1] The instrument measures the time from the start of the test until the aperture is completely occluded and registers the