Biosci Rep (2006) 26:1–6
DOI 10.1007/s10540-006-9004-1

ORIGINAL PAPER

Anticoagulant Modulation of Blood Cells and Platelet Reactivity by Garlic Oil in Experimental Diabetes Mellitus

O.C. Ohaeri · G. I. Adoga

Published online: 9 June 2006
© Springer Science+Business Media, Inc. 2006

Abstract Multiple blood cell types and metabolic pathways involved in the modulation of platelet reactivity were investigated in streptozotocin-induced diabetic rats treated with garlic oil. Platelet counts of diabetic rats treated with garlic oil were significantly ($P<0.01$) reduced as compared to diabetic control rats. Garlic oil also increased the leucocyte counts of diabetic rats as compared to diabetic control rats. The significant ($P<0.001$) decreases by garlic oil of plasma concentration factors, V, VII, VIII: C, IX and X in diabetic rats may be interpreted to mean that there was a modulation of factor VII similar to that brought about by thrombin on factors V and VIII: C. This reversal of hypercoagulation through integrated biochemical reaction is suggestive of multicellular modulation of platelet reactivity, erythrocytes and neutrophils and the functional interactions between plasma coagulation factors and platelet cofactors.

Keywords Platelets · Garlic oil · Coagulation and diabetes

Introduction

The use of garlic for treatment of various diseases dates back to thousands of years. Garlic extract has been known to inhibit platelet aggregation, a first step in the genesis of any thrombotic episode (Gerstein and Yusuf 1996). Garlic has also been used to prevent the development of arteriosclerosis, reduce high blood pressure and to improve prothrombin, thrombin and partial thromboplastin times (Makheja and Bailey 1990; Adoga and Ohaeri

O.C. Ohaeri · G. I. Adoga
Department of Biochemistry, Faculty of Medical Sciences, University of JOS, JOS, Nigeria

O.C. Ohaeri (✉)
Department of Chemical Sciences, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia state, Nigeria
E-mail: ohaeric2000@yahoo.co.uk

1991). Chronic hyperglycaemia in diabetes is considered a causative factor in the numerous vascular complications associated with this disease. Suitable/Pertinent animal models are now available to delineate mechanisms whereby hyperglycaemia promotes atherothrombosis. It becomes necessary therefore to challenge the disease and elucidate those vascular causes that have a serious effect on prognosis.

Motivated by the anticoagulant potentials of garlic oil from previous studies (Adoga and Ohaeri 1991; Ohaeri 1999; Ohaeri 2001), the current work was therefore designed to further characterize how atypical platelet function affects blood vascular disease in proximity with other causative factors such as modulations due to blood cells, plasma factors and viscosity alterations in streptozotocin-induced diabetes mellitus in rats. Given the fact that activated factor X is usually bound to the platelet surface in the presence of factor V and that factor VII of the extrinsic coagulation system is also platelet surface activated (Marcus 1990). Nonetheless, the activation of factor X requires assembly of the tenase complex [Ca^{2+}, activated factors VIII, IX and X] on the surface of platelets (Broze 1995), as these interactions culminate in the formation of thrombin which catalyzes fibrinogen transformation to fibrin making platelet count and evaluation of the above coagulation factors relevant in this study.

This will be an important contribution of information that can be obtained by systemic and in-depth studies of garlic and diabetes mellitus.

Materials and methods

Male albino rats, weighing 130–160 g were purchased from the Animal House Unit, University of Jos and housed in metallic cages. The rats were divided into four groups of four rats per cage. Fresh garlic cloves (*Allium sativum*) bought from Jos main market were used to prepare garlic oil according to the method described by Ohaeri (2001), (Ohaeri 1999). Garlic oil supplementation of 50 mg kg^{-1} body weight was given daily intragastrically through stomach tubes to Group 1 (test diabetic) and Group 3 (excipient control) animals for 28 days. All rats were fed ad libitum on normal rat pellets (Pfizer, Kaduna, Nigeria) and had free access to water throughout the experimental period of 28 days. Rats in Group 1 (test diabetic) and Group 2 (diabetic control) were made diabetic by a single peritoneal injection (10 mg/ml) of streptozotocin (a gift from UpJohn Co. Kalamazoo, USA) at 60 mg kg^{-1} body weight dissolved in freshly prepared 0.1 M citrate buffer pH 4.5.

The induction and confirmation of diabetes mellitus was as described previously (Ohaeri 2001). Group 3 (excipient control) animals were intubated with the same 0.1 M citrate buffer pH 4.5 used to dissolve the streptozotocin while Group 4 rats were the normal control rats.

The animals were sacrificed on the 29th day and blood for coagulation studies was collected by cardiac puncture into 3.8% sodium citrate (9:1 v/v). The blood was collected quickly but mixed carefully with the anticoagulant before transferring into a centrifuge tube to avoid haemolysis. From this blood, platelet rich plasma (PRP) was obtained by centrifugation at 150 g for 8 min while platelet poor plasma (PPP) was obtained by centrifugation at 1,200 g for 15 min. All blood for coagulation studies were stored at −20°C until required. Coagulation reagents used were purchased from Sigma Chemical Company USA. The activity of coagulation factors V, VII, VIII: C, IX and X assayed according to the general principles of parallel line bioassays for coagulation factors as described by Brozovic and Mackie (1991). Dilutions were first made in buffered saline in plastic tubes in the ice-bath to obtain 1 in 10, 1 in 20, 1 in 100 and 1 in 200 of the test and standard plasma. About 0.1 ml of each dilution was placed in glass tube. To each of these dilutions was added 0.1 ml of freshly reconstituted substrate

deficient plasma and warmed in a water-bath to 37°C. About 0.1 ml of thromboplastin reagent was added and the content mixed. At exactly 30 s, 0.1 ml 0.25 M $CaCl_2$ was added and the clotting time recorded. Each dilution of the test and standard plasma were repeated. The clotting times of the test and standard plasma were plotted against the concentration of each factor on 2 cycle×2 cycle log graph paper. Statistical analysis was by the Student's *t*-test.

Blood was collected into dipotassium EDTA and used for erythrocytes (RBC), leucocytes (WBC) and platelet (PLT) counts as well as differential leucocyte counts according to the methods of Dacie and Lewis (1991).

Results

Haematopoietic cell counts are as shown in Fig. 1. No variations in reticulocyte estimations were observed in both diabetic and normal rats treated with or without garlic oil supplementation. The treatment of diabetic rats with garlic oil showed noteworthy reduction of

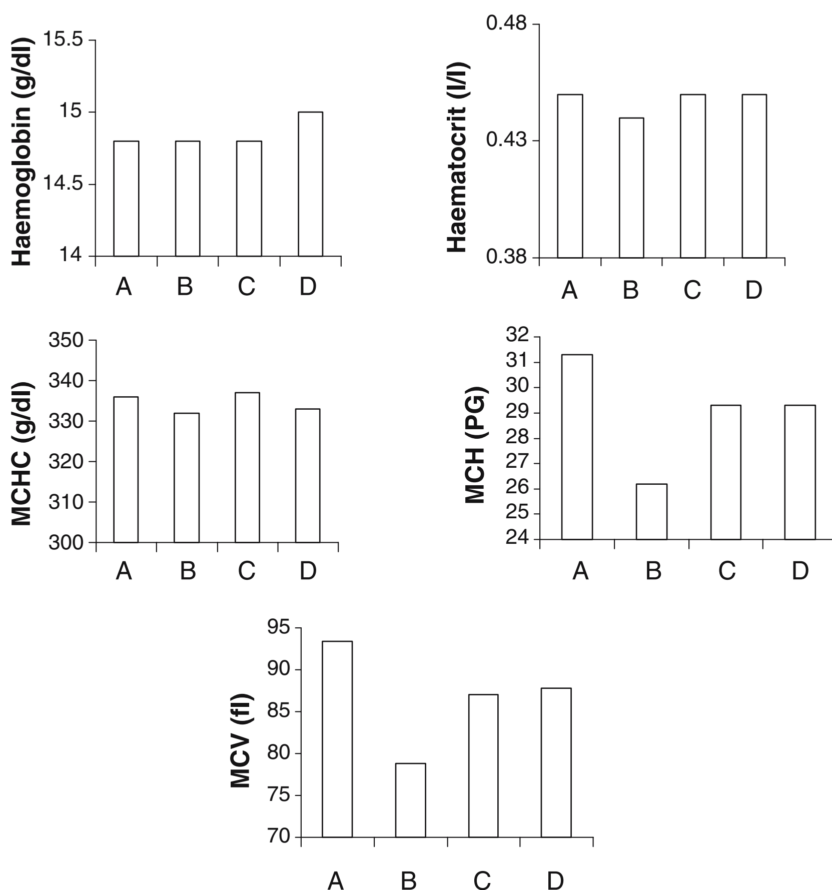

Fig. 1 Effects of garlic oil on haemoglobin, haematocrit and absolute indexes in streptozotocin-induced diabetic rats. (**a**) Diabetic rats on garlic oil; (**b**) diabetic control rats; (**c**) normal rats on garlic oil; (**d**) normal control rats

erythrocyte (RBC), leucocytes (WBC) and platelet (PLT) counts when compared with diabetic control rats ($P<0.01$).

The effect of garlic oil on blood coagulation factors V, VII, VIII: C, IX, X are summarized in Fig. 2. There were significant increases ($P<0.001$) in clotting times of factors V, VII, VIII: C, IX, X levels of diabetic rats treated with garlic oil in contrast to diabetic control rats (Table 1).

Discussion

The progress of vascular disease in diabetes mellitus could have important implications that may require operative surgery, amputation, blood transfusion or haemodialysis. Several cell types in the microvasculature can modulate the growth of platelet thrombi. Platelet function affects blood vascular disease and an increase in platelet number above normal serves as a marker of vascular diseases such as microangiopathy and macroangiopathy (Kwaan 1992). The observed increase in platelet count of diabetic rats in this study is reminiscent of hypercoagulation. Although atypical platelet function affects blood vascular disease, it is possible

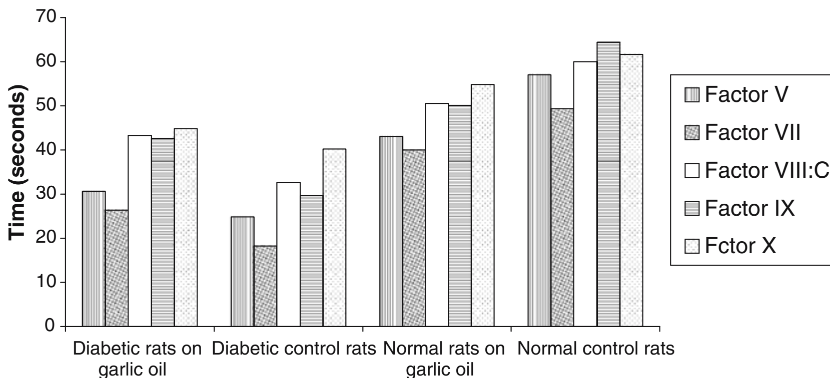

Fig. 2 Effect of Garlic oil on activity of some coagulation factors in streptozotocin-induced diabetic rats

Table 1 Effects of garlic oil on haematopoietic cells in streptozotocin diabetic rats (Values are mean±SEM for four determinations)

	Diabetic rats on garlic oil	Diabetic control rats	Normal rats on garlic oil	Normal control rats
Reticulocyte count ($\times 10^9$/l)	33.00±6.30	35.00±6.50	35.00±6.50	33.00±11.0
Erythrocyte count ($\times 10^{12}$/l)	5.10±0.20^b	4.80±0.01	5.10±0.18	5.20±0.23^c
Leucocyte count ($\times 10^9$/l)	10.20±0.31^b	9.03±0.20	9.40±0.18	9.60±0.30
Platelet count ($\times 10^9$/l)	345.0±26.3^a	602.5±44.8	211.3±20.0^a	236.0±24.6^{a,d}
Neutrophil count ($\times 10^9$/l)	2.95±0.10^b	2.18±0.28	3.52±0.09^f	2.73±0.22
Lymphocytes ($\times 10^9$/l)	7.89±0.32^c	6.39±0.28	5.78±0.18^g	6.83±0.14^e
Eosinophils ($\times 10^9$/l)	0.11±0.06	0.00	0.28±0.22^g	0.01±0.03^d
Monocytes ($\times 10^9$/l)	0.05±0.05	0.00	0.23±0.23^g	0.01±0.03

As compared with diabetic control rats $^aP<0.001$, $^bP<0.01$, $^cP<0.02$

As compared with diabetic rats on garlic oil $^dP<0.05$, $^eP<0.01$

As compared with normal control rats $^fP<0.05$, $^gP<0.01$ (Student's *t*-test)

that other causative factors such as rheological disturbances due to blood cells, plasma factors and viscosity alterations are involved. Platelet thrombus gradually becomes mixed with erythrocytes and neutrophils. Functional interactions between platelets and other cells have been demonstrated in-vitro and there is a strong evidence for occurrence in-vivo (Marcus 1990). In a related study it was discovered that in-vivo circulating macroparticles became procoagulants' due to exposure of phosphatidylserine and tissue factor, which were the initiators of in-vivo coagulation (Broze 1995). These small vesicles called microparticles are released from blood and endothelial cells during activation and apoptosis (Nemerson 1992). Since these interactions enhance or inhibit platelet reactivity, there could be circumstances whereby transcellular metabolism occurs and one cell is transformed by another or there is formation of a metabolite not synthesized by either cell alone (Kannel et al. 1990; Rapaport and Rao 1995). For example releasates produced by the mixtures of erythrocytes and platelets contain higher concentration of secreted ADP that induces stronger platelet aggregation than platelets alone (Miller et al. 1994).

The other observation of this study is the decrease of both intrinsic and extrinsic coagulation factors through garlic oil supplementation. Again this is suggestive of lower numbers of platelet possibly through diminution of cells as well as the low affinity of platelets for fibrinogen since fibrinogen receptors are crucial for the binding of fibrinogen to platelets (Muntean et al. 1985). Fibrinogen receptor sites on the platelet membrane glycoprotein complex undergoes change during platelet activation to assume its receptor function (Osterman and van de Loo 1986). In addition the proximity of platelets and neutrophils allow for modulation of each other's activity during hemostasis, thrombosis and inflammatory response. Since platelet reactivity was inhibited by garlic oil this may well be interpreted as a direct effect mediated by functional fibrinogen receptors that do not require metabolically active platelets (Laasko and Lehto 1997). Marcus and Safier (1993) were pragmatic about the binding of fibrinogen and other macromolecules that constitute one of the final steps in the irreversible aggregation of platelets. If this were so, it could as well mean that the garlic oil exerts its anticoagulant effect by acting on the glycoprotein molecule of fibrinogen which functions as a platelet cofactor. ATIII is crucial in neutralizing the coagulation enzymes as it inactivates all serine proteases of the cascade by forming high molecular weight complexes (Brozovic and Mackie 1991; Morrissey et al. 1993). In an interrelated study we reported that garlic oil treatment of diabetic rats enhanced the reduction of hypercoagulation (Adoga and Ohaeri 1991).

The contention that the anticoagulant action by garlic oil in diabetic rats was mediated within cellular proximity or direct contact emanates from the fact that antithrombin III inactivates thrombin and thus inhibits coagulation. In addition, a normal balance between ATIII and thrombin creates hemostasis while on the contrary ATIII deficiency increases coagulation (Ceriello et al. 1988; Adoga and Ohaeri 1991; Brozovic and Mackie 1991; Rapaport and Rao 1995). Given that the effect of the oil was more pronounced on the partial thromboplastin time (Adoga and Ohaeri 1991), it was conceivably stated that the effect of the oil was more evident in the intrinsic pathway which involves factors IX, X and XI. However, assays of factors V, VII, VIII: C, IX and X (both intrinsic and extrinsic pathways) show decreased concentrations of these factors in garlic oil treated diabetic rats. Garlic oil was also found to decrease intact erythrocytes without any evidence of anemia while increasing the neutrophils within acceptable limits. The occurrence of this incident in garlic oil treated diabetic rats coupled to the decreases in platelets and erythrocyte counts suggests that garlic oil interface occurred within cell proximity. Neutrophil reticence of platelet reactivity is enhanced when adhesion was blocked (Rapaport and Rao 1995). Interestingly, modified factor VII is more active when

complexed with thromboplastin and Ca^{2+} than its unmodified form (Ceriello et al. 1988, 1995). The lowering of plasma factors, V, VII, VIII: C, IX and X in diabetic rats by garlic oil may be accounted by a modification of factor VII similar to that brought about by thrombin on factors V and VIII: C.

This study concludes by suggesting that the anticoagulant effect of garlic oil on diabetic rats was based on multicellular modulation of platelet reactivity in proximity with erythrocytes, neutrophils and the biochemical and functional interactions between plasma coagulation factors and platelet cofactors.

Acknowledgment The author acknowledges the financial contribution of the Federal Ministry of Health and Social Welfare Nigeria to O.C. O haeri. The data in this paper formed part of a PhD thesis submitted to the University of Jos, Nigeria. We are grateful to Professor Z.S.C. Okoye for useful comments and advice.

References

Adoga GI, Ohaeri OC (1991) Effects of garlic oil on prothrombin, thrombin and partial thromboplastin times in streptozotocin-induced diabetic rats. Med Sci Res 19:407–408

Broze GJ Jr (1995) Tissue factor pathway inhibitor and the current concept of coagulation. Blood Coagul Fibrinolysis 6:S7–S12

Brozovic M, Mackie I (1991) Investigation of acute haemostatic failure. In: Dacie JV, Lewis SM (eds) Practical haematology 7th edn. Churchill Livingstone, London, pp 281–282

Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Torella R (1988) Blood glucose may condition FVII levels in diabetic and normal subjects. Diabetilogia 31:889–891

Ceriello A,Giacomello R, Colatutto A, Taboga C, Gonano F (1995) Hyperglycaemia induced thrombin formation in diabetes: the possible role of oxidative stress. Diabetes 44:924–928

Dacie JV and Lewis SM (1991) Basic haematological techniques. Diabetes 44:924–928

Gerstein HC, Yusuf S (1996) Dysglycaemia and risk of cardiovascular disease. Lancet 347:949–950

Kannel WB, D'Agostino RB, Wilson PW, Belanger AJ, Gagnon DR (1990) Diabetes, Fibrinogen, and risk of cardiovascular disease: the Framingham experince. Am Heart J 120:672–676

Kwaan HC (1992) Changes in blood coagulation, platelet function, and plasminogen-plasmin system in diabetes. Diabetes 41(Suppl. 2):32–35

Laasko M, Lehto S (1997) Epidemiology of macrovascular disease in diabetes. Diabetes Rev 5:294–315

Makheja AN, Bailey JM (1990) Antiplatelet constituent of garlic and onion. Agents Act 29:360–363

Marcus AJ (1990) Thrombosis and inflammation as multicellular processes. Pathophysiolological significance of transcellular metabolism. Blood 76:1903–1907

Marcus AJ, Safier LB (1993) Thromboregulation: multicellular modulation of platelet reactivity in haemostasis and thrombosis. Fed Am Soc Exp Biol J 7(6):516–522

Miller GJ, Stirling Y, Esnouf MP, Heinrich J, van de Loo J, Kienast J, Wu KK, Morrissey JH, Meade TW, Martin JC (1994) FVII-deficient substrate plasmas depleted of protein C raise the sensitivity of the FVII bioassay to activated FVII: an international study. Thromb Haemost 71:38–48

Morrissey JH, Macik BG, Neuenschwander PF, Comp PC (1993) Quantitation of activated FVII levels in plasma using a tissue factor mutant selectivity deficient in promoting FVII activation. Blood 81:743–744

Muntean WE, Borkenstein MH, Haas J (1985) Elevation of FVIII over FVIII coagulant antigen in diabetic children without vascular disease: a sign of activation of the FVIII coagulant moiety during poor diabetes control. Diabetes 34:140–144

Nemerson Y (1992) Tissue factor pathway of blood coagulation. Semin Haematol 2:170–176

Ohaeri OC (1999) Renal function and calcium metabolism in streptozotocin-diabetic rats treated with garlic oil. Med Sci Res 27:443–444

Ohaeri OC (2001) Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Biosci Rep 21(1):19–24

Osterman H, van de Loo J (1986) Factors of the hemostatic system in diabetic patients. Haemostasis 16:386–416

Rapaport SI, Rao LVM (1995) The tissue factor pathway: how it has become a ''prima ballerina''. Thromb Haemost 74:7–17