European Journal of Pharmacology 678 (2012) 26–31

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Treatment with dipyridamole improves cardiac function and prevent injury in a rat model of hemorrhage

Mona M. Soliman [a,*], Maha M. Arafah [b]

[a] Department of Physiology, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia
[b] Department of Pathology, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia

ARTICLE INFO

Article history:
Received 6 June 2011
Received in revised form 12 December 2011
Accepted 18 December 2011
Available online 29 December 2011

Keywords:
Hemorrhage
Heart
Inflammation

ABSTRACT

Hemorrhagic shock is a leading cause of death despite the improvement in emergency services. One reason is that the resuscitation policies are designed to reestablish tissue perfusion, but not to prevent the inflammatory response to shock that cause myocardial dysfunction and injury. Dipyridamole is a platelet inhibitor that promotes anti-inflammatory effects. The present study investigated the therapeutic value of treatment with dipyridamole before resuscitation from hemorrhagic shock on myocardial injury and protection. Male Sprague–Dawley rats were assigned to 3 experimental groups (n = 6 per group): 1) hemorrhage, 2) hemorrhage treated with dipyridamole, and 3) sham hemorrhage. Rats were hemorrhaged over 60 min to reach a mean arterial blood pressure of 40 mm Hg. After 60 min hemorrhagic shock, rats were treated or not by injection of 1 mL of (20 μg/L) dipyridamole intra-arterially. Resuscitation was made *in vivo* by reinfusion of the shed blood to restore norm tension for 30 min. Arterial blood samples were collected for measurements of TNF-α. Left ventricular generated pressure and +dP/dtmax was significantly higher in dipyridamole treated rats compared to the untreated group. Myocardial biopsy samples were taken for light and electron microscopy. Dipyridamole decreased the number of inflammatory cells and mitochondrial swollen. Dipyridamole also decreased the plasma levels of TNF-α. Our results demonstrate that treatment with dipyridamole before *in vivo* resuscitation of hemorrhagic shock protect the myocardium against post-resuscitation myocardial dysfunction by decreasing the inflammatory response to shock.

1. Introduction

Despite the recent advances in resuscitation and emergency services, hemorrhagic shock remains one of the leading causes of death (Heckbert et al., 1998; Keel and Trentz, 2005; Tripodi and Mannucci, 2007). Clinical and experimental studies have shown that hemorrhagic shock and resuscitation will cause cardiac dysfunction (Keel and Trentz, 2005; Yang et al., 2004). Recent studies have shown that inflammatory response to hemorrhagic shock and resuscitation may cause myocardial damage and cardiac dysfunction (Frangogiannis et al., 2002; Martin et al., 1997). The release of cytokines have been shown to an immune response to hemorrhagic shock and injury (Hierholzer and Billiar, 2001). Elevated tumor necrosis factor TNF-α levels have been shown following hemorrhagic shock (Ayala et al., 1991; Beiser et al., 2010). TNF is one of the most characteristic inflammatory and cardio-depressant factors contributing to cardiovascular shock in hemorrhage and trauma (Tracey and Cerami, 1993b; 1994; Ulloa and Tracey, 2005).

Dipyridamole is a platelet inhibitor drug that has been shown to exert anti-inflammatory effects (Chello et al., 1999; Weyrich et al., 2009; Zhao et al., 2006). Dipyridamole increase intracellular levels of adenosine that inhibit neutrophil activation and reduce the expression of adhesion molecules on neutrophils and polymorphonuclear cells (Wollner et al., 1993). Dipyridamole treatment before elective coronary bypass surgery significantly reduced postsurgical neutrophil superoxide anion generation and the extent of neutrophil adhesion to endothelium after surgery (Chello et al., 1999). Several studies showed the effects of dipyridamole on inflammation by assessing the impact on circulating levels of inflammatory markers (Libby and Theroux, 2005; Weyrich et al., 2005; Zhao et al., 2006). Several studies have shown that dipyridamole decreased the levels of TNF-α (Chakrabarti et al., 2007; Poturoglu et al., 2009).

Nucleoside transport inhibitors, including dipyridamole (Amrani et al., 1992; Auchampach and Gross, 1993), reduce ischemia-reperfusion injury when infused immediately before experimental myocardial infarction. In recent studies, elevation of serum adenosine by administration of dipyridamole improved cardiac function in patients with heart failure (Bedetti et al., 2005; Strauer et al., 1996). However, the cardioprotective effects of nucleoside transport inhibitors following resuscitation of hemorrhagic shock is unknown.

* Corresponding author at: Department of Physiology, College of Medicine, King Saud University, P.O. Box 29825 (29), Riyadh 11461, Saudi Arabia. Tel.: +966 505468581; fax: +966 14672567.
E-mail address: monaslmn@yahoo.com (M.M. Soliman).

0014-2999/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.12.038

The purpose of the present study was to investigate the ability of dipyridamole to protect the heart against post resuscitation myocardial dysfunction and injury by decreasing the inflammatory response in a rat model of hemorrhagic shock and resuscitation.

2. Materials and methods

This study was approved by the *Deanship of Scientific Research*, *King Saud University* and the ethical committee.

2.1. Experimental protocol

Male Sprague–Dawley rats (350–400 g) were assigned to 3 groups: 1) hemorrhage, 2) hemorrhage treated with dipyridamole, and 3) sham hemorrhage (n = 6 per group). Rats were hemorrhaged over 60 min to reach a mean arterial blood pressure of 40 mm Hg. After 60 min hemorrhagic shock, rats were treated or not by injection of 1 mL of (20 μg/L) dipyridamole intra-arterially. Resuscitation was made *in vivo* by reinfusion of the shed blood to restore norm tension for 30 min. In the sham hemorrhage group, rats underwent the same surgical procedure and experimental protocol, except rats were not hemorrhaged (Fig. 1).

2.2. Rat hemorrhagic shock model

Rats were injected intra-peritonealy (i.p.) with heparin sodium 2000 I.U. 15 min prior to anesthesia. The rats were then anesthetized using urethane 125 mg/kg intra-peritonealy. The left carotid artery was cannulated using polyethylene tubing size 60, and connected to an in-line pressure transducer for continuous blood pressure monitoring. Animals were allowed to stabilize for a period of 30 min. The animals were assigned randomly in experimental groups: 1) Hemorrhagic shock, 2) Hemorrhage + dipyridamole, and 3) sham hemorrhage (n = 6 per group). Rats were hemorrhaged using a reservoir (a 10 mL syringe), to maintain a mean arterial pressure of approximately 40 mm Hg, which was connected to the arterial (carotid artery) three way stopcocks. Opening the stopcock and aspirating gently and gradually with the syringe induced hemorrhage. Blood was aspirated at a rate of approximately 1 mL/min. Blood was continuously withdrawn or re-infused to the animal to maintain a mean arterial pressure of approximately 30–40 mm Hg. The same surgical procedure was performed as for the sham hemorrhage groups except rats were not hemorrhaged and rats were injected with 1 mL of normal saline (placebo) instead of the 1 mL of treatment received by the dipyridamole treated group. After 60 min hemorrhagic shock, rats were treated or not by injection of 1 mL of (20 μg/L) intra-arterially. Resuscitation was made *in vivo* by reinfusion of the shed blood to restore norm tension for 30 min.

2.3. Blood pressure measurements

Mean arterial blood pressure (MAP) of all rats were monitored throughout the experimental time by pressure transducer connected to the intra-carotid artery cannula and data was collected using the BIOPAC system and the Acknowledge software.

2.4. Measurement of myocardial contractile function in the isolated hearts

Hearts were excised quickly and rapidly placed onto a Langendorff apparatus, and perfused at a flow rate of 10 mL/min with Krebs Hanseleit Bicarbonate Buffer (KHB, in mM: NaCl 118, $CaCl_2$ 1.25, KCl 4.7, $NaHCO_3$ 21, $MgSO_4$ 1.2, glucose, 11, KH_2PO_4 1.2, and EDTA 0.5). Perfusate temperature was maintained at 37 °C and was gassed with a mixture of 95% O_2 and 5% CO_2 at a pH of 7.4 as described previously (Soliman, 2009). Hearts were stimulated electrically at 5 Hz using electrical stimulator (6020 Stimulator from Harvard Apparatus). Left ventricle pressure was measured by the use of a saline-filled cellophane balloon-tipped catheter which was placed into the left ventricle via the mitral valve and inflated to maintain an end diastolic pressure at 5 mm Hg. Left ventricular generated pressure was calculated as the difference between the left ventricular systolic pressure and the end diastolic pressure. + dP/dtmax was calculated by the Acknowledge software as the first derivative of pressure over time. Blood pressure was continuously measured by the left carotid artery cannula . Perfusion pressure was continuously recorded using a pressure transducer. Data were collected and analyzed using the BIOPAC system and the Acknowledge software.

2.5. Pathology

To perform myocardial pathology of the myocardium, rat hearts were harvested and stored in 10% formalin solution (n = 6 in each group). We obtained 2 transverse sections per heart for histopathological examination and sections were stained with HE and trichome. The number of infiltrating cells in myocardium was counted as the sum of the cell counts on three fields at ×200 magnification in the HE staining (Yamashiro et al., 1998). The area of the myocardium affected by hemorrhagic shock and resuscitation consisting of inflammatory cells, myocardial necrosis, and fibroblast, was determined in the trichome staining. All data were analyzed in a blind fashion.

2.6. Electron microscopy

Myocardial biopsy samples were obtained by cutting 1 mm thick samples with a surgical blade from the left ventricles' walls within 60 s of isolation of hearts. The tissue obtained at biopsy was cut into 1 mm^3 pieces and fixed in 3% buffered glutaraldehyde for transmission electron microscopy. Tissue were fixed with 1% osmium

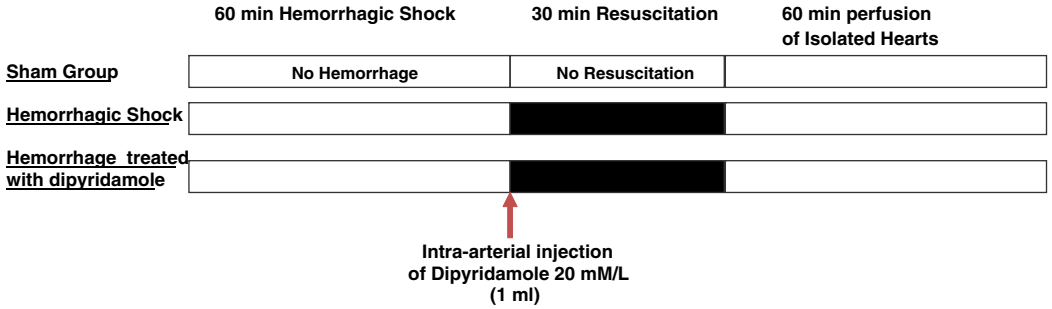

Fig. 1. Experimental protocol. Rats were assigned to 3 groups: 1) hemorrhage, 2) hemorrhage treated with dipyridamole, and 3) sham hemorrhage (n = 6 per group). Rats were hemorrhaged over 60 min to reach a mean arterial blood pressure of 40 mm Hg. After 60 min hemorrhagic shock, rats were treated or not by injection of 1 mL of (20 μg/L) dipyridamole intra-arterially. Resuscitation was made *in vivo* by reinfusion of the shed blood to restore norm tension for 30 min.

tetraoxide in 0.1 M cocodylate buffer, dehydrated, and embedded in epon. Thin sections were stained with uranyl acetate and lead citrate, and photographed with JEOL-JEM 1010 transmission electron electron microscopy.

2.7. Blood samples for enzyme-linked immunosorbent assay and plasma pH

Blood (0.5 mL) was collected from the left carotid artery cannula before hemorrhage, before resuscitation and 30 min after resuscitation and centrifuged at 2500 g for 10 min and plasma was stored at −80 °C until analysis for TNF-α measurement. Serum samples were analyzed by ELISA (R&D systems) according to the manufacturer's instructions.

Blood samples were analyzed for plasma pH.

2.8. Statistical analysis

Data were presented as means ± S.D. Data was analyzed with one way (ANOVA). The values of $P<0.05$ were considered significant. The student *t* test was used to compare mean values between the experimental groups.

3. Results

3.1. Blood pressure

Hemorrhagic shock required withdrawing 15 ± 2.3 mL blood/kg body weight. Treatment with dipyridamole did not cause any significant difference in mean arterial blood pressure at the end of the experimental period (90 min) compared to the sham and the hemorrhagic shock resuscitated group (Fig. 2).

3.2. Dipyridamole prevented myocardial contractile dysfunction after hemorrhagic shock

The measurement of myocardial contractile function in the isolated hearts using the Langendorff system after treatment and resuscitation of the hemorrhagic shocked animals showed improved myocardial function compared to non-treated hemorrhagic shock group (Fig. 3). Exposure to hemorrhagic shock resulted in myocardial contractile dysfunction as compared to controls. Left ventricular generated pressure was significantly lower in hemorrhagic shock (44.4 ± 17.9 mm Hg) compared to shams (87.0 ± 15.8 mm Hg) (Fig. 3A). Left ventricular generated pressure was significantly higher in animals treated with dipyridamole than values measured in hemorrhage non-treated animals (143.0 ± 19.2 mm Hg) ($P<0.05$) (Fig. 3A). Left ventricular +dP/dtmax was significantly lower in hemorrhagic shock (139.6 ± 67.9 mm Hg/s) compared to shams (313.1 ± 53.0 mm Hg/s) (Fig. 3B). +dP/dtmax was significantly higher in animals treated with dipyridamole than values measured in hemorrhage non-treated animals (589.6 ± 110.8 mm Hg) ($P<0.05$) (Fig. 3B).

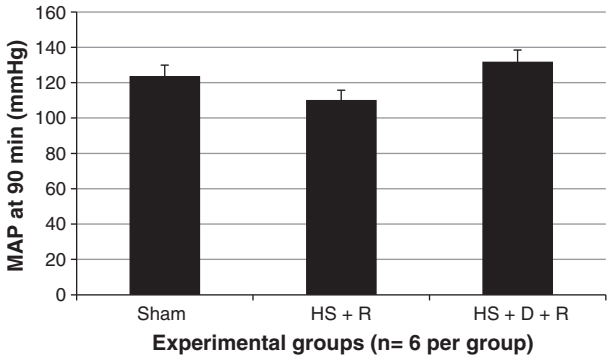

Fig. 2. Recording of mean arterial blood pressure (MAP) after one hour hemorrhages and 30 min of resuscitation in the sham hemorrhage group (Sham), hemorrhage group (HS + R), without dipyridamole and hemorrhage treated with dipyridamole (HS + D + R).

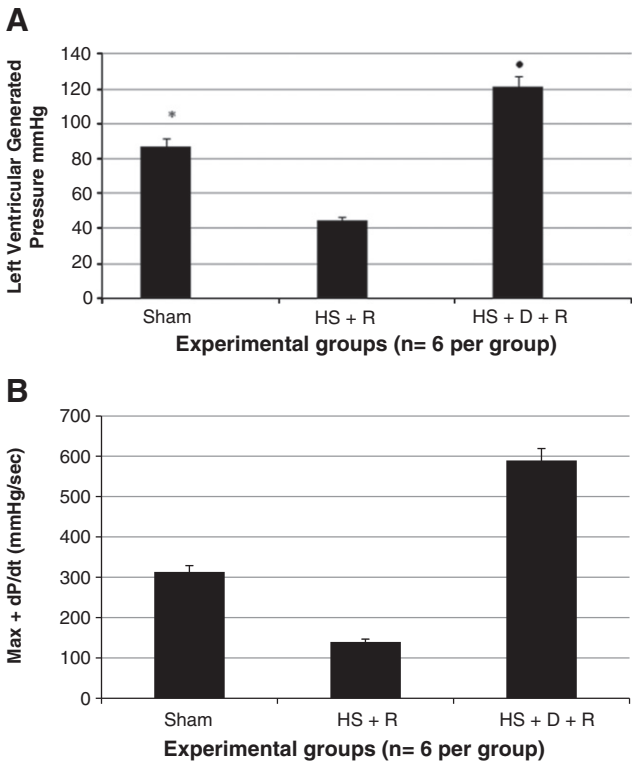

Fig. 3. Effect of dipyridamole treatment before resuscitation of hemorrhagic shock on left ventricular function. (*A*) Left ventricular generated pressure in the sham, hemorrhagic shock (HS + R) and hemorrhagic shock + dipyridamole (HS + D + R) and (*B*) positive change in pressure over time (+dP/dtmax) (n = 6 per group). In *A* and *B*, left ventricular generated pressure and +dP/dtmax was significantly improved in dipyridamole treated rats compared to untreated. All values are means ± S.D. * represents $P<0.05$ versus hemorrhagic shock group, • represents $P<0.05$ versus hemorrhagic shock group (n = 6 per group).

3.3. Myocardial pathology

The number of infiltrating inflammatory cells, including neutrophil and macrophages, were increased in the hemorrhagic non-treated group (Fig. 4A). Dipyridamole treatment before resuscitation decreased the number of infiltrating inflammatory cells (Fig. 4B and C).

3.4. Electron microscopy

Electron microscopy was performed in randomly selected hearts from the *in vivo* hemorrhage treated and untreated groups. The hemorrhage group showed prominent I bands and swollen mitochondria with disrupted cristae and amorphous matrix densities (Fig. 5A). However, no such changes were seen in the dipyridamole treated group (Fig. 5B).

3.5. Blood pH

Blood was collected before hemorrhage, before resuscitation and 30 min after resuscitation. The blood pH after hemorrhage was significantly decreased (pH 7.24 ± 0.04) ($P<0.01$) compared to the sham group (pH 7.42 ± 0.02) . This drop in the pH was not changed by treatment with dipyridamole (pH 7.32 ± 0.04) (Fig. 6).

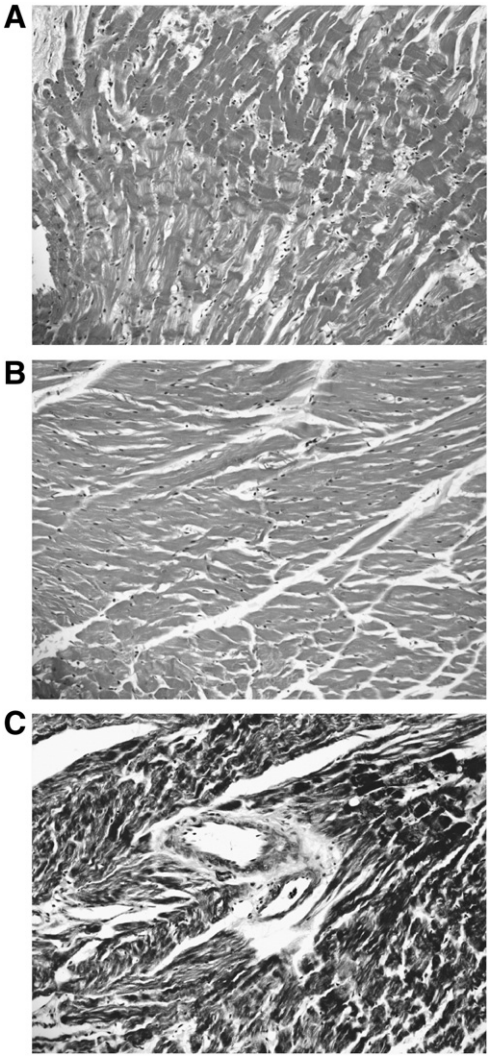

Fig. 4. *A.* HE 200 magnification showing hemorrhagic shock untreated group with inflammatory cells, myocardial necrosis, and fibroblast. *B.* HE 200 magnification. *C.* Trichome staining 200 magnification showing dipyridamole treatment before resuscitation following hemorrhagic shock with attenuated infiltration of inflammatory cells and LV fibrosis.

3.6. Dipyridamole decreased TNF-α levels

TNF-α levels were significantly elevated following hemorrhagic shock compared to sham hemorrhage animals (Fig. 5). Treatment with dipyridamole before resuscitation following hemorrhagic shock significantly lowered the levels of TNF-α (Fig. 7).

4. Discussion

The present study demonstrated that the certain dosage of dipyridamole improved post-resuscitation myocardial dysfunction (Fig. 3). This myocardial protective effect of dipyridamole is due to the anti-inflammatory effects that causes the significant decrease in TNF-α levels (Fig. 7). Dipyridamole also decreased the number of inflammatory cells in the postresuscitated hearts (Fig. 4). The myocardial protective effects of dipyridamole also involved the ultra structures as dipyridamole decreased the mitochondrial swelling when administered before resuscitation (Fig. 5).Therefore, our data suggest that one of the mechanisms by which dipyridamole protect the myocardium against dysfunction and injury when administered before resuscitation following hemorrhagic shock in rats, by decreasing the inflammatory response to shock and resuscitation. The myocardial protective effects of dipyridamole may provide a new spectrum for myocardial protection following hemorrhagic shock.

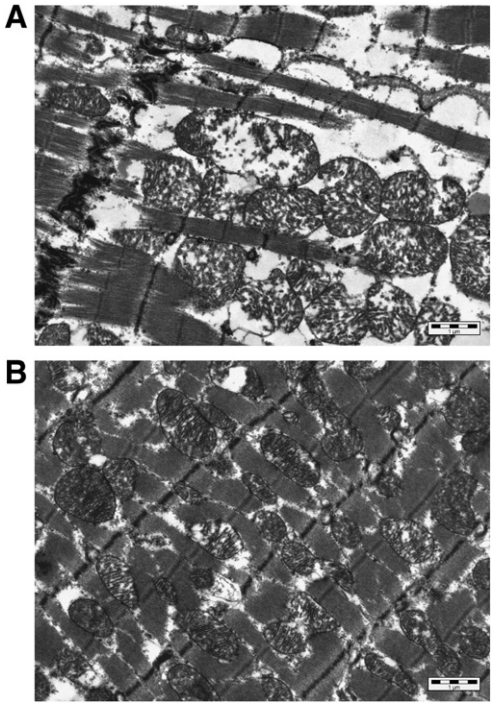

Fig. 5. Electron microscopic analyses. *A.* Hemorrhage untreated hearts showing mitochondrial swelling with disrupted cristae. *B.* Dipyridamole-treated hearts showing mild mitochondrial swelling with clear cristae (original magnification ×30,000).

Dipyridamole has been shown to possess anti-inflammatory properties (Jain and Ridker, 2005). Dipyridamole has been shown to increase extracellular adenosine levels that inhibit neutrophil activation (Wollner et al., 1993). In cocultures of human platelets and monocytes, dipyridamole attenuated nuclear factor $_{\kappa}$B transcription in monocytes (Weyrich et al., 2005). Evidence from previous studies suggested that dipyridamole exhibits anti inflammatory effects on all stages of atherosclerotic plaque progression (Libby and Theroux, 2005). Based on the results of several basic, preclinical and clinical studies, there is evidence that dipyridamole exert anti-inflammatory response. However, the myocardial protective effects of dipyridamole following shock and resuscitation, by decreasing the inflammatory response, has not been investigated. In our study, dipyridamole significantly lowered the levels of TNF-α when administered before resuscitation of hemorrhagic shock (Fig. 7).

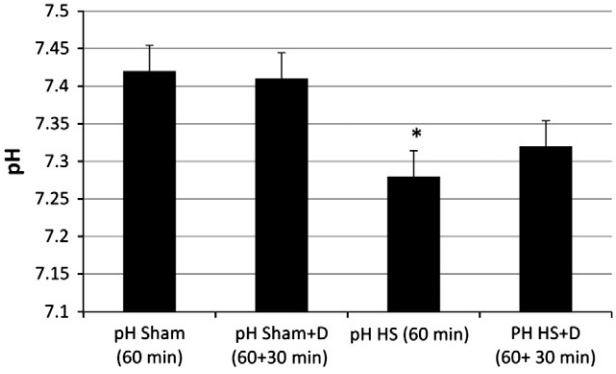

Fig. 6. Arterial pH in two blood samples: 60 min following hemorrhagic shock and 60 min hemorrhagic shock + 30 min resuscitation in the sham hemorrhage and hemorrhage treated with dipyridamole (n = 6 per group). * represents $P<0.05$ versus sham group. (n = 6 per group).

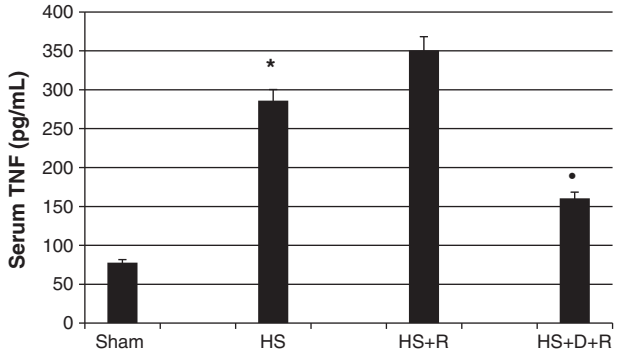

Fig. 7. Hemorrhage increased TNF-α levels. * represents P<0.05 versus sham group. (n=6 per group), and • represents P<0.05 versus hemorrhage group (n=6 per group).

Another mechanism by which dipyridamole improved post resuscitation myocardial function is increasing the blood flow and the vascular volume within the ventricular wall which in turn leads to stretching of myocardial fibers and to an increase in contractility through the Frank–Starling effect (Poli et al., 1996). This could be one of the reasons for that the higher left ventricular generated pressure in rats treated with dipyridamole (143.03 mm Hg) than that of sham group (87.04 mm Hg).

Resuscitation following hemorrhagic shock has been reported to cause a systemic inflammatory response that can be more dangerous than the original hemorrhage (Cai et al., 2009). Several previous studies have shown that TNF-α and other inflammatory markers are elevated in the plasma following trauma or hemorrhagic shock (Heckbert et al., 1998; Keel and Trentz, 2005; Shahani et al., 2000; Soliman, 2011; Yang et al., 2004). TNF is a characteristic cardiodepressant factor contributing to myocardial dysfunction and injury in hemorrhage and trauma (Soliman, 2011; Tracey and Cerami, 1993a; 1994; Ulloa and Tracey, 2005). Moreover, TNF neutralization prevents cardiovascular dysfunction (Cai et al., 2011; Soliman, 2009). Therefore, there is an increasing interest in developing new resuscitation therapeutics that are capable of lowering the TNF-α levels by decreasing the inflammatory response to shock. This is consistent with the data from the present study that showed that lowering the TNF-α levels by administering dipyridamole prevent the myocardial dysfunction and injury following hemorrhagic shock and resuscitation in rats.

Previous studies have shown that dipyridamole therapy protect against ischemia-reperfusion injury in guinea pigs (Figueredo et al., 1999) . Another study showed that intracoronary dipyridamole improves systolic and diastolic ventricular performance in humans during coronary angioplasty (Strauer et al., 1996). However, the protective effects of dipyridamole following hemorrhagic shock and resuscitation is unknown.

Though, the present study is limited by the fact that it examined one certain dosage of dipyridamole that did not cause any further drop in blood pressure (Fig. 2). Figueredo et al. (1999) showed that dipyridamole therapy, 11.4 mg/L (4 $mg.kg^{-1}.day^{-1}$) given in water for 2–6 weeks produced sustained protection against ischemia-reperfusion injury in guinea pigs. Phillis et al. (1998) showed that dipyridamole 1 μM doubled coronary flow rates during hypercapnic acidosis. However, the results of the present study is limited by only one dosage of dipyridamole (20 μg/L). Further studies are needed to examine the dose–response effects. Another limitation of the use of dipyridamole following hemorrhagic shock is the drop in blood pressure and antiplatelet properties.

In conclusion, the present study demonstrated that dipyridamole protect the myocardium against post resuscitation myocardial dysfunction injury following hemorrhagic shock. This may be partially explained by the anti inflammatory effects of dipyridamole that lowered the levels of TNF. Further studies are needed to measure other inflammatory markers (ex. PGE2, IL, COX2, etc.). Thus, treatment with dipyridamole before resuscitation of hemorrhagic shock may be considered in the certain examined dose for prevention of post resuscitation myocardial dysfunction and injury.

Acknowledgments

This work was supported by a grant from the Deanship of Scientific Research, King Saud University. Technical help from Mr. Sabirine is acknowledged.

References

Amrani, M., Shirvani, R., Allen, N., Ledingham, S., Yacoub, M., 1992. Enhancement of low coronary reflow improves postischemic myocardial function. J. Thorac. Cardiovasc. Surg. 104, 1375–1382.
Auchampach, J., Gross, G., 1993. Adenosine A1 receptors, KATP channels, and ischemic preconditioning in dogs. Am. J. Physiol. 264, H1327–H1336.
Ayala, A., Wang, P., Ba, Z.F., Perrin, M.M., Ertel, W., Chaudry, I.H., 1991. Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. Am. J. Physiol. 260, R167–R171.
Bedetti, G., Pizzi, C., Gabrieli, L., Varga, A., Pasanisi, E., Bugiardini, R., Picano, E., 2005. The beneficial effect of insulin, glucose, and dipyridamole on regional left ventricular function early after acute myocardial infarction. Int. J. Cardiol. 102, 255–258.
Beiser, D.G., Wang, H., Li, J., Wang, X., Yordanova, V., Das, A., Mirzapoiazova, T., Garcia, J.G., Stern, S.A., Vanden Hoek, T.L., 2010. Plasma and myocardial visfatin expression changes are associated with therapeutic hypothermia protection during murine hemorrhagic shock/resuscitation. Resuscitation 81, 742–748.
Cai, B., Deitch, E.A., Grande, D., Ulloa, L., 2009. Anti-inflammatory resuscitation improves survival in hemorrhage with trauma. J. Trauma 66, 1632–1639 Discussion 1639–1640..
Cai, B., Dong, W., Sharpe, S., Deitch, E.A., Ulloa, L., 2011. Survival and inflammatory responses in experimental models of hemorrhage. J. Surg. Res. 169 (2), 257–266.
Chakrabarti, S., Blair, P., Wu, C., Freedman, J.E., 2007. Redox state of dipyridamole is a critical determinant for its beneficial antioxidant and antiinflammatory effects. J. Cardiovasc. Pharmacol. 50, 449–457.
Chello, M., Mastroroberto, P., Malta, E., Cirillo, F., Celi, V., 1999. Inhibition by dipyridamole of neutrophil adhesion to vascular endothelium during coronary bypass surgery. Ann. Thorac. Surg. 67, 1277–1282.
Figueredo, V.M., Diamond, I., Zhou, H.Z., Camacho, S.A., 1999. Chronic dipyridamole therapy produces sustained protection against cardiac ischemia-reperfusion injury. Am. J. Physiol. 277, H2091–H2097.
Frangogiannis, N.G., Smith, C.W., Entman, M.L., 2002. The inflammatory response in myocardial infarction. Cardiovasc. Res. 53, 31–47.
Heckbert, S.R., Vedder, N.B., Hoffman, W., Winn, R.K., Hudson, L.D., Jurkovich, G.J., Copass, M.K., Harlan, J.M., Rice, C.L., Maier, R.V., 1998. Outcome after hemorrhagic shock in trauma patients. J. Trauma 45, 545–549.
Hierholzer, C., Billiar, T.R., 2001. Molecular mechanisms in the early phase of hemorrhagic shock. Langenbecks Arch. Surg. 386, 302–308.
Jain, M.K., Ridker, P.M., 2005. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat. Rev. Drug Discov. 4, 977–987.
Keel, M., Trentz, O., 2005. Pathophysiology of polytrauma. Injury 36, 691–709.
Libby, P., Theroux, P., 2005. Pathophysiology of coronary artery disease. Circulation 111, 3481–3488.
Martin, C., Boisson, C., Haccoun, M., Thomachot, L., Mege, J.L., 1997. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. Crit. Care Med. 25, 1813–1819.
Phillis, J.W., Song, D., O'Regan, M.H., 1998. The role of adenosine in rat coronary flow regulation during respiratory and metabolic acidosis. Eur. J. Pharmacol. 356, 199–206.
Poli, A., Previtali, M., Lanzarini, L., Fetiveau, R., Diotallevi, P., Ferrario, M., Mussini, A., Specchia, G., Montemartini, C., 1996. Comparison of dobutamine stress echocardiography with dipyridamole stress echocardiography for detection of viable myocardium after myocardial infarction treated with thrombolysis. Heart 75, 240–246.
Poturoglu, S., Kaymakoglu, S., Gurel Polat, N., Ibrisim, D., Ahishali, E., Akyuz, F., Badur, S., Demir, K., Mungan, Z., 2009. A new agent for tumour necrosis factor-alpha inhibition: in vitro effects of dipyridamole in Crohn's disease. Scand. J. Clin. Lab. Invest. 69, 696–702.
Shahani, R., Marshall, J.G., Rubin, B.B., Li, R.K., Walker, P.M., Lindsay, T.F., 2000. Role of TNF-alpha in myocardial dysfunction after hemorrhagic shock and lower-torso ischemia. Am. J. Physiol. Heart Circ. Physiol. 278, H942–H950.
Soliman, M., 2009. Dimethyl amiloride, a Na+–H+ exchange inhibitor, and its cardioprotective effects in hemorrhagic shock in *in vivo* resuscitated rats. J. Physiol. Sci. 59, 175–180.
Soliman, M.M., 2011. Na(+)–H(+) exchange blockade, using amiloride, decreases the inflammatory response following hemorrhagic shock and resuscitation in rats. Eur. J. Pharmacol. 650 (1), 324–327.
Strauer, B.E., Heidland, U.E., Heintzen, M.P., Schwartzkopff, B., 1996. Pharmacologic myocardial protection during percutaneous transluminal coronary angioplasty by intracoronary application of dipyridamole: impact on hemodynamic function and left ventricular performance. J. Am. Coll. Cardiol. 28, 1119–1126.

Tracey, K.J., Cerami, A., 1993a. Tumor necrosis factor, other cytokines and disease. Annu. Rev. Cell Biol. 9, 317–343.
Tracey, K.J., Cerami, A., 1993b. Tumor necrosis factor: an updated review of its biology. Crit. Care Med. 21, S415–S422.
Tracey, K.J., Cerami, A., 1994. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. Annu. Rev. Med. 45, 491–503.
Tripodi, A., Mannucci, P.M., 2007. Abnormalities of hemostasis in chronic liver disease: reappraisal of their clinical significance and need for clinical and laboratory research. J. Hepatol. 46, 727–733.
Ulloa, L., Tracey, K.J., 2005. The "cytokine profile": a code for sepsis. Trends Mol. Med. 11, 56–63.
Weyrich, A.S., Denis, M.M., Kuhlmann-Eyre, J.R., Spencer, E.D., Dixon, D.A., Marathe, G.K., McIntyre, T.M., Zimmerman, G.A., Prescott, S.M., 2005. Dipyridamole selectively inhibits inflammatory gene expression in platelet–monocyte aggregates. Circulation 111, 633–642.
Weyrich, A.S., Skalabrin, E.J., Kraiss, L.W., 2009. Targeting the inflammatory response in secondary stroke prevention: a role for combining aspirin and extended-release dipyridamole. Am. J. Ther. 16, 164–170.
Wollner, A., Wollner, S., Smith, J.B., 1993. Acting via A2 receptors, adenosine inhibits the upregulation of Mac-1 (Cd11b/CD18) expression on FMLP-stimulated neutrophils. Am. J. Respir. Cell Mol. Biol. 9, 179–185.
Yamashiro, S., Takeya, M., Kuratsu, J., Ushio, Y., Takahashi, K., Yoshimura, T., 1998. Intradermal injection of monocyte chemoattractant protein-1 induces emigration and differentiation of blood monocytes in rat skin. Int. Arch. Allergy Immunol. 115, 15–23.
Yang, S., Zheng, R., Hu, S., Ma, Y., Choudhry, M.A., Messina, J.L., Rue III, L.W., Bland, K.I., Chaudry, I.H., 2004. Mechanism of cardiac depression after trauma-hemorrhage: increased cardiomyocyte IL-6 and effect of sex steroids on IL-6 regulation and cardiac function. Am. J. Physiol. Heart Circ. Physiol. 287, H2183–H2191.
Zhao, L., Gray, L., Leonardi-Bee, J., Weaver, C.S., Heptinstall, S., Bath, P.M., 2006. Effect of aspirin, clopidogrel and dipyridamole on soluble markers of vascular function in normal volunteers and patients with prior ischaemic stroke. Platelets 17, 100–104.