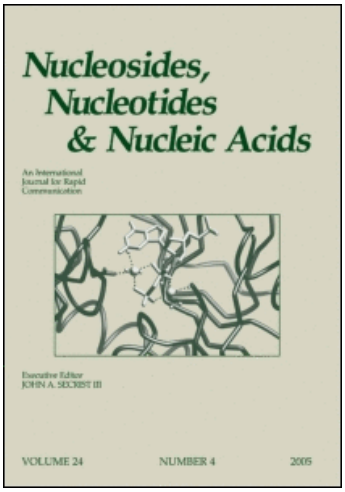

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Extracellular-Purine Metabolism in Blood Vessels (Part I). Extracellular-Purine Level in Blood of Patients with Abdominal Aortic Aneurysm

Joanna Lecka[a]; Stanislaw Molski[b]; Michal Komoszynski[c]
[a] Department of Biochemistry Collegium Medicum Bydgoszcz, N. Copernicus University, Torun, Poland [b] Department of Vascular Surgery, Collegium Medicum Bydgoszcz, N. Copernicus University, Torun, Poland [c] Department of Biochemistry, N. Copernicus University, Torun, Poland

Online publication date: 11 August 2010

To cite this Article Lecka, Joanna , Molski, Stanislaw and Komoszynski, Michal(2010) 'Extracellular-Purine Metabolism in Blood Vessels (Part I). Extracellular-Purine Level in Blood of Patients with Abdominal Aortic Aneurysm', Nucleosides, Nucleotides and Nucleic Acids, 29: 9, 647 — 657
To link to this Article: DOI: 10.1080/15257770.2010.502164
URL: http://dx.doi.org/10.1080/15257770.2010.502164

Nucleosides, Nucleotides and Nucleic Acids, 29:647–657, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2010.502164

Taylor & Francis
Taylor & Francis Group

EXTRACELLULAR-PURINE METABOLISM IN BLOOD VESSELS (PART I). EXTRACELLULAR-PURINE LEVEL IN BLOOD OF PATIENTS WITH ABDOMINAL AORTIC ANEURYSM

Joanna Lecka,[1] Stanislaw Molski,[2] and Michal Komoszynski[3]
[1]*Department of Biochemistry Collegium Medicum Bydgoszcz, N. Copernicus University, Torun, Poland*
[2]*Department of Vascular Surgery, Collegium Medicum Bydgoszcz, N. Copernicus University, Torun, Poland*
[3]*Department of Biochemistry, N. Copernicus University, Torun, Poland*

□ *Adenosine and adenosine derivatives are the main regulators of purinoceptors (P1 and P2) mediated hemostasis and blood pressure. Since impaired hemostasis and high blood pressure lead to atherosclerosis and to the development of aneurysm, in this study we tested and compared the concentration of extracellular purines (e-purines) in the blood in of patients having abdominal aortic aneurysm with that from healthy volunteers. Whereas adenine nucleosides and nucleotides level in human blood plasma was analysed using reverse phase high performance liquid chromatography (HPLC), cholesterol concentration was estimated by an enzymatic assay. We did not find any correlation between e-purines concentration and the age of healthy volunteers. Furthermore, the sum level of e-purines (ATP, ADP, AMP, adenosine, and inosine) in the control group did not exceed 70 μM, while it was nearly two-fold higher in the blood of patients having abdominal aortic aneurysm, (123 μM). In a special case of people with Leriche Syndrome, a disease characterized by deep atherosclerotic changes, the e-purines level had further increased. Additionally, we also report typical atherosclerotic changes in the aorta using histological assays as well as total cholesterol rise. The significant rise in cholesterol concentration in the blood of the patients with abdominal aortas aneurysm, compared with the control groups, was not unique since 23% of the healthy people also exceeded the normal level of cholesterol. Therefore, our results strongly indicate that the estimation of e-purines concentration in the blood may serve as another indicator of atherosclerosis and warrant further consideration as a futuristic diagnostic tool.*

Keywords Atherosclerosis; extracellular-purines; aorta aneurysm; blood

Received 1 December 2010; accepted 14 June 2010.
The authors are grateful to Prof. Karel P. M. Heirwegh (Catolic University of Leuven, Belgium), Dr. Manjit Singh Rana, and Dr. Jean Sévigny (University Laval, Québec, Canada) for a critical review of this article.

Address correspondence to Joanna Lecka, Le Centre Hospitalier Universitaire de Québec (CHUQ), Centre de recherché du pavillon CHUL, 2705 boulevard Laurier, local T1-49, Québec, Qué G1V 4G2, Canada. E-mail: joanna.lecka@crchul.ulaval.ca

INTRODUCTION

Mechanical weakening of the aortic wall caused by degenerative ischemic changes in atherosclerosis may induce aortic aneurysm. Although there is no clear evidence of association between aneurysm and atherosclerosis, however, the inflammation and abdominal aortic aneurysm (AAA) following the development and buildup of fatty plaques on interior walls of blood vessels have been reported.[1,2] Leriche Syndrome (LS), besides being marked by partial occlusion or complete occlusion as in case of the infrarenal aorta, is also characterized by development of thrombus or changes in blood pressure,[5,6] similar to artherosclerosis. We hypothesized that since high blood pressure, thrombus development, and accompanying changes in hemostasis that are common in atherosclerosis, AAA, and LS, these pathologies may also have been driven by similar biochemical and molecular events.

At the molecular level both blood pressure and hemostasis are regulated by extracellular purines (e-purines) such as e-ATP, e-ADP, and e-adenosine in the blood via their receptors expressed on endothelial cells and platelets in the blood vessels in cell specific manner.[3,4] E-adenosine activated P1 receptors present on platelets inhibit hemostasis, while endothelium born P1 receptors decrease blood pressure.[5–7] On the other hand e-ATP activated P2 receptors at the neuromuscular junction mediate a high blood pressure,[8] while activated endothelial cells P2 receptors relax blood vessels.[5,9–12] In a nonpathological homeostasis state, the concentration of e-ADP and e-adenosine is upregulated by the e-ATP degradation[13] and platelet degranulation (α-granules) that are rich sources of ATP and ADP (together 1M).[14,15] Adenosine, ADP and ATP released by exocytosis or by the injured blood and endothelial cells may also add to the repertoire of these e-purines.[16–18] Serum obtained from both pregnant women with high or normal blood pressure also showed higher concentration of some oxypurines or e-adenosine respectively.[19,20] On the other hand, some animal studies have demonstrated that chronic blockage of P1 receptors (activated by adenosine) may also cause high blood pressure.[21] The e-ADP, an agonist of P2 ($P2Y_1$ and $P2Y_{12}$) receptors expressed on the surface of platelets, initiates and amplifies the hemostasis[22,23] On the contrary, hemostasis is inhibited by e-ATP, an antagonist of $P2Y_1$ and $P2Y_{12}$ receptors and by e-adenosine via $A2_a$ receptors.[24–26] There is much evidence in recent literature of involvement of $P2Y_1$ and $P2Y_2$ receptors in atherosclerosis.[27,28] Furthermore, although e-ATP induces high blood pressure and in reciprocation e-edenosine leads to its relaxation, there is no unambiguous evidence showing a link between artherosclerotic changes and altered levels of extracellular purines. Therefore, we hypothesized that there is a correlation between nucleotide signalling pathway and etiology of atherosclerosis and in this work we show unequivocal changes in the level of e-purines in the blood of the patients with AAA caused by atherosclerosis.

MATERIAL AND METHODS

Study Group

The study was approved by the Ethics Committee of the Medical Academy, Bydgoszcz, Poland. All subjects were duly informed about the purpose of project and their consent to collect blood samples was obtained prior to the investigation. The analyzed material (4 mL of blood for every investigated case) was obtained from patients with AAA who qualified for surgery at the Department of Vascular Surgery, Medical Academy, Bydgoszcz and were free from diabetes. All subjects, patients, and volunteers forming a control group, comprising 90% men, were administered a routine medical interview while measuring their blood pressure (group characteristics are presented in the Table 1). There was no increase in platelet activation time in any analyzed blood sample. Patients prepared for surgery were asked to observe overnight fast and were taken to the hospital laboratory. Blood samples from subjects were obtained in heparinazed and precooled tubes on the day of medical examination preceding their surgery. Blood samples were also collected in the laboratory from healthy volunteers under similar conditions and following matching modalities (overnight fasting).

Study Protocol

For the analysis of e-purines in blood, the subjects were segregated in three groups; groups 1 and 2 constituted control groups. Group I ($n = 33$), included the cases with the lowest risk of atherosclerosis and ranged between newborn to 20-year-old persons. The second group (II; $n = 28$) was created with blood samples from healthy people between 40 and 70 years of age. The third group (III; $n = 37$) represented the patients whose age matched with that of Group II and were prepared for the surgical removal of aortic aneurysms. Additionally we analyzed the e-purine level in the blood of patients with the Leriche Syndrome ($n = 2$).

Adenine Nucleosides and Nucleotides in Human Blood Plasma

Each collected blood sample, containing heparin and 100 μM dipiridamol, was immediately centrifuged at 4°C to collect the plasma. The protein

TABLE 1 Physical group characterization (all the data presented in percentage)

	High blood pressure (above 140/90 mmHg)	Smoking	High total cholesterol level (above 200 mg/dL)	Anti-hypertensive and decreasing cholesterol drug intake
Groups			[%]	
Control volunteers	29	14	23	0
AAA patients	30	10	61	60

were removed from the plasma with ice-cold 1 M $HClO_4$ and centrifuged. The supernatant was neutralized with 1 M KOH and centrifuged. Lipids were removed by extraction with n-heptane (5:1 v/v). During these preparatory steps the temperature was maintained at 4°C. The concentration of e-purines in the supernatant was assayed by using high performance liquid chromatography (HPLC) method. Briefly, 20 μL samples were injected on a SuperPack ODS × 250 mm (LKB Sweden) column and products were separated by isocratic elution with: 0.1 M KH_2PO_4/K_2HPO_4 buffer pH 7.0, 5 mM EDTA, 2.5% MeOH and 25mM tetra-n-butyl ammonium hydrogen sulfate (TBA).

Samples of plasma having low e-purines concentration (less than 1 μM) were lyophilized and dissolved in 1/10 initial volume of running buffer preceding their HPLC analyses. Identification of e-purines was carried out by HPLC (both under lyophilized and nonlyophilized conditions). The internal standards were used to quantify these compounds. The e-ATP concentration measured via Luciferase Assay using spectrofluorophotometer RF-5001PC SHIMADZU served as a positive control.

Cholesterol Level

Total cholesterol levels were estimated by using ANALCO-GBG spectrophotometer. The presence of 200 mg/dl (5.2 mM) of cholesterol defined the upper limit of its concentration in healthy human blood samples.

Cross-Sections of Human Abdominal Aortas

Paraffin-embedded sections of human abdominal aortas were fixed in 10% formalin. Tissues were stained with haematoxyline/eosine.[31]

Statistics

Means and standard deviations of extracellular purines concentration were calculated. Comparison between groups was done using one-way analysis of variation (ANOVA) test with Bonferroni posttest. A value of $P < 0.05$ was considered significant.

RESULTS

Blood Plasma Nucleotides/E-Purines Level

Some characteristic of the study groups (I–III) in regard to their blood pressure, smoking habits and cholesterol level are presented in Table 1. In order to estimate the changes in e-purines that might have led to the development of AAA we measured concentrations of ATP, ADP, AMP, adenosine and inosine in blood plasma of patients with aneurysm. We did not find

TABLE 2 Concentration of some purines in human blood plasma

Groups	n	E-purines concentration [μM] ±SD				
		Inosine	Adenosine	AMP	ADP	ATP
I. Control group, age:0–20	33	57.89 ± 26.7	3.93 ± 4.13	2.40 ± 2.92	2.10 ± 2.40	1.16 ± 1.09
II. Control group, age: 40–70	28	58.36 ± 36.25	3.01 ± 2.19	3.77 ± 4.07	2.75 ± 3.23	0.88 ± 1.41
III. AAA group age: 40–70	37	97.53 ± 58.8	8.56 ± 7.38	7.61 ± 7.38	4.97 ± 5.11	4.08 ± 7.90
Statistical differences	I:III	$p = 0.0002$	$p = 0.001$	$p = 0.0002$	$p = 0.006$	$p = 0.015$
	II:III	$p = 0.001$	$p = 0.0001$	$p = 0.01$	NS	$p = 0.015$

any correlation between the purine levels obtained from healthy individuals and their ages (Table 2), although most of neonates did measure traces of e-ADP and e-ATP in their blood (data not shown). The total concentration of the e-purines in control groups (I, II) was estimated to be 70 μM, while in the investigated group III at 123 μM, that is almost double and statistically significant ($p = 1{\cdot}10^{-6}$). The concentration of inosine ranging between 58 and 97 μM was the highest in all the three investigated groups, while ATP between 1 and 4 μM was the lowest (Table 2).

The comparison between two control groups and the group representing AAA patients showed raised level of e-purines in their blood. The blood samples from the AAA patients measured 4 times higher ATP, 2.5 times higher adenosine and around 2 times higher concentration of each of ADP, AMP, and inosine. The differences were statistically significantly except for ADP second control group and third (patients) group (Table 2).

The differences in the level of e-ATP were further verified by luciferase assay in 5 blindly chosen samples from each group and were found to be similar (data not shown).

Total Cholesterol Concentration and Histological Assays on Aorta Fragments

Since the presence of enhanced level of cholesterol is often a risk factor in development of athrosclerosis, we sought to verify this dogma. We found that the average value of cholesterol concentration in control groups were 152 ± 54 mg/dL and 170 ± 40 mg/dL for I ($n = 14$) and II ($n = 14$) group respectively, while for the investigated AAA group ($n = 26$) it was 222 ± 53 mg/dL (Figure 1), which is statistically significantly higher compare with the control groups. Furthermore, not only people with aneurysms (61%) surpassed the upper normal limit of 200 mg/dL cholesterol but 23% healthy persons also exceeded this accepted norm (Figure 1).

Additionally, the histological pictures of blood vessels from healthy and AAA patients are different from the control groups (Figure 2A, 2B, and 2C). The endothelial cells in case of AAA patients (Figure 2C) are nearly

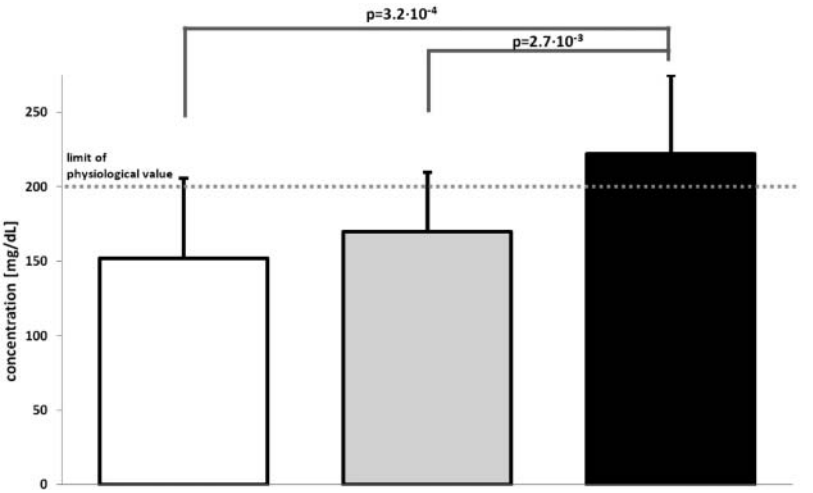

FIGURE 1 Total cholesterol concentration in serum of healthy subjects (white bar = up to 20 years old (n = 14), gray bar = above 40 years old (n = 14), and black bar = atherosclerotic (n = 26). The horizontal dotted line indicates the concentration of cholesterol considered as "normal" for homeostasis.

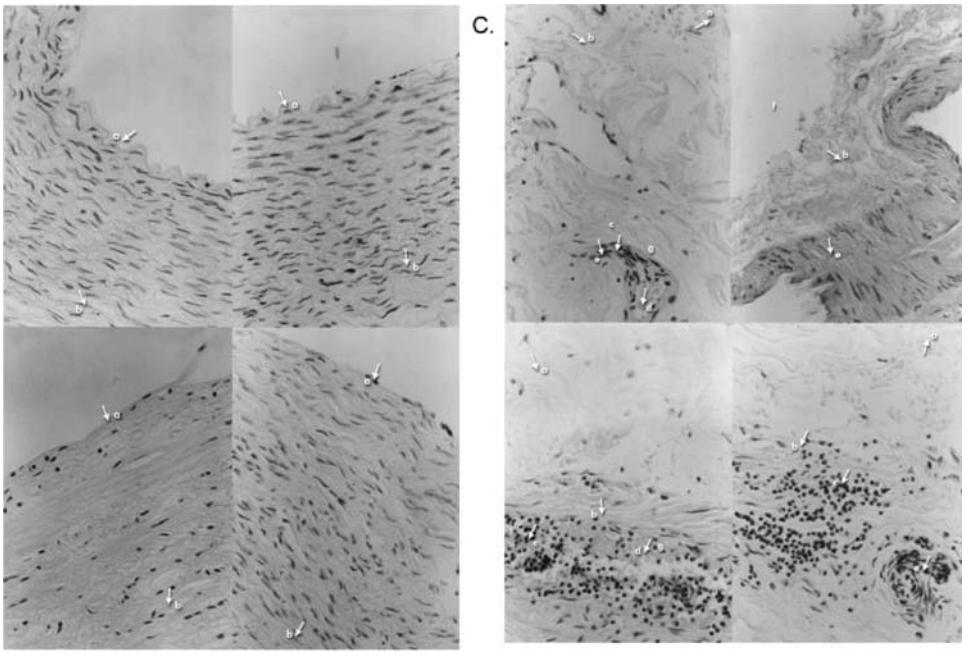

FIGURE 2 Cross-sections of human abdominal aortas. A) A 29-day-old child; B) A 29-year-old man died in an accident (without any symptoms of atherosclerosis); C) A 49-year-old patient with aneurysm of abdominal aorta. The arrows show: a) endothelial cells; b) muscle cells; c) red blood cells; d) white blood cells; e) calluses in intima; f) lumen; g) inflammations.

TABLE 3 Concentration of e-purines in blood vessels of people with Leriche Syndrome (LS)

	E-purines concentration [μM] ±SD LS, $n = 2$
Inosine	276.6
Adenosine	20.4
AMP	33.6
ADP	22.2
ATP	11.1

completely destroyed as a likely consequence of exacerbated high blood pressure.

E-Purine Concentrations in the Blood of Leriche Syndrome (LS) Patients

Next we compared the levels of e-purines of both AAA and LS. We showed that similar to a substantial increase in level of e-purines in the blood samples of AAA patients, LS patients also measured enhanced e-purine levels compared to the control groups (I and II),

Furthermore, concentrations of inosine and adenosine in LS group were 2.5 times higher than those found in AAA group and 5 times higher than that in control groups (Tables 2 and 3). The concentrations of AMP and ADP were 4 times higher in blood samples from LS patient than in the patient representing AAA group and 10 times higher than what was observed in control groups. And finally, the concentration of ATP was 3 times higher in LS subjects than in AAA group and 10 times higher when compare with control groups (Tables 2 and 3).

DISCUSSION

In this article, we show that e-purines level increases in AAA and LS patients' blood. Aside from atherosclerosis, patients with AAA infliction, LS and some pregnant women are also characterized by high blood pressure and a higher risk of platelet aggregation.[19,20] Both high blood pressure and tendency to blood clot formation can disrupt the normal hemostasis in these physiological or pathological states. Among other variables e-purines in the blood are also known to play a role in maintaining hemostasis and regulate blood pressure. The concentration of e-purines is modulated by vessel stage (e.g., the level/size of pathological changes leading to cells damage and releasing the components to the blood stream) and controlled by the activity of several ecto-enzymes that we described in a separate article.[42] With the realization that perturbations in the hemostasis contributes to the evolution of atherosclerosis, we had hypothesized that the levels of e-purines

may in part be responsible for this etiology. Our results strongly suggest that there is direct correlation between high level of e-purines and development of both AAA and LS.

Several recent studies clearly show strong associations between total cholesterol level, the traditional risk factor, the risk of incident abdominal aortic aneurysms and development of atherosclerosis.[32–34] Here, we for the first time suggest that the alterations in e-purine levels also lead to development of atherosclerosis. Even though total cholesterol level in the blood of patients with AAA is statistically significantly higher but the fact that as many as 39% of the patients have a normal level of blood cholesterol in their blood while 20% healthy people may have elevated total cholesterol level argues against the total blood cholesterol level as a pertinent indicator of development of athrosclerosis. However, the raised e-purine levels seem to reflect disease initiation and progression better.

To our knowledge till date there are no reports on the analysis of purines levels in human blood of atherosclerotic patients. A concentration of 1.5–2 μM e-ADP is required to activate $P2Y_{12}$ purinoceptors and initiating hemostasis.[25,29–33] Again a very low concentration of this nucleotide in the blood of children (up to 14 years) and young people (up to 20) suggests that under physiological conditions initiation of platelet aggregation is present only as a defense mechanism (Table 2). The presence of $P2Y_{12}$ ligand, e-ADP at concentrations higher than 2 μM in the blood of AAA patients suggests that the hemostasis process is always active. However, the existence of e-ATP and e-adenosine, the antagonists of ADP, may delay or inhibit this process.[5,29,30,32,33] Hall et al.[34] previously had shown that as much as 10-fold higher concentration of ATP than ADP was needed to inhibit platelet aggregation.

E-ATP, a natural ligand of P2X receptors, may contribute to the high blood pressure[12,30,35,36] that in turn may damage endothelial cells,[5,37] resulting in increased levels of ATP and ADP. The raised level of these nucleotides inhibit ecto-5′-nucleotidase activity[38,39] that leads to decreased production of adenosine (relaxation factor) in relation to ATP implying still higher blood pressure in the patients. Some recent studies suggest that AMP does not have a direct influence on homeostasis in the circulatory system, however, it may modulate the amount of e-adenosine.[30,35,40,41] Here, we show that the level of AMP in the blood of AAA patients increases (Table 2).

As a result of exacerbated high blood pressure, the endothelial cells of aneurystic vessels are nearly completely destroyed (Figure 2C) allowing e-adenosine and e-ATP to trigger their respective receptors chaotically and destroying the ectonucleotidase composition that may further lead to alteration in e-purines level. Therefore, our data demonstrating the raised level of e-purines in AAA patient's blood may inform about the impairment of both blood pressure and platelet aggregation found in AAA, LS and atherosclerosis development.[30,35,36,40] This is in agreement with the fact that

people with AAA have consistently high blood pressure. Analyses of blood plasma of patients with very chronic changes as in LS, strongly confirmed the conclusion that e-purine concentrations in the blood signify the physiological condition of blood vessels that precede development of atherosclerosis.

Altogether we have shown that blood concentrations above 4 μM of ATP and ADP may indicate pro AAA and LS changes in the blood vessels. Alteration in e-purine levels in the AAA patients are another, beside cholesterol, clear indicator of disease progression than cholesterol. Finally, e-purine levels may additionally inform about physiological status/conditions of the blood vessels. Therefore, it is tempting to suggest that plasma e-purine levels play significant role in the etiology of atherosclerotic diseases including AAA and LS and in the least may serve as diagnostic tool and therapeutic targets.

REFERENCE

1. Killion, S.L.; Hunter, G.C.; Eskelson, C.D.; Dubick, M.A.; Putnam, C.W.; Hall, K.A.; Luedke, C.A.; Misiorowski, R.L.; Schilling, J.D.; McIntyre, K.E. Vitamin E levels in human atherosclerotic plaque: the influence of risk factors. *Atherosclerosis* **1996**, 126(2), 289–297.
2. Yamagishi, M.; Higashikata, T.; Ishibashi-Ueda, H.; Sasaki, H.; Ogino, H.; Iihara, K.; Miyamoto, S.; Nagaya, N.; Tomoike, H.; Sakamoto, A. Sustained upregulation of inflammatory chemokine and its receptor in aneurysmal and occlusive atherosclerotic disease: results form tissue analysis with cDNA macroarray and real-time reverse transcriptional polymerase chain reaction methods. *Circ. J.* **2005**, 69(12), 1490–1495.
3. Tolhurst, G.; Vial, C.; Leon, C.; Gachet, C.; Evans, R.J.; Mahaut-Smith, M.P. Interplay between P2Y(1), P2Y(12), and P2X(1) receptors in the activation of megakaryocyte cation influx currents by ADP: evidence that the primary megakaryocyte represents a fully functional model of platelet P2 receptor signaling. *Blood* **2005**, 106(5), 1644–1651.
4. Skinner, M.R.; Marshall, J.M. Studies on the roles of ATP, adenosine and nitric oxide in mediating muscle vasodilatation induced in the rat by acute systemic hypoxia. *J. Physiol.* **1996**, 495(Pt 2), 553–560.
5. Sévigny, J.; Beaudoin, A.R. An open window on the world of extracellular nucleotides and the potential role of the ATP-diphosphohydrolase [Review]. *M. S-Médecine Sciences* **1994**, 10(8–9), 836–844.
6. Abebe, W.; Makujina, S.R.; Mustafa, S.J. Adenosine receptor-mediated relaxation of porcine coronary artery in presence and absence of endothelium. *Am. J. Physiol.* **1994**, 266(5 Pt 2), H2018–2025.
7. Soslau, G.; Parker, J. Modulation of platelet function by extracellular adenosine triphosphate. *Blood* **1989**, 74(3), 984–993.
8. Smyth, L.M.; Bobalova, J.; Mendoza, M.G.; Lew, C.; Mutafova-Yambolieva, V.N. Release of beta-nicotinamide adenine dinucleotide upon stimulation of postganglionic nerve terminals in blood vessels and urinary bladder. *J. Bio. Chem.* **2004**, 279(47), 48893–48903.
9. Rummery, N.M.; Brock, J.A.; Pakdeechote, P.; Ralevic, V.; Dunn, W.R. ATP is the predominant sympathetic neurotransmitter in rat mesenteric arteries at high pressure. *J. Physiol.* **2007**, 582(Pt 2), 745–754.
10. Lazarowski, E.R.; Boucher, R.C.; Harden, T.K. Constitutive release of ATP and evidence for major contribution of ecto-nucleotide pyrophosphatase and nucleoside diphosphokinase to extracellular nucleotide concentrations. *J. Bio. Chem.* **2000**, 275(40), 31061–31068.
11. Hara, Y.; Nakaya, H. Dual effects of extracellular ATP on the muscarinic acetylcholine receptor-operated K+ current in guinea-pig atrial cells. *Eur. J. Pharmacol.* **1997**, 324(2–3), 295–303.
12. Kwan, Y.W.; To, K.W.; Lau, W.M.; Tsang, S.H. Comparison of the vascular relaxant effects of ATP-dependent K+ channel openers on aorta and pulmonary artery isolated from spontaneously hypertensive and Wistar-Kyoto rats. *Eur. J. Pharmacol.* **1999**, 365(2–3), 241–251.
13. Lecka, J.; Molski, S.; Komoszynski, M. Alteration in ecto-purines metabolism in vascular disease. Paper presented at: Second international workshop on ecto-ATPases and related ectonucleotidases; **2000**, Diepenbeek, Belgium.

14. Eltzschig, H.K.; Eckle, T.; Mager, A.; Kuper, N.; Karcher, C.; Weissmuller, T.; Boengler, K.; Schulz, R.; Robson, S.C.; Colgan, S.P. ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ. Res.* **2006**, 99(10), 1100–1108.
15. Li, Z.; Zhang, G.; Marjanovic, J.A.; Ruan, C.; Du, X. A platelet secretion pathway mediated by cGMP-dependent protein kinase. *J. Bio. Chem.* **2004**, 279(41), 42469–42475.
16. Goldsmith, H.L.; Bell, D.N.; Braovac, S.; Steinberg, A.; McIntosh, F. Physical and chemical effects of red cells in the shear-induced aggregation of human platelets. *Biophys. J.* **1995**, 69(4), 1584–1595.
17. Morrell, C.N.; Matsushita, K.; Chiles, K.; Scharpf, R.B.; Yamakuchi, M.; Mason, R.J.; Bergmeier, W.; Mankowski, J.L.; Baldwin, W.M., 3rd, Faraday, N.; Lowenstein, C.J. Regulation of platelet granule exocytosis by S-nitrosylation. *PNAS* **2005**, 102(10), 3782–3787.
18. Luthje, J. Origin, metabolism and function of extracellular adenine nucleotides in the blood [published erratum appears in Klin Wochenschr 1989 May 15;67(10):558]. [Review]. *Klin. Wochenschr.* **1989**, 67(6), 317–327.
19. Szczesniak, A.; Klejewski, A.; Banaszak, F. [Monitoring oxypurines levels in women with pregnancy induced hypertension and in women with physiological pregnancy]. *Ginekol. Pol.* **1997**, 68(9), 407–412.
20. Yoneyama, Y.; Suzuki, S.; Sawa, R.; Otsubo, Y.; Power, G.G.; Araki, T. Plasma adenosine levels increase in women with normal pregnancies. *Am. J. Obstet. Gynecol.* **2000**, 182(5), 1200–1203.
21. Guimaraes, S.; Albino-Teixeira, A. Hypertension due to chronic blockade of P1-purinoceptors. *J. Auton. Pharmacol.* **1996**, 16(6), 367–370.
22. Gachet, C. ADP receptors of platelets and their inhibition. *Thromb Haemost* **2001**, 86(1), 222–232.
23. Hechler, B.; Cattaneo, M.; Gachet, C. The P2 receptors in platelet function. *Sem. Thromb. Hemost.* **2005**, 31(2), 150–161.
24. Motte, S.; Communi, D.; Pirotton, S.; Boeynaems, J.M. Involvement of multiple receptors in the actions of extracellular ATP: the example of vascular endothelial cells. [Review]. *Int. J. Bioch. Cell B* **1995**, 27(1), 1–7.
25. Hourani, S.M. Purinoceptors and platelet aggregation. *J. Auton. Pharmacol.* **1996**, 16(6), 349–352.
26. Born, G.V.R. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* **1961**, 194, 927–929.
27. Wang, Z.X.; Nakayama, T.; Sato, N.; Izumi, Y.; Kasamaki, Y.; Ohta, M.; Soma, M.; Aoi, N.; Matsumoto, K.; Ozawa, Y.; Ma, Y.T.; Doba, N.; Hinohara, S. Association of the purinergic receptor P2Y, G-protein coupled, 2 (P2RY2) gene with myocardial infarction in Japanese men. *Circ. J.* **2009**, 73(12), 2322–2329.
28. Hechler, B.; Freund, M.; Ravanat, C.; Magnenat, S.; Cazenave, J.P.; Gachet, C. Reduced atherosclerotic lesions in P2Y1/apolipoprotein E double-knockout mice: the contribution of non-hematopoietic-derived P2Y1 receptors. *Circulation* **2008**, 118(7), 754–763.
29. Hinnen, J.W.; Koning, O.H.; Visser, M.J.; Van, Bockel, H.J. Effect of intraluminal thrombus on pressure transmission in the abdominal aortic aneurysm. *J. Vasc. Surg.* **2005**, 42(6), 1176–1182.
30. Ralevic, V.; Burnstock, G. Receptors for purines and pyrimidines. *Pharmacol. Rev.* **1998**, 50(3), 413–492.
31. Gachet, C.; Hechler, B.; Leon, C.; Vial, C.; Leray, C.; Ohlmann, P.; Cazenave, J.P. Activation of ADP receptors and platelet function. *Thromb. Haemost.* **1997**, 78(1), 271–275.
32. Park, H.S.; Hourani, S.M. Differential effects of adenine nucleotide analogues on shape change and aggregation induced by adnosine 5-diphosphate (ADP) in human platelets. *Br. J. Pharmacol.* **1999**, 127(6), 1359–1366.
33. Côté, Y.P.; Filep, J.G.; Battistini, B.; Gauvreau, J.; Sirois, P.; Beaudoin, A.R. Characterization of ATP-diphosphohydrolase activities in the intima and media of the bovine aorta: evidence for a regulatory role in platelet activation in vitro. *Biochim. et Biophys. Acta* **1992**, 1139(1–2), 133–142.
34. Hall, D.A.; Frost, V.; Hourani, S.M. Effects of extracellular divalent cations on responses of human blood platelets to adenosine 5′-diphosphate. *Biochem. Pharmacol.* **1994**, 48(7), 1319–1326.
35. Windscheif, U. Purinoceptors: from history to recent progress. A review. *J. Pharm. Pharmaco.* **1996**, 48(10), 993–1011.
36. El-Moatassim, C.; Dormand, J.; Mani, J.-M. Extracellular ATP and cell signalling. *Biochim. et Biophys. Acta.* **1992**, 1134, 31–45.
37. Lecka, J.; Komoszynski, M. The role of ecto-purines (e-purines) in regulation of blood pressure, hemostasis and etiology of atherosclerosis. *Prog. Med. Res.* **2003**.
38. Zimmermann, H. 5′-nucleotidase: molecular structure and functional aspects. *Biochem. J.* **1992**, 285, 345–365.

39. Minelli, A.; Moroni, M.; Mezzasoma, I. Human seminal plasma soluble 5′-nucleotidase: regulatory aspects of the dephosphorylation of nucleoside 5′-monophosphates. *Biochem. Mol. Med.* **1997**, 61(1), 95–101.
40. Chinellato, A.; Ragazzi, E. Receptor-mediated pathways of endothelium activity in experimental atherosclerosis. *Pharmacol. Res.* **1995**, 31(3–4), 163–168.
41. Obata, T. Adenosine production and its interaction with protection of ischemic and reperfusion injury of the myocardium. *Life Sci.* **2002**, 71(18), 2083–2103.
42. Lecka, J.; Bloch-Boguslawska, E.; Molski, S.; Komoszynski, M. Extracellular purine metabolism in blood vessels (part II): activity of ecto-enzymes in blood vessels of patients with abdominal aortic aneurysm. *Clin. Appl. Thromb. Hemost.* **2010**, May 11 [Epub].