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Effects of adenosine and dipyridamole on serum levels of IL-6, TNF - α, and some antioxidants in rabbits

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Abstract:

Nearly 30 years of research findings stated that adenosine adjust ischemia. Although, adenosine use for treatment has not been accepted clinically in wide manner, also there have no many clinical trials. Aims: study the effects of adenosine and dipyridamole on total Antioxidant Capacity (TAC), Adenosine Deaminase (ADA), GSH (Glutathione), TNF - α (tumor necrosis factor Alpha) and IL-6 (Interleukin 6) in rabbits. Material and methods: Thirty five male rabbits were included in the study. The animals were divided into 3 groups: Group one(5 animals): injected (i.p) with 2 ml of distilled water/day (control group). Group two (15 animals): were treated by intraperitoneal injection of adenosine, they were divided into 3 sub groups (5 animals) according to adenosine dose:1 mg/kg, 2mg/kg and 4 mg/kg.Group 3 (15 animals): were treated by dipyridamole orally, they were divided into 3 sub groups (5 animals) according to dipyridamole dose:4 mg/kg, 8 mg/kg and 12 mg/kg. Total Antioxidant Capacity Assay kit, Adenosine Deaminase (ADA) colorimetric Assay kit, Rabbit IL-6 (Interleukin 6) ELIISA kit, Rabbit TNF - α (tumor necrosis factor Alpha), GSH (Glutathione) ELISA kits were used. Results: ANOVA Test and Duncan's Multiple Range Test were applied between study groups. In comparison among adenosine, dipyridamole and control groups, highly significant differences were found among them in TAC,ADA and GSH levels while no significant differences were found in TNF -α and IL-6 levels among all groups. Conclusion: both adenosine, dipyridamole cause reduction in total Antioxidant Capacity together with increase in glutathione levels and Adenosine Deaminase.

Keywords: adenosine, dipyridamole, antioxidant.

تأثير الادينوسين والديبيريدامول على مستويات المصل من IL_6 وTNF_alpha وبعض مضادات الأكسدة في الارانب

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ملخص البحث:

ما يقارب ٣٠ عاما من النتائج التجريبية التي تدعم الفرضية التي تثبت بان الادينوسين يعدل الاصابة بنقص اعادة ضخ التروية الدموية ,على الرغم من هذا الدليل لم يتم قبول استخدام هذا الدواء ومعدلاته سريريا على نطاق واسع كما لم يتم اجراء ما يكفي من التجارب السريرية .وتهدف الدراسة الحالية الى دراسة تأثير كل من الادينوسين و**الديبيريدامول** على مستويات مصل الدم من حيث القدرة الكلية المضادة للأكسدة والكلوتاثايون وانزيم الادينوسين دي امينيز و الفاTNF. المواد وطرائق العمل: اشتملت الدراسة على خمسة وثلاثون من ذكور الارانب بعمر(١٠_١٢) شهر ووزن (١,٠_١,٥) كغم. قسمت الحيوانات عشوائيا إلى ثلاث مجموعات: المجموعة الاولى(٥حيوانات): حقنت(I.p) ب ٢مل من الماء المقطر يوميا طوال فترة التجربة،(مجموعة السيطرة)،المجموعة الثانية(١٥ حيوان): عولجت بالحقن داخل الصفاق بمادة الادينوسين ،وقسمت إلى ٣ مجموعات فرعية(٥حيوانات)حسب جرعة الادينوسين ١ ملغم/كغم،٢ملغم / كغم،و ٤ملغم/ كغم. المجموعة الثالثة (١٥ حيوان):عولجت بمادة الدايبايريدامول عن طريق الفم بواسطة أنبوب التزقيم، قسمت إلى ٣مجموعات فرعية(٥حيوانات) حسب جرعة الديبيريدامول:٤ ملغم/كغم،٨ملغم/ كغم،و ١٢ ملغم /كغم. تم استخدام سعة مضادات الأكسدة الكلية ،مجموعة الفحص اللوني لانزيم Adenosine Deaminase،مجموعة الElisa لعامل نخر الورم_الفا في الارانب،مجموعة الElisa ل انترليوكين_٦ في الارانب، ومجموعة الElisa لانزيم الكلوتاثيون لتحليل المصل.النتائج:عند تطبيق اختبارات ANOVAو Duncan's متعدد المدى بين مجموعات الدراسة تم العثور على فروق ذات دلالة احصائية فيما بينها في مستويات TACو ADAوGSH بينما لم يتم العثور على ذلك في مستويات الفاTNF و IL6 . الاستنتاج:يتسبب كل من الادينوسين و**الديبيريدامول** في انخفاض مستوى ال TACمع زيادة مستويات ال GSH وADA كانزيم مسؤول عن ايض الادينوسين.

الكلمات المفتاحية: الادينوسين، الدايبايريدامول، مضادات الاكسدة

INTRODUCTION

Adenosine is a nucleoside which occurs naturally in a diverse forms in all cells of the body. It is an vital constituent of the energy production and usage of the body (Mahler,1998). Adenosine is a purine nucleoside consists of a molecule of adenine connected to ribose sugar molecule (ribofuranose) moiety. of Adenosine derivatives are commonly found in nature and has a significant position in biochemical processes. Adenosine thiphosphate (ATP) and adenosine diphosphate (ADP) acting on energy transfer while cyclic adenosine monophosphate (cAMP) has a role in signal transduction. Also adenosine itself is a neuromodulator substance, supposed to have a major role in sleep promotion and arousal suppression. Adenosine also has an action on regulation of blood flow to a variety of organs throughout vasodilation. All these functions and others need specific signal through adenosine receptors. Dipridamole was introduced as a coronary vasodilator, given orally (Melani et al., 2020). It inhibits the enzyme phosphodiesterass due to decrease of the adenosine transporter and elevates cAMP and eGMP levels. (Yip and Benavente, 2011). Moreover, dipyridamol has platelet inhibitory actions and interfere with metabolism of adenosine, also related to platelet aggregation, through potential stimulation of the adenlyl cyclase in platelets, resulting in elevated cAMP (Verro et al., 2008). Studies suggest that dipridamol gives good useful direct and indirect action into the vasculature, including the endothelium including inhibition of proliferation, antioxidant, and anti-inflammatory properties (Chakrabarti and Freedman,2008). Dipyridamol inhibits adenosine reuptake by erythrocytes, endothelial cells and platelet increasing plasma levels of adenosine (Halkes et al.,2006). This vasodilator activity of dipyridamole leads to improved tissue perfusion (chakyabarti et al.,2005). Dipyridamole increase the aggregation inhibiting effects of adenosine and prostaglandine E1, reduce platelets uptake of adenosine, serotonin and glucose. At greater level dipridamole decrease platelet aggregation provoked by ADP and/or collagen (Steele et al., 1981).

MATERIALS AND METHODS

Thirty five apparently local healthy mature male rabbits of (10-12) months old and body weight of 1.0-1.5 Kg were involved in the study. Animals were housed in animal house of college of Dentistry / University of Mosul.

Dose calculations

According to references, both adenosine((MACKLIN CAS: 58-61-7/CHINA) in powder form with a capacity of (25 g) and with a concentration of (99.5%) and molecular weight (267.24)and Dipyridamole(tablet (75 mg), European origin (CYPRUS) are mainstay drugs used for treatment of human diseases at a wide range of dosing intervals depending on patient condition and type of drug combined with them to produce effect. (Katzung, 2017) They are available mainly at 6mg, 12mg and 30mg for adenosine and 25mg, 50mg and 75mg for Dipyridamole. (Kleinman, et al., 2010; DeCaen, et al., 2010; Lewis et al., 2017). In this study the doses of rabbit were calculated according to human Equivalent doses using the following formula: animal dose(mg/kg) = human dose (mg/kg) x conversion factor(3.08 for rabbit) (Shine, et al., 2010) According to our experimental protocol, a pilot study was carried out using rabbit doses that result from conversion of human doses (t.i.d. /for adult human of 60 kg body weight) (Belardinelli, et al., 2001; Shine, et al., 2010), doses results from this calculation were 0.924,1.848 and 4.620 mg/kg for adenosine, and 3.85, 7.70 and 11.55 mg/kg for Dipyridamole. After that we use up and down method on these calculated doses to find the doses that start to produce histological changes in tissue examined which are 1, 2, and 4 mg/kg/day for adenosine and 4, 8 and 12 mg/kg/day for Dipyridamole which were also chosen because their uppermost safety limit (Honur et al., 1976, Murday et al., 1984,Radojkovic et al., Browse et al., 2003).

Experimental design

The animals were randomly divided into 3 groups: Group one(5 animals): was injected (i.p) with 2 ml of distilled water per day throughout the trial period. (control group). Group two (15 animals): were treated by intraperitoneal injection of adenosine, they were divided into 3 sub groups (5 animals) according to adenosine dose:1 mg/kg, 2mg/kg and 4 mg/kg. Group 3 (15 animals): were treated by dipyridamole orally by gavage tube, they were divided into 3 sub groups (5 animals) according to dipyridamole dose:4 mg/kg, 8 mg/kg and 12 mg/kg. All these groups were received their treatments once daily for 30 days.

Blood samples collection

Fresh blood was drawn from each rabbit for the analysis of biochemical parameters,. Samples were collected after animals starvation for 12 hr before blood sampling, then the serum was then separated by centrifuge (Volker's optical Gmll, Germany), and stored at (-20C°) till analysis by using total Antioxidant Capacity Assay kit, Adenosine Deaminase (ADA) colorimetric Assay kit, Rabbit IL-6 (Interleukin 6) ELIISA kit, Rabbit TNF - α (tumor necrosis factor Alpha) ELISA kit, GSH (Glutathione) ELISA kit. All kit measurement were carried out in college of Dentistry / University of Mosul.

RESULTS

In this study ANOVA Test and Duncan's Multiple Range Test were applied between study groups. Highly significant differences were found in TAC, ADA and GSH levels among adenosine, dipyridamole and control groups. On other hand, no significant differences were found in TNF -α and IL-6 levels among all study groups.

Table (1): Comparison in serum biochemical markers among the study sampled groups.

Parameters	Group A Mean ± SD	Group D Mean ± SD	Control group Mean ± SD	P-value*
No. of rabbits	15	15	5	---
TAC (U/ml)	5.15 ± 1.83 ^{B}	3.73 ± 0.77 ^{B}	13.33 ± 2.50 ^{A}	0.000
ADA (U/ml)	13.20 ± 3.14 ^{B}	23.11 ± 4.03 ^{A}	0.00 ± 0.00 ^{C}	0.000
GSH (µg/ml)	22.11 ± 2.79 ^{A}	7.00 ± 2.37 ^{B}	5.54 ± 0.23 ^{B}	0.000
TNF- α (pg/ml)	15.59 ± 0.58 ^{A}	15.48 ± 0.82 ^{A}	15.63 ± 0.03 ^{A}	0.875
IL-6 (pg/ml)	15.37 ± 0.51 ^{A}	15.39 ± 0.52 ^{A}	15.62 ± 0.01 ^{A}	0.589

* One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share a letter are significantly different.

Table (2): The effect of different doses of adenosine on the serum biochemical markers in group A Vs. control group.

Parameters	Dosages in group A [Adenosine]			Control group	P-value*
	1 mg/kg Mean ± SD	2 mg/kg Mean ± SD	4 mg/kg Mean ± SD		
No. of rabbits	5	5	5	5	---
TAC (U/ml)	3.63 ± 1.03 ^{C}	4.67 ± 1.60 ^{BC}	7.14 ± 0.25 ^{B}	13.33 ± 2.50 ^{A}	0.000
ADA (U/ml)	10.03 ± 0.60 ^{C}	12.63 ± 1.85 ^{B}	16.93 ± 0.67 ^{A}	0.00 ± 0.00 ^{D}	0.000
GSH (µg/ml)	22.97 ± 1.66 ^{AB}	23.56 ± 2.22 ^{A}	19.80 ± 3.07 ^{B}	5.54 ± 0.23 ^{C}	0.000
TNF- α (pg/ml)	15.89 ± 0.80 ^{A}	15.35 ± 0.52 ^{A}	15.53 ± 0.29 ^{A}	15.63 ± 0.03 ^{A}	0.423
IL-6 (pg/ml)	15.81 ± 0.54 ^{A}	15.23 ± 0.49 ^{AB}	15.06 ± 0.13 ^{B}	15.62 ± 0.01 ^{AB}	0.018

* One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share a letter are significantly different.

Table (3): The effect of different doses of dipyridamole on the serum biochemical markers in group D Vs. control group

Parameters	Dosages in group D [Dipyridamole]			Control group	P-value*
	4 mg/kg Mean ± SD	8 mg/kg Mean ± SD	12 mg/kg Mean ± SD		
No. of rabbits	5	5	5	5	---
TAC (U/ml)	3.46 ± 0.41 ^{B}	3.38 ± 0.30 ^{B}	4.35 ± 1.04 ^{B}	13.33 ± 2.50 ^{A}	0.000
ADA (U/ml)	25.38 ± 3.58 ^{A}	19.83 ± 4.00 ^{B}	24.13 ± 2.64 ^{AB}	0.00 ± 0.00 ^{C}	0.000
GSH (µg/ml)	9.14 ± 1.89 ^{A}	6.96 ± 2.16 ^{AB}	4.89 ± 0.40 ^{B}	5.54 ± 0.23 ^{B}	0.001
TNF- α (pg/ml)	16.09 ± 1.14 ^{A}	15.14 ± 0.46 ^{A}	15.23 ± 0.39 ^{A}	15.63 ± 0.03 ^{A}	0.122
IL-6 (pg/ml)	15.59 ± 0.07 ^{A}	15.08 ± 0.86 ^{A}	15.49 ± 0.16 ^{A}	15.62 ± 0.01 ^{A}	0.229

*One-way ANOVA-test with Tukey's Pair wise comparisons was used. Means that do not share a letter are significantly different.

DISCUSSION

Results showed that both adenosine and dipyridamole (non selective agonists for the A2A receptor) have no significant effects on IL6 and TNF- α which can be explained by results of many studies that have established the ability of only high-affinity agonists with strong selectivity for the A2A receptor alone to repress the inflammatory mediators in several animal models(Palmer1 TM and Trevethick MA., 2008). Also the lack of significant effect of both adenosine and dipyridamole on IL6 and TNF- α could be due to the fact that NF-_B is a significant regulator of inflammation in mammalians. Also cytokines act throughby signaling pathways other than the NF-_B pathway.(Morello S et al., 2006)

Both pro-inflammatory and anti-inflammatory responses will be generated by activation of adenosine receptors. Adenosine binding to A1AR cause low concentrations of adenosine release which induce neutrophil chemotaxis and adherence to the endothelium and up regulate endothelial P-selectin expression. Accordingly, a high concentration of adenosine will induce antiadhesive effects via A2AR(Effendi WI, et al. 2020) i.e opposite to effect of low concentration this could explain the resuts of this study which show that dose of adenosine used here produce no significant effects on inflammatory mediaters includuning IL6 and TNF-α. Also adenosine as largely known anti-inflammatory agent in the immune system, might be attributable to its downregulation of IL-1β and caspase-1 protein but not to IL6 and TNF- α after adenosine treatment.(Ozel I et L; 2020) So, activation of A2AARs inhibits neutrophil adhesion to endothelial cells and also formation of reactive oxygen Species(Effendi WI, et al. 2020) which explain the significant reduction of TAC in treatment group compared to control group.

Adenosine deaminase can be used as oxidative stress marker. Their increased levels indicate oxidative stress, this could explain the increased ADA levels with decreased in TAC levels in treatment groups.(Dasegowda SM et al. ; 2015) The result of our current study showed that there was a significant increase in the concentration of the enzyme glutathione in the serum of rabbits of the adenosine group, this indicates that adenosine has a stimulating effect of antioxidant enzymes. A2AR has ability to control glutamatergic terminals provide a strong anatomical and molecular basis for A2AR to integrate glutamate signals (Chen JF and Cunha R.; 2020)

Glutathione, a most important antioxidant and regulator of redoex in the cells. It is serum level may be increased as reflex defensive mechanism to reduced total antioxidant capacity and increased redox homeostasis i.e. oxidative stress. Glutathione is also a cofactors for a multitude of enzymes. It has been shown that the glutathione cycle cast the

activity of synaptic glutamate, this relation may explain the increased levels of glutathione in treatment groups compared to control group. Accordingly these agents can affect glutathione-glutamate homeostasis which may influence their therapeutic benefit. (Sedlak TW,2019). Rising evidences indicates that levels of adenosine increase considerably in tissues offered to stressful conditions like inflammation, ischemia, and hypoxia. However, excessive extracellular adenosine accumulation fibrosis. Thus, rising in adenosine production in response to stress might have a dual regulatory position in tissue homeostasis. While adenosine primarily acts as a molecule reporting injury to cells in the surrounding areas for triggering protection, over accumulation of adenosine may lead to maladaption of organs in their responses, as observed in chronic inflammation, fibrosis.(Herman-de-Sousa C et al .,2020)

In the dipyridamole group, the increasing in the proportion of glutathione was slight compared with the control group.

Dipyridamole could be an active quencher of peroxyl radicals at pharmalogical doses, Because of its ability to extend the peroxidation lag stage. In the current study, There is a delay in inducing a change in glutathione concentration compared to adenosine group which may be is due to the fact that this treatment needs time to be absorbed into the membranes in optimal manner. It appears rational to assume that with an earlier drug treatment the preventive action will be more successful. Where dipyridamole acts as a chain- breaking substances once the progression of lipid peroxidation had begun (Kusmic et al. 2000). The action of dipyridamole is extended to cytoplasmic thiol in our experimental condition, despite the inability of dipyridamole to penetrate the cytosol rapidly (Nassar et al., 1997).

In this study dipyridamole's total serum antioxidant capacity is significantly reduced may be as a result to decrease in the amount of radicals formtion at the place of the membrane. In disagreement with our study, some researchers have observations elucidating the antioxidant effects of dipyridamole (Peudlli et al., 1999). The dose selection of dipyridamole was to be considered (Di Perri et al., 1991). In our research therapeutic doses were used, a larger doses be may needed to produce greater effects. Therefore, the dipyridamole antioxidant effect is significant and observable at a doses obtained during medical drug usage. The antiradical effect is accomplished by different biochemical mechanism, all irrespective of the accumulation of adenosine. Elevated dipyridamole concentration, achieved by high parenteral doses, is needed for recruitment of the antioxidant capacity (Kusmic et al., 2000). The results of our current study showed that

there were significant differences in the level of TAC in the serum of rabbits after 30 days of the experiment, as there was a significant decrease in the level of TAC in the adenosine and dipyridamole groups, as its value reached (5.15± 1.83) and (3.73± 0.77) U/ml respectively compared to the control group (13.33± 2.50) u/ml. So the effect of adenosine and dipyridamole on the TAC level is negatives and this explains the disruptive effect of these drugs on tissues (Borea et al., 2017). In disagreement with our results, Sailaja et al., 2003 said that diabetic human have shown elevated lipid peroxidation and reduction in levels of glutathione reductase, glutathione peroxidase, glutathione, reduced glutathione, and G6PDH. Researchs on adenosine needs to set up the function of adenosine receptor agonists in more clinically applicable models.

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