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ARTICLE

The effect of the antioxidant drug U-74389G on endometrial edema during ischemia reperfusion injury in rats

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ABSTRACT The aim of this experiment was to study the effects of the antioxidant drug U-74389G on rat model, particularly in ischemia reperfusion (IR) protocol. The beneficial or other effects of that molecule were studied pathologically using endometrial edema (EE) lesions. Forty rats were used of mean weight 231.875 gr. EE lesions were evaluated 60 min after reperfusion (groups A and C) and 120 min after reperfusion (groups B and D), with administration of the drug U-74389G in groups C and D. Results were that U-74389G administration non-significantly decreased without lesions the EE scores by 0.41 [-1.00 - 0.17] (p= 0.1607). This finding was in accordance with the results of Wilcoxon signed-rank test (p= 0.5229). Reperfusion time non-significantly increased without lesions the EE scores by 0.21 [-0.38 - 0.81] (p= 0.4701), also in accordance with Wilcoxon signed-rank test (p= 0.1022). However, U-74389G administration and reperfusion time together non-significantly decreased without lesions the EE scores by 0.17 [-0.53 - 0.18] (p= 0.3383). Results of this study indicate that U-74389G administration hardly non-significantly decreases without lesions the EE within short-term time context of 2 hours.
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KEY WORDS

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Tissue ischemia and reperfusion (IR) remain one of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. The use of antioxidant substances has been a research subject for many years. However, even if important progress has been made, satisfactory answers have not been given yet to fundamental questions, such as, how much powerful should an antioxidant be, when should it be administered, and in which dosage. The particularly satisfactory action of the antioxidant U-74389G in tissue protection has been noted in several performed experiments. Since a careful literature search (PubMed - Medline) was conducted, it was realized that this certain antioxidant has been tried in IR experiments. However, just few relative reports were found, not covering completely this particular matter. Also, a lot of publications addressed trials of other similar molecules of aminosteroids (lazaroids) to which the studied molecule also belongs to. U-74389G or better 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt (Cayman Chemical Product Catalog) is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation. It protects against IR injury in animal heart, liver, and kidney models. These membrane-associating antioxidants (Shi et al. 1995) are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. The same authors (Tsompos et al. 2014) found a light non-significant decline in chloride serum levels in related IR injury experiments in rats by 0.58%±0.77% (p=0.4533) 1h after reperfusion, by 0.97%±0.53% (p=0.0879) 1.5h after reperfusion, by 0.75%±0.38% (p=0.0159) after time and drug interaction and by 1.36%±0.76% (p=0.1113) 2h after reperfusion.

The aim of this experimental study was to examine the effect of the antioxidant drug U-74389G on rat model and particularly in a uterus IR protocol. The beneficial effect or non-effectiveness of that molecule was studied by evaluating mean endometrial edema (EE) lesions.

Materials and Methods

Animal preparation

This experimental study was approved by Scientific Committee of Ippokrateion General Hospital, Athens University and by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. Institutional and national guide for the care and use of laboratory animals

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was followed. This experimental study was carried out at the Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. All settings needed for the study including consumables, equipment and substances used, were provided by them. Albino female Wistar rats were used in accordance with accepted standards of humane animal care. They spent 7 days in laboratory before experimentation with an easy access to water and food. The experiment was acute, that is, the animal usage was completed by following experimental set of times without awakening and preservation of the rodents. They were randomly assigned to four experimental groups (10 animals in each group). Group A: Ischemia for 45 min followed by reperfusion for 60 min. Group B: Ischemia for 45 min followed by reperfusion for 120 min. Group C: Ischemia for 45 min followed immediately by U-74389G intravenous (IV) administration and reperfusion for 60 min. Group D: Ischemia for 45 min followed immediately by U-74389G IV administration and reperfusion for 120 min. The molecule U-74389G was administered in a dose of: 10 mg/kg body weight of the animal. The experiment was beginning by prenarcosis and general anesthesia administration to the animals. Their electrocardiogram and acidometry were continuously monitored. The inferior aorta was prepared so as its blood flow, it could be excluded by forceps. After exclusion, the protocol of IR was applied, exactly as is described in experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava catheterization, which had been carried out after general anesthesia. The EE evaluation was performed at 60 min after reperfusion for groups A and C and at 120 min after reperfusion for groups B and D.

Protocol of the experiment

The experimental rats were given general anesthesia by initial intramuscular (IM) administration of 0.5 cc of a compound, constituting 0.25 cc xylazine, [25 cc, 20mg/cc] and 0.25 cc ketamine hydrochloride [1000, 100mg/cc, 10cc]. 0.03 cc butorphanol [10mg/cc, 10cc] anesthetic agent was administered subcutaneously (SC) before laparotomy. Continuous oxygen supply was administered during the whole experiment. Ischemia was caused by clamping inferior aorta over renal arteries for 45 min after laparotomic access. Reperfusion was achieved by removing the clamp and inferior aorta patency re-establishment. Forty (40) female Wistar albino rats were used with a mean weight of 231.875 gr [Std. Dev: 36.59703 gr], with min weight ≥ 165 gr and max weight ≤ 320 gr. Rats weight could be potentially a confusing factor, e.g. fatter rats to have greater EE thickness. This suspicion will be investigated. Also, detailed histopathological (Osmanağaoğlu et al. 2012) study (pathology) and grading of edema findings was performed by scores, this is: 0 when lesions were not found, 1 when mild lesions were found, 2 when moderate lesions were found and 3 when serious lesions were found. The previous grading is transformed as follows: (0-0.499) without lesions, (0.5-1.499) the mild lesions, (1.5 -2.499) the moderate lesions and (2.5-3) the serious lesions damage, because the study concerns score ranges rather than point scores.

Table 1. Weight and endometrial edema (EE) score mean levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weigh	243 gr	45.77 gr
	EE	mild 1.2	0.78
B	Weigh	262 gr	31.10 gr
	EE	mild 1.3	1.15
C	Weigh	212.5 gr	17.83 gr
	EE	mild 0.7	0.67
D	Weigh	210 gr	18.10 gr
	EE	mild 1	1.05

Std. Dev: standard deviation

Model of ischemia-reperfusion injury

Control groups: 20 control rats of mean weight 252.5 gr [Std. Dev: 39.31988 gr] were subjected to ischemia for 45 min followed by reperfusion.

Group A: 10 controls rats of mean weight 243 gr [Std. Dev: 45.77724 gr], mean mild EE score 1.2 [Std. Dev: 0.78] were subjected to 60 min reperfusion (Table 1).

Group B: 10 controls rats of mean weight 262 gr [Std. Dev: 31.10913 gr], mean mild EE score 1.3 [Std. Dev: 1.15] (Table 1) were subjected to 120 min reperfusion (Table 1).

Lazaroid (L) group: 20 rats of mean weight 211.25 gr [Std. Dev: 17.53755 gr] were subjected to ischemia for 45 min followed by reperfusion in the beginning of which 10 mg U-74389G/kg body weight were IV administered.

Group C: 10 L rats of mean weight 212.5 gr [Std. Dev: 17.83411 gr], mean mild EE score 0.7 [Std. Dev: 0.67] (Table 1) were subjected to 60 min reperfusion (Table 1).

Group D: 10 L rats of mean weight 210 gr [Std. Dev: 18.10463 gr], mean mild EE score 1 [Std. Dev: 1.05] (Table 1) were subjected to 120 min reperfusion (Table 1).

Results

Every weight group of rats was compared initially with another one from 3 remained groups applying statistical paired t-test (Table 1). Any emerging significant difference among EE scores will be investigated whether owed in the above mentioned significant weight correlations. Every EE scores rats groups initially were compared with other one from 3 remained groups applying Wilcoxon signed-rank test (Table 2). Applying generalized linear models (glm) with dependant variables of the EE scores and with independent variables of the U-74389G administration or without that, the reperfusion time and their interaction, resulted in: U-74389G administra-

Table 2. Statistical significance of mean values difference for groups (DG) after statistical paired t test application for weight and Wilcoxon signed-rank test for scores.

DG	Variable	Difference	p-value
A-B	Weight	-19 gr	0.2423
	EE	without lesions -0.1	0.8728
A-C	Weight	30.5 gr	0.0674
	EE	mild 0.5	0.2212
A-D	Weight	33 gr	0.0574
	EE	without lesions 0.2	0.5164
B-C	Weight	49.5 gr	0.0019
	EE	mild 0.6	0.1573
B-D	Weight	52 gr	0.0004
	EE	without lesions 0.3	0.4935
C-D	Weight	2.5 gr	0.7043
	EE	without lesions -0.3	0.4818

DG: difference for groups

Table 3. The decreasing influence of U-74389G in connection with reperfusion time.

Decrease	95% c. in		p-values	
		Reperfusion time	Wilcoxon	glm
mild 0.5	-1.18 - 0.18	1 h	0.2212	0.1451
without lesions 0.4	-0.99 - 0.19	1.5 h	0.1816	0.1781
without lesions 0.3	-1.34 - 0.74	2 h	0.4935	0.5525

c. in: confidence interval

tion non-significantly decreased without lesions the EE scores by 0.4 [-0.99 - 0.19] (p= 0.1781). This finding was also in accordance with the result of Wilcoxon signed-rank test (p= 0.1816). Reperfusion time non-significantly increased without lesions the EE scores by 0.2 [-0.40 - 0.80] (p= 0.5046), approximately in accordance with Wilcoxon signed-rank test also increased without lesions by 0.3 [-0.87 - 0.27] (p= 0.3330). However, U-74389G administration and reperfusion time together non-significantly decreased without lesions the EE scores by 0.16 [-0.52 - 0.19] (p= 0.3641). Reviewing above and Table 2, Table 3 sums up concerning the decreasing influence of U-74389G in connection with reperfusion time. Inserting the weight of the rats also as an independent variable at glm analysis, a non-significant relation results in (p=0.4934), so further investigation is not needed.

Discussion

The following clinical situations show the association between ischemia and EE. Canisso et al (2013) detected a reduction in endometrial edema during progestagen treatment, which returned to normal after cessation of treatment similarly with effectively abolished estrous behavior response to the stallion within 24h which returned to the control level after cessation of treatment, in mares, being received progestagen treatment when confirmed in estrus. Ludwig (1982) considered difficult to describe the morphology of the endometrial reaction, thus, he evaluated the endometria of women with respect to stromal edema. Sridhar et al. (2000) considered amniotic membrane transplantation in chemical injury with severe limbal ischemia and in severe thermal injury in acute phase, as in an eye with opaque cornea, stromal edema, and scarring within the first few weeks of injury. Batra et al. (1995) observed prominent hypoplasia of the syncytium, stromal edema, ischemia findings, increased basement membrane thickening and high villous edema scores statistically significant in numerous representative samples taken from premature placentas immediately after delivery as compared to controls.

EE is involved in endometrial cancer and infertility. Gaete et al (2012) have previously shown that an estradiol-like hypertrophy of uterine cells may not be associated with cell proliferation, uterine eosinophilia, or endometrial edema. However, estrogen-induced cell proliferation in the uterus increases the risk of cancer development. Gaete et al (2011) found that complete inhibition of estradiol-induced mitoses in uterine luminal epithelium, endometrial stroma, myometrium and RNA content are followed by partial inhibition estradiol-induced uterine eosinophilia and endometrial edema in prepubertal rats. Protection against estrogen-induced cell proliferation in uterus suggests the decrease of the risk for uterine cancer after hormone replacement therapy in climacteric women. Canisso et al. (2013) detected a reduction in endometrial edema during progestagen treatment, which returned to normal after cessation of treatment in cyclic mares. Witte et al. (2012) induced endometritis associated with increased amounts of intrauterine (i.u.) fluid or viscous mucus in estrus detected by ovarian follicle >3.0 cm and endometrial edema, which may contribute to low fertility in mares. Integrity of epithelium was not affected.

Conclusion

U-74389G administration hardly non-significantly decreases the EE within short-term time context of 2 hours. Perhaps, a longer study time may reveal clearer and more significant results. Also, a more detailed study of molecular pathway of antioxidant capacity involvement of U-74389G in edema is required.

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References

Batra S, Sen R, Jain K, Gulati N (1995) Phase contrast microscopic evaluation of placental pathology in premature gestation. Indian J Pathol Microbiol 38(4):407-411.

Canisso IF, Gallacher K, Gilbert MA, Korn A, Schweizer CM, Bedford-Guaus SJ, Gilbert RO (2013) Preovulatory progestagen treatment in mares fails to delay ovulation. Vet J 197(2):324-328.

Canisso IF, Gallacher K, Gilbert MA, Korn A, Schweizer CM, Bedford-Guaus SJ, Gilbert RO (2013) Preovulatory progestagen treatment in mares fails to delay ovulation. Vet J 197(2):324-328.

Cayman Chem Prod Cat https://www.caymanchem.com/app/template/Product.vm/catalog/75860.

Gaete L, Tchernitchin AN, Bustamante R, Villena J, Lemus I, Gidekel M, Cabrera G, Astorga P (2012) Daidzein-estrogen interaction in the rat uterus and its effect on human breast cancer cell growth. J Med Food 15(12):1081-1090.

Gaete L, Tchernitchin AN, Bustamante R, Villena J, Lemus I, Gidekel M, Cabrera G, Carrillo O (2011) Genistein selectively inhibits estrogen-induced cell proliferation and other responses to hormone stimulation in the prepubertal rat uterus. J Med Food 14(12):1597-1603.

Ludwig H (1982) The morphologic response of the human endometrium to long-term treatment with progestational agents. Am J Obstet Gynecol 142(6 Pt 2):796-808.

Osmanağaoğlu MA, Kesim M, Yuluğ E, Menteşe A, Karahan SC (2012) Ovarian-protective effects of clotrimazole on ovarian ischemia/reperfusion injury in a rat ovarian-torsion model. Gynecol Obstet Invest 74(2):125-130.

Shi F, Cavitt J, Audus KL (1995) 21-aminosteroid and 2-(aminomethyl) chromans inhibition of arachidonic acid-induced lipid peroxidation and permeability enhancement in bovine brain microvessel endothelial cell monolayers. Free Rad Biol Med 19(3):349-357.

Sridhar MS, Bansal AK, Sangwan VS, Rao GN (2000) Amniotic membrane transplantation in acute chemical and thermal injury. Am J Ophthalmol 130(1):134-137.

Witte TS, Melkus E, Walter I, Senge B, Schwab S, Aurich C, Heuwieser W (2012) Effects of oral treatment with N-acetylcysteine on the viscosity of intrauterine mucus and endometrial function in estrous mares. Theriogenology 78(6):1199-1208.

Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A (2014) The effect of the antioxidant drug "U-74389G" on chloride during ischemia reperfusion injury in rats. Med Rev 50(2):40-44.