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RESEARCH REPORT

Comparison of the hypoprogesteronaemic effects of erythropoietin and U-74389G in an experimental model of ischaemia–reperfusion in rats

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Abstract: *Background:* The lazaroid U-74389G is an antioxidant whose experimental assessment so far has suggested a wide variety of potential therapeutic applications. Interestingly, U-74389G has also been shown to be able to trigger hypoprogesteronaemia. *Aim:* The aim of this study was to record and compare the effects of U-74389G and of erythropoietin on the serum progesterone levels of rats submitted to ischaemia–reperfusion. *Methodology:* We applied a laparotomic clamping of the inferior aorta over the renal arteries of female Wistar rats in order to cause reversible ischaemia for 45 min. The subsequent clamp removal restored the excluded inferior aorta patency and led to reperfusion, while the assessed agents were administered (both at a dose of 10 mg/kg of body weight) at the beginning of the reperfusion phase, through an inferior vena cava catheter. Serum progesterone levels were determined at 60, 90, and 120 min after the induction of reperfusion. *Results:* The lazaroid U-74389G was found to exert a significant hypoprogesteronaemic effect in rats subjected to ischaemia–reperfusion; in particular, significant hypoprogesteronaemia was noted at 90 and 120 min after the commencement of the reperfusion / U-74389G administration. U-74389G was found to be 5.46-fold more hypoprogesteronaemic than erythropoietin ($p<0.0001$), at least within the timeframe of the herein employed experimental conditions. *Conclusion:* U-74389G exerts a significant hypoprogesteronaemic effect in rats undergoing ischaemia–reperfusion, and this effect is superior to that induced by erythropoietin.

Keywords: erythropoietin; ischaemia; reperfusion; serum progesterone levels; U-74389G

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Despite the fact that we have previously reported the hypoprogesteronaemic capacity of U-74389G (Tsompos *et al.*, 2016), this property is not widely recognized among the characteristic actions of this lazaroid. The effects of U-74389G have been examined in a number of experimental studies employing the ischaemia–reperfusion type of tissue injury, in which the tissue-protective capacity of U-74389G has been established. The lazaroid U-74389G is an antioxidant that can prevent iron-dependent or arachidonic acid-induced lipid peroxidation *in vivo*. Moreover, animal heart models, brain microvascular endothelial cells monolayers, liver, and kidneys have been found to be effectively protected by U-74389G from the effects of an ischaemia–reperfusion type of injury. In fact, U-74389G has anti-shock properties, can protect the endothelium, can enhance mononuclear immunity, can trigger the production of cytokines, can treat the endotoxin-induced

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Table 1. The extent of the erythropoietin-induced hypoprogesteronaemia as a function of the reperfusion time.

Reperfusion time / endpoint	**Hypoprogesteronaemia** (mean ± standard deviation)	***p*-value**
60 min	-0.21% ± 52.57%	0.9904
90 min	-9.33% ± 40.64%	0.3549
120 min	-18.45% ± 31.95%	0.1509
reperfusion (placebo)	+9.33% ± 43.73%	0.3721
interaction (overall)	-5.05% ± 6.73%	0.4430

Table 2. The extent of the U-74389G-induced hypoprogesteronaemia as a function of the reperfusion time.

Reperfusion time / endpoint	**Hypoprogesteronaemia** (mean ± standard deviation)	***p*-value**
60 min	-39.00% ± 59.33%	0.0663
90 min	-49.58% ± 47.51%	0.0001
120 min	-60.15% ± 34.51%	0.0003
reperfusion (placebo)	+9.91% ± 45.07%	0.4103
interaction (overall)	-27.59% ± 7.46%	0.0005

shock, can down-regulate the proinflammatory genes, and can attenuate leukocytes.

Erythropoietin, a cytokine of ambiguous hypoprogesteronaemic capacity (Tsompos *et al.*, 2014), can be considered as a suitable reference agent for comparison with U-74389G in this regard. To this end, the aim of our study was to record and compare the short-term effects of erythropoietin and of U-74389G on the serum progesterone levels of female Wistar rats submitted to ischaemia–reperfusion.

Methodology

The experimental setup described in this article has also been previously described in detail (Tsompos *et al.*, 2014; 2016). The experimental protocol was approved by the Scientific Committee of the Hippocration General Hospital (National and Kapodistrian University of Athens) and by the Veterinary Service of the Prefecture of East Attica (Greece) as defined by law (3693/12-11-2010; 14/10-1-2012). The experiments were carried out at the Experimental Research Center of ELPEN Pharmaceuticals (Pikermi, Attica, Greece).

In brief, 60 female albino Wistar rats (16–18-weeks-old) were randomly assigned into six groups (n=10 rats each). The common stage of a 45-min hypoxia was applied in all six groups. Subsequently, reperfusion was applied: for 60 min (group A), for 120 min (group B), for 60 min with intravenous (i.v.) administration of erythropoietin (group C), for 120 min with i.v. administration of erythropoietin (group D), for 60 min with i.v. administration of U-74389G (group E), and for 120 min with i.v. administration of U-74389G (group F). Both agents were administered at a dose of 10 mg/kg of body weight.

For the herein described ischaemia–reperfusion experiments, we employed the laparotomic clamping of the inferior aorta over the renal arteries of the rats in order to cause reversible ischaemia for 45 min. The subsequent clamp removal restored the excluded inferior aorta patency and led to reperfusion, while the assessed agents were administered at the beginning of the reperfusion phase, through an inferior vena cava catheter. Serum progesterone levels were determined at 60, 90, and 120 min after the induction of reperfusion.

Results

Table 1 presents our findings regarding the extent of the erythropoietin-induced hypoprogesteronaemia as a function of the reperfusion time, while Table 2 presents the respective data for U-74389G. Chi-squared tests have been applied using the odds ratios associated with the U-74389G / erythropoietin hypoprogesteronaemic efficacies at the herein assessed endpoints; the outcomes of these tests are presented in Table 3. In brief, U-74389G has been found to cause a highly significant 182.04-fold hypoprogesteronaemic effect than that caused by erythropoietin at 60 min after the induction of reperfusion (Table 3). Moreover, the hypoprogesteronaemic effect of U-74389G has been found to be 5.31- and 3.26-fold that of erythropoietin at 90 and 120 min after the induction of reperfusion, respectively (Table 3). Overall, the hypoprogesteronaemia caused by U-74389G was found to be 5.46-fold that induced by erythropoietin ($p<0.0001$), at least within the timeframe of the employed experimental conditions (Table 3).

Discussion

To our knowledge, there has been no other study investigating the hypoprogesteronaemic effect of U-74389G

Table 3. The U-74389G / erythropoietin hypoprogesteronaemic efficacies after chi-squared testing.

Reperfusion time / endpoint	Odds ratio [95% confidence interval]	*p*-value
60 min	182.04 [181.64–182.45]	<0.0001
90 min	5.31 [5.30–5.32]	<0.0001
120 min	3.26 [3.26–3.26]	<0.0001
reperfusion (placebo)	1.06 [1.06–1.06]	<0.0001
interaction (overall)	5.46 [5.45–5.46]	<0.0001

besides ours (Tsompos *et al.*, 2016). Apart from the lazaroid's widely-reported neuroprotective and membrane-stabilizing properties (Bimpis *et al.*, 2012; 2013; 2015), U-74389G has been shown to be a promising anti-inflammatory drug for the treatment of the reperfusion syndrome in ischaemia–reperfusion-involving heart injury (Perna *et al.*, 1996), to exert antiproliferative properties on brain cancer cells (Durmaz *et al.*, 1999), to act as an immunosuppressant in a flap survival context (Karamatsoukis *et al.*, 2020), to be beneficial for the management of septic states (Chang *et al.*, 2011), to increase γ-glutamyltransferase, superoxide dismutase, and glutathione (GSH) levels in hyperoxia-exposed cells (van Klaveren *et al.*, 1997), and to protect against ototoxicity (Hori and Kanno, 1999).

Güleç Başer *et al.* (2018) have concluded that a pre-operative treatment with progesterone might exert anti-apoptotic and antioxidative effects on ovarian tissue ischaemia–reperfusion injury, based on experimental findings in female rats. About the same time, Faheem *et al.* (2019) have shown that progesterone administration can reduce the white matter injury, the microglial activation, and the infarct size after focal cerebral ischaemia–reperfusion injury in male Wistar rats. Moreover, Keshavarzi *et al.* (2018) have reported that the administration of oestradiol alone or combined with progesterone can significantly increase GSH levels in male Wistar rats exposed to gastric ischaemia–reperfusion, while Vahidinia *et al.* (2017) have reported that a steroid hormone (oestrogen and progesterone) treatment can significantly reduce the neurological deficits, the infarct volume, the extent of the penumbra's pathology, and the induction of proteases on both extrinsic and intrinsic apoptotic signalling pathways in male Wistar rats after transient middle cerebral artery occlusion. A few years earlier, Remus *et al.* (2015) had conducted a study in male Sprague-Dawley rats that had also undergone transient middle cerebral artery occlusion, and had shown that progesterone administration was able to protect endothelial cells after cerebrovascular occlusion by decreasing macrophage infiltration.

In experiments examining the structure and function of autografted ovaries in mice, progesterone levels have been found to decrease as a result of the ovarian tissue autografting (Shojafar *et al.*, 2018; Noori Hassanvand *et al.*, 2019); the experimental simulation of a fertility restoration technique that is commonly employed in clinical practice, particularly in young patients who undergo radio- and / or chemotherapy due to cancer. As the ischaemia–reperfusion-induced injury is a critical limiting factor for the success of this technique, maintaining high progesterone levels could be particularly beneficial.

Unfortunately, our experiments have shown that the lazaroid U-74389G can exert a significant hypoprogesteronaemic effect in female rats undergoing ischaemia–reperfusion, and that this effect is superior to that induced by an equal dose of erythropoietin (Table 3). However, this finding should be assessed along with the plethora of the biochemical effects that U-74389G has been shown to exert under the same experimental conditions (Tsompos *et al.*, 2024).

Conclusion

Our study confirms that U-74389G can exert a short-term hypoprogesteronaemic effect in rats undergoing ischaemia–reperfusion, and that this effect is superior to that of erythropoietin. Future studies should pursuit the biochemical elucidation of how U-74389G is able to exert this effect.

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Conflicts of interest statement

None to declare.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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