The effect of the antioxidant drug “U-74389G” on phosphorus during ischemia reperfusion injury in rats

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Key words: U-74389G, phosphorus, reperfusion

Abstract
the aim of this experiment study was the U-74389G testing, on rat model and particularly in ischemia reperfusion (IR) protocol. The beneficial or non effect of that molecule were studied biochemically on using mean blood phosphorus.

Material and methods
40 rats were used of mean weight 231.875 gr. Phosphorus were measured on these time points: on 60 min after reperfusion (groups A and C), and on 120 min after reperfusion (groups B and D), A and B without but C and D with U-74389G administration.

Results
were that 1) U-74389G administration decreased non significantly the phosphorus by 0.180 mg/dl [-0.9077379 mg/dl - 0.5477378 mg/dl] (P=0.6195), in accordance also with paired t-test (P=0.5383), 2) reperfusion time decreased non significantly the phosphorus by 0.2 mg/dl [-0.9271745 mg/dl - 0.5271746 mg/dl] (P=0.5809), in accordance also with paired t-test (P=0.5446), and 3) interaction of U-74389G administration and reperfusion time decreased non significantly the phosphorus by 0.1218182 mg/dl [-0.5602848 mg/dl - 0.3166483 mg/dl] (P=0.5771).

Conclusions
are that U-74389G administration, reperfusion time and their interaction have a general but not significant short – term effects on phosphorus levels within time context of 2 hours. Perhaps, longer experiment times may reveal some possible significant decreasing effect of U-74389G on blood phosphorus.
Introduction

Tissue ischemia and reperfusion (IR) remain out of main causes of damage (permanent or transient) with serious implications on near organs and certainly on patients’ health. The use of antioxidant substances has been a research subject a lot of years ago. However, even if important progress has been made, satisfactory answers have not been given yet in fundamental questions, as, how much powerful should an antioxidant be, when should it be administered, and in which dosage. The particularly satisfactory action of antioxidant U-74389G in tissue protection was noted in performed experiments. It was realized that this certain antioxidant has been tried in IR experiments, after international literature (PubMed – medline) careful examination. However, just few relative reports were found, not covering completely this particular matter. Also, a lot of publications concerned trials of such similar other molecules of aminosteroids (lazaroids) suppressor in which the studied molecule also belongs to.

Aim of the experiment

Aim of present experimental study was the trial of U-74389G in rat animal model and precisely in IR protocol. The benefit or not of that particular molecule was studied measuring blood phosphorus.

Experimental groups

This experimental study was laid out by Experimetal Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki, and all of settings including of consumables, equipment and substances used, were a courtesy of that S. A. Wistar rats were used in accordance with accepted standards of humane animal care. They spent in laboratory 7 days before experimentation with easy access in water and food. They were randomly assigned into the following experimental groups (10 animals in each group). The experiment was acute, that is, the animal usage was completed by following experimentation times expiring as awakening and preservation did not exist.

1 - Ischemia for 45 min and afterwards reperfusion for 60 min (group A).
2 - Ischemia for 45 min and afterwards reperfusion for 120 min (group B).
3 - Ischemia for 45 min and afterwards immediate U-74389G intravenous (IV) administration and reperfusion for 60 min (group C).
4 - Ischemia for 45 min and afterwards immediate U-74389G IV administration and reperfusion for 120 min (group D).

The molecule U-74389G dose was 10 mg/Kg body weight of animals.

The experiment started with prenarcosis and general anaesthesia administration in animals. Their electrocardiogram and acidometry were continuously monitored. The vessels concerning blood supply, were prepared so as their flow to be excluded by forceps. After exclusion, the protocol of IR was applied, described more in experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava (catheterization had been preceded at experiment beginning, after general anaesthesia establishment).

The phosphorus measurement was performed on these time points:

1 - on 60 min of reperfusion (groups A and C),
2 - on 120 min of reperfusion (groups B and D).

Protocol of the experiment

Rats were introduced into general anaesthesia by initial intramuscular (IM) administration of 0,5 cc compound, constituted by 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] anaesthesia was administered
subcutaneous (s.c.) before laparotomy. Continuous oxygen supply was administered during whole experiment performance. Ischemia was caused by clapping inferior aorta for 45 min after laparotomic access. Reperfusion was achieved by removing clapping and inferior aorta patency re-establishment.

40 Wistar rats of mean weight 231.875 gr [Std. Dev: 36.59703 gr] were used, min weight ≥ 165 gr and max weight < 320 gr.

**Control groups**

20 control rats mean weight 252.5 gr [Std. Dev: 39.31988 gr] suffered by ischemia for 45 min and then reperfusion.

**Group A**

Reperfusion which lasted 60 min concerned 10 controls rats of mean weight 243 gr [Std. Dev: 45.77724 gr], mean phosphorus 11.29 mg/dl [Std. Dev: 1.341185 mg/dl] (Table 1).

**Group B**

Reperfusion which lasted 120 min concerned 10 controls rats of mean weight 262 gr [Std. Dev: 31.10913 gr], mean phosphorus 11.02 mg/dl [Std. Dev: 1.329829 mg/dl] (Table 1).

**Lazaroid group**

20 rats of mean weight 211.25 gr [Std. Dev: 17.53755 gr] suffered by ischemia for 45 min and then reperfusion in the beginning of which 10 mg U-74389G /kg body weight were IV administered.

**Group C**

Reperfusion which lasted 60 min concerned 10 L rats of mean weight 212.5 gr [Std. Dev: 17.83411 gr], mean phosphorus 11.04 mg/dl [Std. Dev: 1.224019 mg/dl] (Table 1).

**Group D**

Reperfusion which lasted 120 min concerned 10 L rats of mean weight 210 gr [Std. Dev: 18.10463 gr], mean phosphorus 10.91 mg/dl [Std. Dev: 0.5839522 mg/dl] (Table 1).

Weight comparison of each one from 4 rats groups initially was performed with other one from 3 remained groups applying statistical paired t-test. (Table 2). Any emerging significant difference among phosphorus, will be investigated whether owed in the above mentioned significant weight correlations. Phosphorus comparison of each one from 4 rats groups initially was performed with other one from 3 remainder groups applying statistical paired t-test. (Table 2). Applying generalised linear models (glm) with dependant variable the phosphorus levels and independent variables the U-74389G administration or no, the reperfusion time and their interaction, results in: 1) U-74389G administration decreased non significantly the phosphorus by 0.180 mg/dl [-0.9077379 mg/dl - 0.5477378 mg/dl] (P=0.6195), in accordance also with paired t-test (P=0.5383), 2) reperfusion time decreased non significantly the phosphorus by 0.2 mg/dl [-0.9271745 mg/dl - 0.5271746 mg/dl] (P=0.5809), in accordance also with paired t-test (P=0.5446), and 3) interaction of U-74389G administration and reperfusion time decreased non significantly the phosphorus by 0.1218182 mg/dl [-0.5602848 mg/dl - 0.3166483 mg/dl] (P=0.5771).

Reviewing the above and table 2, the table 3 sums up concerning the decreasing influence of U-74389G in connection with reperfusion time. Inserting the rats weight also as an independent variable at generalised linear models analysis, a non significant relation results in phosphorus (p=0.1636), so as to further investigation does not need.
Table 1: Weight and phosphorus (P) mean levels and Std. Dev. of groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variable</th>
<th>Mean</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight</td>
<td>243 gr 11.29 mg/dl</td>
<td>45.77724 gr 1.341185 mg/dl</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>262 gr 11.02 mg/dl</td>
<td>31.10913 gr 1.341185 mg/dl</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>P</td>
<td>210 gr 10.91 mg/dl</td>
<td>18.10463 gr 0.5839522 mg/dl</td>
</tr>
</tbody>
</table>

Table 2: Statistical significance of mean values difference for groups (DG) after statistical paired t test application.

<table>
<thead>
<tr>
<th>DG</th>
<th>Variable</th>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>Weight</td>
<td>-19 gr 0.27 mg/dl</td>
<td>0.2423 0.6186</td>
</tr>
<tr>
<td>A-C</td>
<td>Weight</td>
<td>30.5 gr 0.25 mg/dl</td>
<td>0.0674 0.5932</td>
</tr>
<tr>
<td>A-D</td>
<td>Weight</td>
<td>33 gr 0.38 mg/dl</td>
<td>0.0574 0.4566</td>
</tr>
<tr>
<td>B-C</td>
<td>Weight</td>
<td>49.5 gr -0.02 mg/dl</td>
<td>0.0019 0.9704</td>
</tr>
<tr>
<td>B-D</td>
<td>Weight</td>
<td>52 gr 0.11 mg/dl</td>
<td>0.0004 0.7781</td>
</tr>
<tr>
<td>C-D</td>
<td>Weight</td>
<td>2.5 gr 0.13 mg/dl</td>
<td>0.7043 0.7587</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Decrease</th>
<th>95% c. in</th>
<th>Reperfusion time</th>
<th>p-values</th>
<th>glm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 mg/dl</td>
<td>-1.456339 mg/dl - 0.956339 mg/dl</td>
<td>1h</td>
<td>0.5932</td>
<td>1.0000</td>
</tr>
<tr>
<td>0.18 mg/dl</td>
<td>-0.9077379 mg/dl - 0.5477378 mg/dl</td>
<td>1.5h</td>
<td>0.5383</td>
<td>0.6195</td>
</tr>
<tr>
<td>0.11 mg/dl</td>
<td>-1.074926 mg/dl - 0.8549259 mg/dl</td>
<td>2h</td>
<td>0.7781</td>
<td>0.8477</td>
</tr>
</tbody>
</table>
Discussion

Unpleasantly, there are not described situations concerning whether ischemia can influence the phosphorus levels in bibliography. On the contrary, there are a lot of cases reporting how the phosphorus levels fluctuations affect the function of various organs. Such examples are described herein. Since isolated phosphorus administration is impossible, it is meant that, phosphorus was associated by another drug or a factor influencing the phosphorus levels was administered. Karabulut K et al found1 the increase in the levels of serum phosphorus in New Zealand rabbits acute mesenteric ischemia (AMI) group, to be statistically significant compared to control and sham groups (p<0.05). The levels of phosphorus were detected to increase from the 1st hour after ischemia onset continued for the following 6 hours (p<0.05). Smyrniotis V et al included2 patients that underwent liver resection and experienced low postoperative phosphorus levels. The lowest levels were observed on the second postoperative day, when 40% developed life-threatening hypophosphatemia (< 1.1 mg/dl). Warm ischemia (vascular exclusion) and major resections aggravated hypophosphatemia < 1.5 mg/dl being more prone to complications compared with counterparts who had serum phosphorus levels > 1.6 mg/dl. O’Hare AM et al associated3 previous diagnosis of peripheral vascular disease (PVD), and elevated serum phosphorus level with the outcome of lower-extremity amputation within 2 years in hemodialysis patients. Wilmer WA et al investigated4 the extent of skin ischemia in patients with calciphylaxis by means of transcutaneous oxygen tension (TCPO(2)) measurement, a noninvasive test that accurately assesses skin oxygenation. Compared with controls, patients with calciphylaxis showed significantly lower TCPO(2) levels at each body region. No correlation with serum parathyroid hormone (PTH), or serum phosphorus values was present. In conclusion, TCPO(2) levels are abnormally low in patients with calciphylaxis, indicating that severe and diffuse skin ischemia exists, even at areas free of skin lesions. Unpleasantly, there is not bibliography concerning the study of phosphorus alteration along with U-74389G administration. So, any conclusion emerges regarding this substances combination, will be based exclusively on this study.

Conclusion

U-74389G administration, reperfusion time and their interaction have a general decreasing but not significant short – term effect on phosphorus levels within time context of 2 hours. Perhaps, longer experiment times may reveal some possible significant decreasing effect of U-74389G on blood phosphorus.

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References


