Drotaverinum – a new modality of prevention and treatment in cerebral vasospasm after subarachoidian hemorrhage?

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Abstract: *Aim*: This study want to demonstrate the efficaciousness of drotaverinum as a replacer of papaverine in the prevention and treatment of vasospasm. *Material and method*: In this study were used 20 albino Wistar male rats. Rats were divided in two groups and vasospasm was induced to the both femoral artery and after that irrigation of the femoral arteries with drotaverinum was performed to demonstrate the vasodilatation that can appear (group A). In the group B after the obtaining of vasospasm irrigation of the femoral arteries with saline solution was performed and this group was used as witness. The length of the vessels was measured when was achieved the vasospasm and also before and after the administration of the solutions. Pictures were taken at every step of dissection and solutions administration to can measure the length of arteries before and after the administration of drotaverinum and the saline solution. *Results*: In all rats was obtained vasospasm at the femoral artery after clipping the artery and after we irrigate with adrenaline. In the group with drotaverinum we obtained the vasodilatation and in the witness group the caliber of the vessels remain the same. The statistical analysis of the data demonstrate a significant differences between the group were was used drotaverinum and the witness group were was used the saline solution ($p<0,001$). *Conclusions*: Drotaverinum has a good vasodilatative effect on arteries and he can prevent the apparition of vasospasm and it can even treat vasospasm if occur by producing local vasodilatation and a good circulation in the area where is administrated. This study showed quantitatively that drotaverinum can treat the experimental peripheral vasospasm in rats.

Key words: vasospasm, drotaverinum, experimental study

Introduction

Cerebral vasospasm can occur after subarachnoidian hemorrhage and is one of the leading causes of morbidity and mortality in this type of pathology (1-3). Subarachnoidian hemorrhage represent the bleeding in the subarachnoidian space and the most frequent pathology that can determine that bleeding are the ruptured aneurysms (4).

Cerebral vasospasm after subarachnoidian hemorrhage represent a challenge problem from the prevention and treatment point of view and contributes to the most devastating injury: delayed cerebral ischemia (2, 5, 6). When delayed cerebral ischemia occur the patient outcome is poor and this can lead even to death (6).

The objective of our study was to demonstrate on rats models the benefits of drotaverinum when is applied on a spastic artery and to demonstrate the efficacy instead of papaverine. Also intraoperatory cisternal irrigation with drotaverinum of the arteries after the aneurysm is clipped is performed to prevent the apparition of the vasospasm or even to treat this if already has developed. In our statistics the incidence of cerebral vasospasm is lower than the data from the literature and we thought that is happening because of the use of drotaverinum.

In the literature are studies about cisternal irrigation after clipping the aneurysm but with papaverine not with drotaverine (7). In literature are studies about the resistance against intra-arterial papaverine in cerebral vasospasm or about the efficacy of papaverine in this type of pathology but about drotaverinum there's no study to see if is a good option to use it instead of papaverine (8, 9).

Materials and methods

Animals and housing

Twenty (n=20) male HsdOla:WI rats weighing between 300 and 350 grams were used in the present study. The animals were housed in polysulfone type III open-top cages (Tecniplast, Italy) and had access to filtered tap water in bottles and peletted feed (Nutret combinat granulat, Cantacuzino Institute, Romania) ad libitum. The rats were kept in the Laboratory Animal Facility of the „Iuliu Hatieganu" University of Medicine and Pharmacy at a standard temperature of 24 ± 2 °C, a relative humidity of 55 ± 10%, 12:12-h light:dark cycle (lights on, 0700 to 1900). All experimental protocols were approved by the Ethics Comittee of the University (no. 301/29.05.2015) and were conducted in accordance the EU Directive 63/2010, as per which, the experimental procedure severity was classified as moderate, as the animals fully recovered and were not sacrificed after the procedure.

Experimental procedure

The animals were randomly assigned into two (n=2) groups of ten (n=10) individuals per group. Group A was used to assess the efficiency of drotaverinum for preventing the occurrence of vasospasm, while group B was used as a control group. The rats were anesthetized by a intramuscular injection of a Xylazine:Ketamine cocktail in a dosage of 8 mg/kg Xylazine to 80 mg/kg Ketamine. After

anesthesia, the animals were positioned in a dorsal decubitus.

After positioning the rats was performed shaving the medial part of the posterior legs and was make a skin incision about 2 cm and dissect the anatomical structures till was discovered the femoral artery and of course the femoral vein and sciatic nerve in the medial and respectively lateral side of artery. The dissection was continued till the femoral artery was isolated (figure 1). After the preparation of the femoral artery a vascular clip was put (figure 2) and maintained it for 2 minutes and at the same time the artery was irrigated with adrenaline (0,1 ml with concentration of 1/1000). After 2 minutes the clip was removed and the narrowing of the lumens artery was obtained (figure 3). When the experimental vasospasm was obtained the irrigation of the femoral artery with drotaverinum (0,1 ml) was performed and after another 2 minutes the vessel was dilated (figure 4). This protocol was applied for all the 10 animal models from the group A and the experiment was made on the both posterior legs.

In the witness group was used the same protocol as was mentioned above with the exception that the irrigation of the femoral artery, after the experimental vasospasm, was made with saline solution not with drotaverinum. In this group was observed that the saline solution does not produce vasodilatation and the vessels remains narrowed were was put the vascular clip.

The dissections were performed with microsurgical instruments under the microscope (Leika). Each step of the study protocol with the femoral artery was photographed. After finishing the study measurements on arteries after clipping, after tamponing with drotaverinum and with saline solution was achieved. The measurements were performed with a special soft of morphometry (Axiovision Rel 4.6.) (figures 5 and 6).

After the experimental study the animal models were observed till 7 days to see if they develop some complications after the vasospasm that was induced. In group A doesn't occur any complication due to vasospasm, but in the group B occurred 2 posterior limb ischemia that maybe was produced by the narrowing of the femural artery.

The statistical analysis was performed using MedCalc soft. The statistical tests which were applied were T-test and correlation coefficient that will be shown in the results.

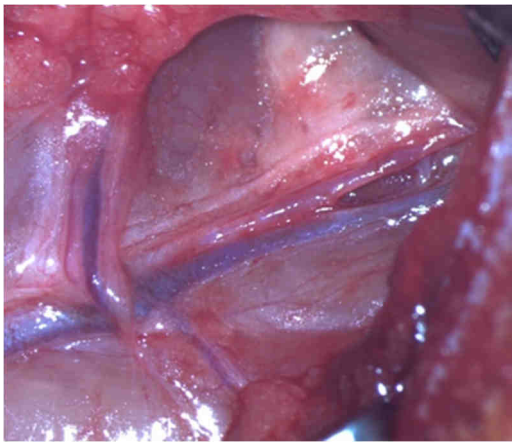


Figure 1 - Femoral artery of the right posterior limb and its relationship with the sciatic and femoral vein

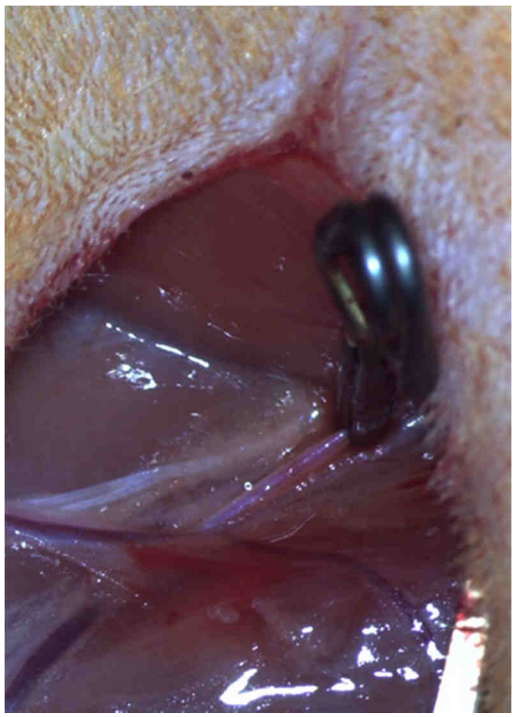


Figure 2 - The vascular clip on the femoral artery

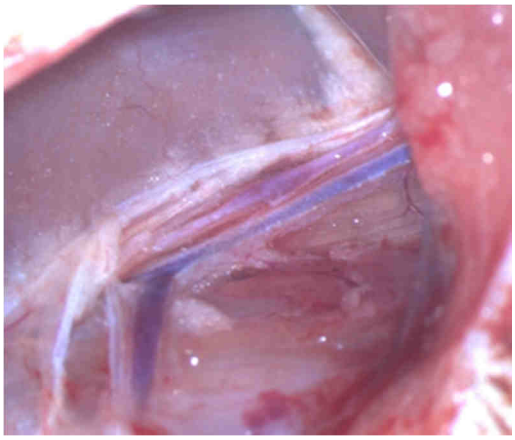


Figure 3 - We can observe the experimental vasospasm obtained on femoral artery that appeared after clipping and irrigate the artery with adrenaline

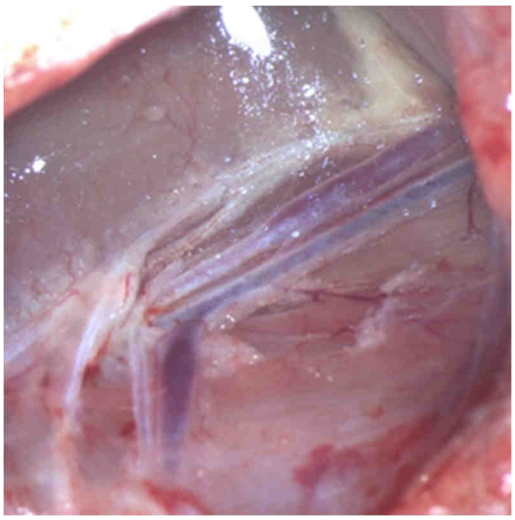


Figure 4 - The dilated artery after the irrigation with drotaverinum

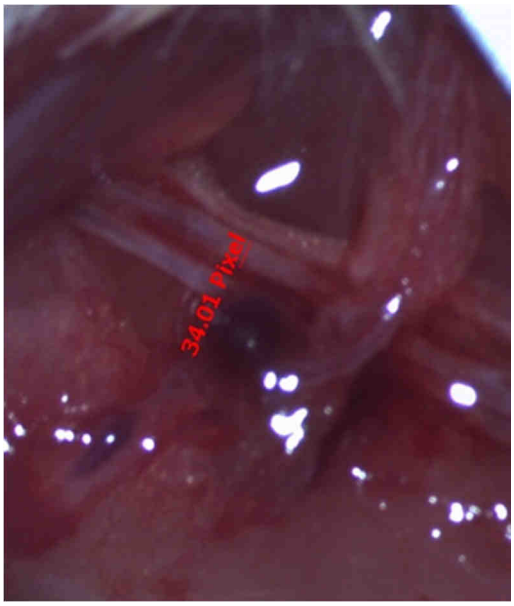


Figure 5 - Measurement of the femoral artery after clipping and irrigation with adrenaline

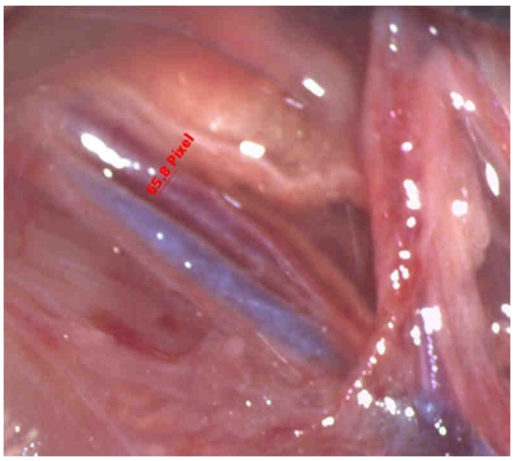
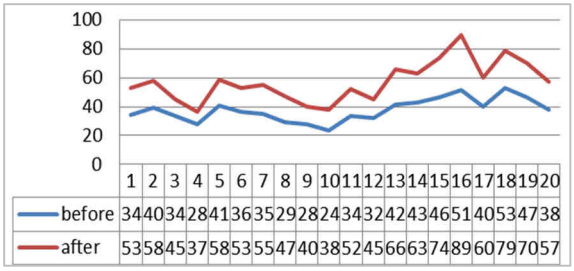


Figure 6 - Measurement of the femoral artery after irrigation with drotaverinum



Graph no.1 The diameter of the vessel is depicted before and after the irrigation of the arteries with drotaverinum

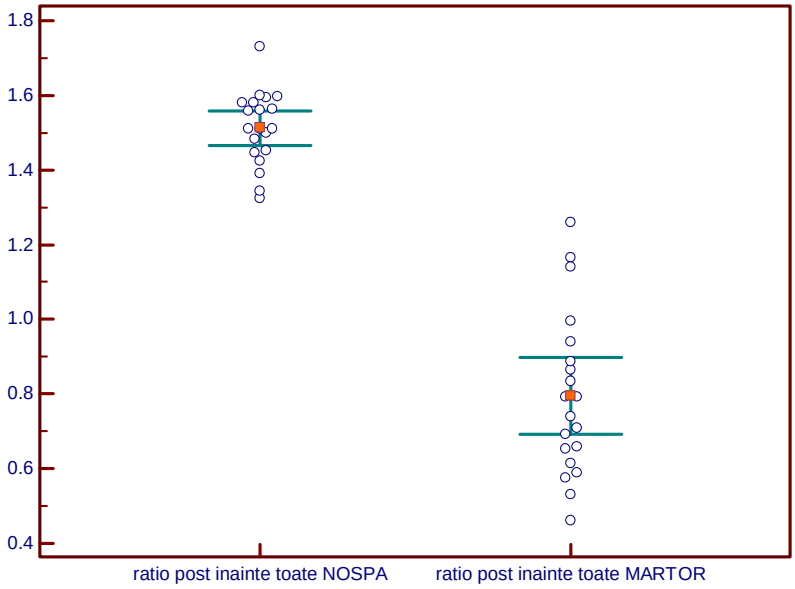


Figure 7 - Data platted for group A and B. Error bars drown at the 95% confidence interval. Red points representing the means

Results

After the dissections and the measurements that were performed on the femoral arteries we analyzed all the data using MedCalc soft.

The first statistical tests was applied to see if there is a significant difference between group A were was used the drotaverinum and the group B were was not administrated the drotaverinum. After the statistical analysis we obtained a significant difference between the

two groups with p<0,0001 and that suggest the benefits of the irrigation with drotaverinum when vasospasm is occurred.

In Graph no.1 the diameter of the vessel is depicted before and after the irrigation of the arteries with drotaverinum. The mean of the vessels before irrigation with drotaverinum was 37,66 and after the administration of drotaverinum the mean was 56,97. The statistical analysis depicted a significant difference between the two means (p<0,05).

Statistical tests in the group B were applied before and after the irrigation with saline solution. The mean before irrigation was 45,44 and after irrigation with saline solution the mean was 44,23. In this group we observe the saline solution has no effect on the vessels.

Between the two groups there was no differences in the diameter of the vessels before the irrigation with drotaverinum or with the saline solution and the standard deviation was 7,83±1,75.

After the irrigation with drotaverinum in the group A and the irrigation with saline solution the group B the mean of the vessels diameter in the first group was 52,02 and in the group B the mean was 44,23. The statistical analysis depicted a significant difference between the two means (p<0,001).

All the data were analyzed and we obtained a statistically significant difference between the two groups after the irrigation with drotaverinum (p<0,001) with a standard deviation 13,76±3,07.

In figure 7 we can see the data platted for group A and B. The red points represent the means. p<0.001.

Discussion

Cerebral vasospasm is a difficult pathological entity to treat or to deal with it and also in the literature are a lot of studies and trials about the controversies concerning the prevention and the treatment of the cerebral vasospasm that can occur after subarachnoidian hemorrhage (10-13).

Cerebral vasospasm after subarahnoidian hemorrhage produced by ruptured aneurysms remains still a very controversial topic (14). Cerebral vasospasm represent the leading cause of mortality and morbidity in subarachnoidian hemorrhage and can lead to delayed cerebral ischemia (1, 2). In the treatment of cerebral vasospasm the only medication that was proven to reduce delayed cerebral ischemia is the nimodinipine (1, 15-17).

About 30% of patients who suffered nontraumatic subarachnoidian hemorrhage develop cerebral vasospasm and secondary to this they develop ischemia (10). Cerebral vasospasm occur often in the third day after the hemorrhage and in the day 5 to 7 riches the maximum (11).

In the treatment of cerebral vasospasm are included the triple H therapy (hypertension, hypervolemia, hemodilution), balloon angioplasty, intra-arterial vasodilatators, administration of substances like statins, endothelina-1 antagonists and magnesium sulfate (2, 5, 11).

Conclusions

Our study demonstrate the importance of using the drotaverinum after the vasospasm occurrence and is very important to perform

irrigation with drotaverinum on the cerebral arteries after clipping the aneurysm because this will prevent the occurrence of vasospasm or will treat it if occurred.

Drotaverinum has no complication if we use it to irrigate the cerebral arteries and the vasodilatation that appears after using drotaverinum is useful because is achieved a better blood circulation in the area and prevent the ischemia occurrence.

In our opinion after the aneurysm is clipped the irrigation of the vessels, surrounding the aneurysm, with drotaverinum can decrease the incidence of vasospasm. Also if, intraoperative, we find a visible vasospasm the irrigation of the arteries with drotaverinum will help the vessel to dilate and to return to optimal caliber for the blood flow.

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