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ORIGINAL ARTICLE

Male Reproduction

Morphological and microcirculatory evaluation of the rat testis after detorsion with or without a capsular release with a tunica vaginalis flap

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Testicular torsion may lead to serious ischemia, and the viability depends on the duration of torsion and the effect of ischemia-reperfusion. Testicular decompression and tunica vaginalis flap application technique were introduced in 2008 by Kutikov *et al*. We aimed to examine the impact of this method on the testicular microcirculation and hemorheological parameters in a rat model. Six adult rats underwent bilateral scrotal exploration. Intravaginal torsion of the testis was created by 720° rotation on both sides for 2 h. After detorsion, the right testes underwent tunica albuginea incision and tunica vaginalis flap application. Testicular microcirculation was monitored and hematological parameters, erythrocyte deformability, and aggregation were determined. Measurements were performed before and after torsion, directly after detorsion, on the 1st–2nd and 8th postoperative day. After the last sampling, testicles were removed to determine their volume for histological examinations. The microcirculatory parameters demonstrated slight differences between testicles. Apical zone of the left (nondecompressed) testicles had elevated compared to the middle zone ($P < 0.05$). On the 2nd and 8th day, the microcirculation of the testes normalized but not equally. The erythrocyte aggregation and deformability decreased by the 8th day. Both testicles underwent atrophy and epithelial necrosis, but the volume of the decompressed ones was lower (1.07 ± 0.08 *vs* 1.25 ± 0.31). Histologically, there was no significant difference in epithelial damage score between decompressed and nondecompressed testes. In conclusion, 2-h ischemia led to alteration in testicular microcirculation, reduction in volume, changes in hemorheological parameters and serious epithelial necrosis both in decompressed and nondecompressed testicles without remarkable differences.

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INTRODUCTION

Torsion of the testis by twisting of the spermatic cord is one of the most common urological emergencies occurring in young males. The annual incidence of this condition is 1 in 4000 males younger than 25 years.[1] Torsion initially causes obstruction in venous return, subsequently, as venous and arterial pressures equalizes; it also compromises arterial flow, resulting in testicular ischemia and uncorrectable testicular damage if it is without a quick and accurate therapy. The severity of ischemia depends on the duration of torsion, the degree of rotation of the spermatic cord[2] and according to recent published articles, also the effect of ischemia-reperfusion on intra-testicular pressure after detorsion plays an important role as well.[3,4] In clinical cases, when detorsion occur <6 h from the onset of symptoms, the testicular salvage rate is about 90%; this rate fell to 50% after 12 h and to <10% after 24 h.[5]

The recent and widely accepted surgical therapy includes detorsion and fixation if the testis is considered viable. In nonviable testis, orchidectomy is recommended. In 2008, Kutikov *et al*.[3] demonstrated that testicular compartment pressures appear elevated after prolonged torsion, secondary to ischemia-reperfusion. By addressing this tissue, the group introduced a new therapeutic concept that could increase the viability of testes after a long period of ischemia. Based on the theory that increased pressure during reperfusion leads to increased testicular deterioration, a novel surgical technique was introduced that included testicular capsulotomy for decreasing the intra-testicular pressure and coverage of the exposed seminiferous tubules was performed with a tunica vaginalis patch. The first retrospective clinical study – comparing detorsion and capsulotomy (DC) (tunica albuginea decompression with tunica vaginalis flap coverage method) with detorsion of the spermatic cord alone (DA), without decompression; in the surgical management of prolonged testicular ischemia – suggested that this new approach is a hopeful alternative for the management of clinically badly affected testes with an increased salvage rate after prolonged ischemia.[6] However, the experimental background of this technique is poor, and many questions are still unanswered regarding the usefulness of tunica albuginea decompression. Recent experimental studies highlighted the possible effects of increased

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intra-testicular pressure and effect of capsulotomy after testicular torsion to subsequent spermatogenesis and degree of histological alterations, but the results, which can determine the usefulness of the new approach are remained disputable.[7–9] Nonetheless of the favorable initiative clinical results, Oktar *et al*.[8] did not find correlation between decreased intra-testicular pressure, histopathological and functional changes in a tunica albuginea decompression model in rat. Alterations of testicular microcirculation and micro-rheological parameters after prolonged testicular ischemia are also subject to substantial interest,[4,10] but correlations of these changes after testicular decompression has not been studied.

In this present study, we aimed to examine and follow-up the impact of the Kutikov's method on testicular microcirculation, hemorheological parameters and signs of testicular atrophy after detorsion in an experimental a rat model.

MATERIALS AND METHODS

The experiment was approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 8/2011. UD CAR) in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998) and EU Directives (EEC 63/2010).

Six healthy male CD rats (bodyweight: 400–450 g) were subjected to the study. The experimental animals were anesthetized using sodium-thiopental (60 mg kg^{-1} i.p., Thiopenthal, Biocheme GmbH, Austria).

Following bilateral scrotal exploration on both sides, 720° (medial) intravaginal rotation was done and atraumatically fixed for 2 h (**Figure 1a**). After detorsion, an incision was made on the tunica albuginea of the right testis, then tunica vaginalis flap was created and sutured continuously using 6/0 polyglycolic acid suture material (DC operation) (**Figure 1b**). The left testis, after detorsion, was left intact (DA operation). The scrotum was closed with 4/0 polyamide-6 suture material at the end of the procedure.

During the operation, on the anterior surface of the upper pole, middle region and lower pole of the testis microcirculatory parameters were recorded by a laser Doppler (LD) tissue flowmetry (LD-01, Experimetria Ltd.) using a standard pencil probe (MNP100XP, Oxford Optronix Ltd., UK) before torsion (base), during testicular torsion and directly after detorsion and macroscopic observations (change in color of the organs) were carried out. Those intraoperative data of native, pretorsed testes were used as control data in our examinations, so we could follow and compare the changes individually within the same testes. On the 2nd postoperative (PO-2) and 8th postoperative (PO-8) days, animals were reanesthesized, and the scrotum was reopened with partial removal of suture line and closure, in order to measure the microcirculatory parameters on the anterior surface of the middle region of both testicles.

In order to evaluate the effect of testicular ischemia based on the micro-rheological alterations – which also determines the microcirculatory flow – we measured red blood cell deformability (elongation index in the function of shear stress) by a LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands), together with red blood cell aggregation with a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) before torsion (base) and on the 8th postoperative. A semi-automated microcell counter (Sysmex F-800, TOA Medical Electronics Co., Japan) was used to determine the general hematological parameters.

The testes were removed on the 8th postoperative day, and changes in testicular volume were measured. H and E staining was used for determination of the degree of testicular atrophy. Individually, two pathologists evaluated the samples using the following score system: (1) point: necrosis of inner 1/3 of germinal epithelium, (2) points: necrosis of 2/3 of germinal epithelium, (3) points: necrosis of the whole germinal epithelium, additional 1 point each if there were an inflammation and if there were a coherent region showing complete necrosis of the tubules in the testes.

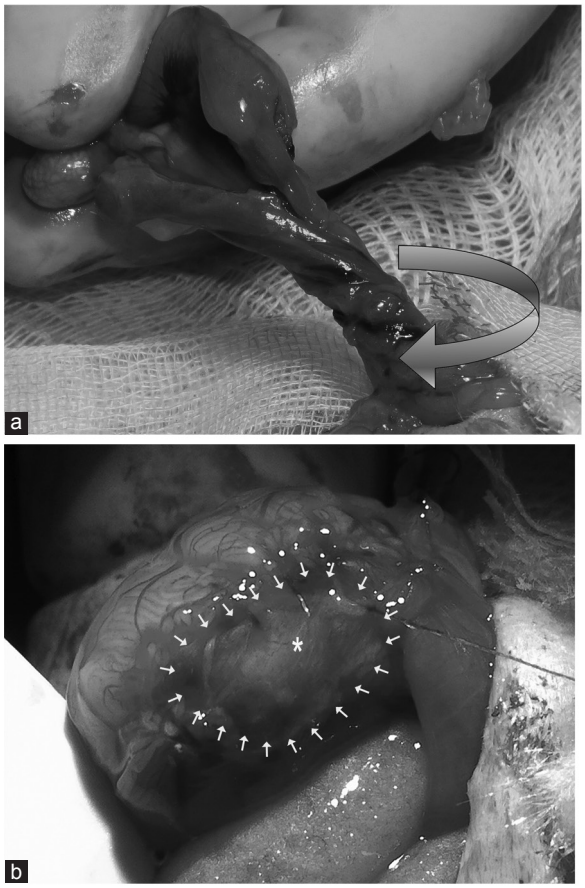

Figure 1: (**a**) Intravaginal rotation of the testis. Following bilateral scrotal exploration on both sides 720° (medial) intravaginal rotation was done and atraumatically fixed for 2 h (Arrow pointing at the torsed funicular components of the right testis). (**b**) Tunica vaginalis flap. After detorsion, an incision was made on the tunica albuginea of the right testis, then tunica vaginalis flap was created and sutured using 6/0 polyglycolic acid suture material (Asterisk pointing at the tunica vaginalis flap and suture line between the tunica vaginalis and the tunica albuginea marked with a dashed line/circle).

Data were presented as mean ± standard deviation (s.d.). The comparison of intraoperative laser Doppler flowmetry data obtained from various measurement sites were carried out using unpaired Student's *t*-test or Mann–Whitney rank sum test, while changes during the time-frame of experiment within measurement sites were analyzed by one-way ANOVA methods (Bonferroni's or Dunnett's test), depending on the data distribution. The significance level was considered when $P < 0.05$.

RESULTS

Macroscopic findings

During the artificial torsion, the color of the testes changed as they became pale, and during the reperfusion they turned edematous. In the reoperation on the 2nd postoperative day, all testes demonstrated a pale pink color and their consistency was maintained. By the

8th postoperative day, signs of testicular atrophy were present. With volumetric measurement, the left testes measured 1.25 ± 0.31 ml and the right (with the tunica vaginalis flap) 1.07 ± 0.08 ml, respectively.

Microcirculatory investigation

The intraoperative microcirculatory parameters of the testes (**Figure 2**) on both sides showed significant deterioration ($P < 0.02$ *vs* base) due to the ischemic injury resulted by the testicular torsion.

In case of the left testes (DA), the values were significantly decreased in the upper pole and lower region after the detorsion ($P < 0.05$ *vs* base) and similarly before the wound closure in the upper pole and lower region ($P < 0.05$ *vs* base) compared to the values before the exploration, while this could not been observed in the parameters of the right testes (DC).

Although differences were visible among the various measurement sites along the same time, most of the parameters did not show any statistical significance. In case of the both testes, the middle region and the lower pole base values were significantly lower than their upper pole. In the left testes, the lower pole value was significantly lower ($P < 0.05$) compared to the upper pole after 1-h ischemia, at the 5th min or reperfusion and before the wound closure. Similarly, the middle region's value at 2-h ischemia and before wound closure was significantly reduced compared to the upper pole. The right testes showed similar characteristics. The lower pole value was significantly lower ($P < 0.05$) compared to the upper pole at I-0', after 1-h and 2-h ischemia, and before the wound closure.

On the postoperative follow-up (PO-2 and PO-8) period, the parameters were normalized. Microcirculatory parameters in the middle regions on the 2nd postoperative day were measured 20.42 ± 3.13 blood flux unit (BFU) in the left and 17.97 ± 6.32 BFU in the right testes, respectively. By 8th postoperative day, the microcirculation of the left and right testes was 18.82 ± 7.19 BFU and 21.32 ± 3.48 BFU, respectively.

Red blood cell deformability

The preoperative and 8th postoperative days' sample did not differ; at most of the shear stress values the parameters were overlapping (**Figure 3**). The calculated parameters ($EI_{max}/SS_{1/2}$) did not show significant difference either.

Red blood cell aggregation

Red blood cells' aggregation index M1 5 s and M1 10 s values (tested with Myrenne aggregometer) represent the magnitude of the aggregation at the 5th and 10th s of the process measured at the shear rate of 3 s^{-1}. By the 8th postoperative day, there were significant differences compared to the base in the M1 values (**Figure 4**).

Histological findings

In the histological slides stained with H and E, we observed necrotic areas in both experimental groups. There was no significant difference between the left and right testes. However, in the testis with tunica vaginalis flap, the Sertoli cells and spermatogonii looked more intact and less necrotic. There was not a significant difference between the average histological score of DC testes (2,83) and DA testes (2,5), but the individual score of DC testes (**Figure 5a**) was higher in five rats than DA (**Figure 5b**) testes.

DISCUSSION

With the introduction of the theory of an intra-testicular compartment syndrome,[3,11] ischemia-reperfusion changes following torsion/detorsion of the testes have become an important issue. Based on this theory, detorsion, decompression with capsulotomy by incision of the tunica albuginea and covering with a tunica vaginalis flap can be a treatment option to rescue testis with questionable viability in the future.[3]

The effect of the tunica albuginea decompression method was primarily investigated by measuring of changes in intra-testicular pressure, testicular volume and weight and as well as with histological examination. In summary, these studies endeavored positive effect on survivability of the testis;[3,8,9] however, the results were not clearly

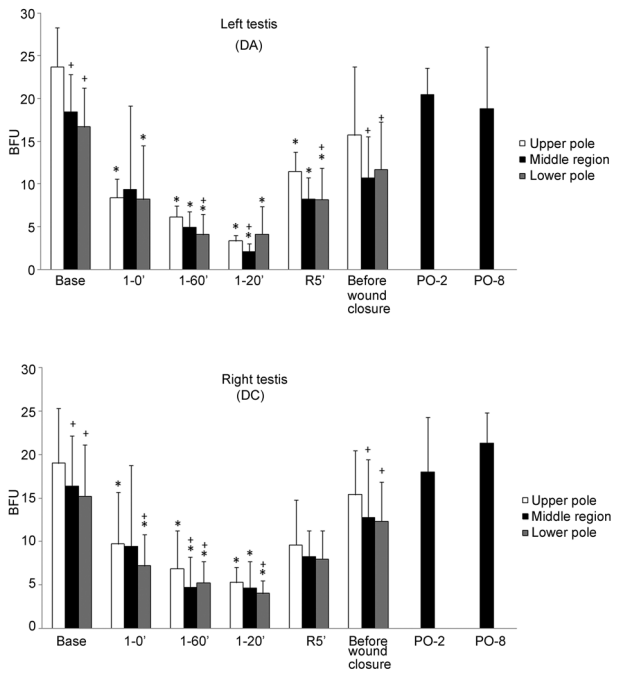

Figure 2: Microcirculation of the testes. On the anterior surface of upper pole, middle region and lower pole of the testis microcirculatory flow parameters (blood flux unit, BFU) were recorded with laser Doppler (LD) tissue flowmetry (LD-01, Experimetria Ltd.). The measurements were carried out before the intravaginal torsion (Base), at the beginning of torsion (1-0′), at the 60th (1-60′), 120th (1-20′) min of torsion and at the end of the procedure (Before wound closure). In the right (capsulotomized, DC) testes recording were done after the completion of tunica vaginalis flap (R5′). On the 2nd (PO-2) and 8th postoperative (PO-8) days, reoperation was done, when the parameters were also measured on the anterior surface of the middle region. *$P < 0.05$ versus Base; +$P < 0.05$ versus Upper pole.

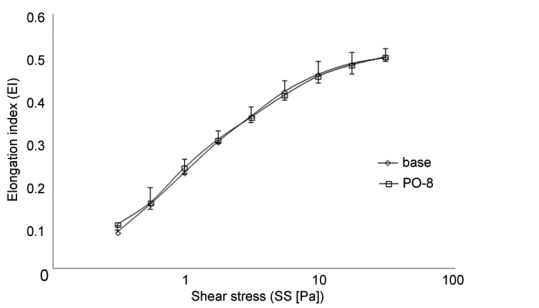

Figure 3: Deformability of red blood cells. The curve shows the elongation index (EI) of the red blood cells in the function of shear stress (SS). The preoperative and 8th postoperative days' parameters did not differ; at most of the shear stress values, the parameters were overlapping.

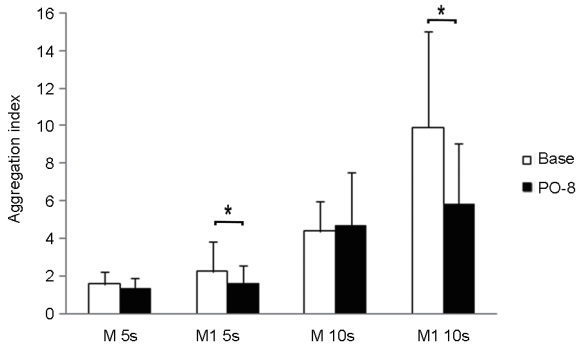

Figure 4: Aggregation of red blood cells. Red blood cell aggregation index M1 5 s and M1 10 s values (tested with Myrenne aggregometer) represent the magnitude of the aggregation at the 5th and 10th s of the process measured at the shear rate of 3 s^{-1}. By the 8th postoperative day, there were significant differences compared to the base. *$P < 0.05$.

supportive. Besides controversial results in animal models, the clinical experiences can be regarded as promising.[6] Although Oktar *et al.*[8] confirmed the effect of DC on the reduction in intra-testicular pressure, they could not show any unequivocal beneficial effect on testicular function. In a recent study, Quintaes *et al.*[9] investigated the affectivity of the DC method after 6 and 12 h of torsion in a rat model with the determination of testicular weight and volume, and histological changes. They found that there was no significant difference in the observed parameters between the DA and DC groups after 6 h of torsion while in the group, where 12 h of torsion was induced and the testis underwent capsulotomy, the volume and the weight of the DC testis were larger, and favorable histological alterations were observed.

Because of these inconclusive results from previous experimental studies, we aimed to investigate another aspect of Kutikov's method, and we examined microcirculation of testes after DC. Testicular microcirculation was measured in three different places of testes in order to exclude the influence of possible collateral blood supply or artery-artery communication, which features in the testicular parenchyma of rats. According to the anatomy of the arterial vasculature of the rat testis that main capsular artery arises from the arterial system of the rat testis epididymis, and the upper pole has stronger arterial supply.[12] In accordance to this, we also measured elevated testicular microcirculation in the apical zone of testes before torsion compared to the middle region or lower pole. These differences were detected after detorsion and tunica vaginalis flap application. Nevertheless, we found only a nonsignificant, slight improvement with the DC method regarding microcirculation parameters and detectable decrease of blood supply after the 2-h ischemic interval. On the 2nd and 8th postoperative days, the microcirculation of the testes normalized and became similar to the preoperative, base values. In other words, we could not support the favorable effect of DC to testicular blood supply in our rat model.

During tissue or organ ischemia-reperfusion process, the hemorheological parameters (i.e., blood and plasma viscosity, hematocrit, red blood cell deformability as well as red blood cell aggregation) may show alteration in different manners. The changes are mainly due to free-radical-caused damages, inflammatory processes and metabolic changes as well as acid-base alterations.[13–17] A previous study pointed out that these are changes after testicular ischemia-reperfusion causes significant effects on hemorheological parameters in the early postoperative period, and further lead to harmful microcirculatory consequences.[4,16] In our results, the

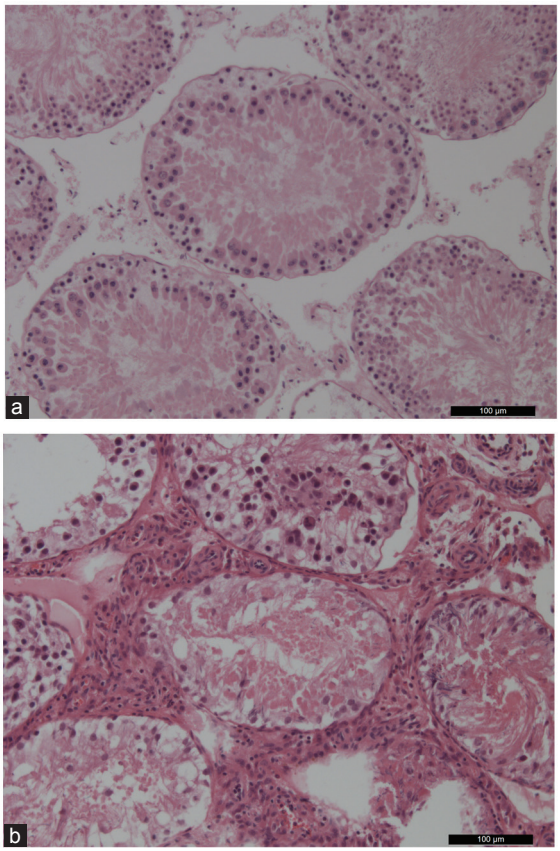

Figure 5: Histologic findings. (**a**) Demonstrates histological features of DC testis with necrosis of the whole germinal epithelium and additional inflammation. Score: 4 (H and E stain ×20 magnification). (**b**) Demonstrates histological features of DA testis with necrosis of inner 2/3 of the germinal epithelium. Score: 2 (H and E stain ×20 magnification). Scale bars = 100 µm.

hematological and micro-rheological parameters accompanied the acute phase reaction during the early postoperative period, and interestingly the erythrocyte aggregation decreased by the 8th day. These changes do not show any remarkable different between DC and DA testes and suggest that intra-testicular compartment syndrome did not affect the appearance of hemorheological parameters.

Changes of testicular volume may also be a predictive factor of noxious effect of testicular ischemia. Quintaes *et al.*[9] reported larger testicular volume after 12 h torsion in DC testes compared to the DA testes. In contrast, our observations demonstrated signs of testicular atrophy on the 8th postoperative day in both DC and DA testes presented by decreased testicular volume with even more intensive atrophy of DC testes. While the average testicular volume in rats within the same age group is 1.6–1.7 ml, the volume of DC testes on the 8th postoperative day was 1.07 ± 0.08 ml, and remarkable higher (1.25 ± 0.31 ml) without significance compared to the DA testes. However, this difference in the testicular volume can be the result of inflammatory changes in DC testes, which appeared in the histological examinations.

Damage of germinal epithelium was determined in our histopathological examinations. The thickness of necrotic epithelium was measured and scored together with signs of inflammation. After 2-h spermatic cord torsion, serious epithelial damage was detected in both DC and DA testes without significant difference. Although epithelial fibrosis was not detectable (8 days are considered not enough for fibrosis formation), our histopathological examinations

did not reveal any histological benefit similarly with the findings of Oktar *et al*.[8] and therefore the DC operation did not support favorable effect. However, 2-h torsion did not cause remarkable macroscopic circulation disturbances and testes seemed viable at inspection. These results together with the decreased testicular volume suggest that a 2-h ischemic period in rat is probably too long for the survival of testes. Reviewing the recent data, most researchers used 1 or 2 h artificial spermatic cord torsion in rat model to analyze the ischemia-reperfusion alterations with different follow-up period, and unequivocal circulatory damage was detected after 2-h ischemia. In comparison, humans were testicular salvage significantly worsens after 6–8 h of ischemia.[5]

The main limitations of animal models are the large differences between visible circulations disturb, duration of the acceptable ischemic period and variability of testicular blood supply compared to the human testes. Also, the pathophysiology of artificial testicular torsion during general anesthesia might be different from the clinical cases occurring in humans.

CONCLUSION

Two-hour artificial spermatic cord torsion in rat leads to alteration in testicular microcirculation, reduction in volume, slight change in hemorheological parameters and results in serious epithelial necrosis in both DC and DA testes, without remarkable differences. Despite that DC flap has favorable effect clinically and experimentally for decreasing intra-testicular pressure after testicular torsion, neither the histological data as other researchers also reported, nor alteration in microcirculation show clear beneficial effect of this technique in rat model. According to these findings, we cannot support the theory of an intra-testicular compartment syndrome to cause an increased injury to a testicle after torsion. Neither the reperfusion injury be decreased by the action of lowering the intratesticular pressure. Further investigations will be needed to create a more accurate experimental model to verify the usefulness of DC method and to find suitable predictive factors, which can determine salvageability of torsed testis.

AUTHOR CONTRIBUTIONS

TJ involved in planning and conducting experiment, performing surgeries, evaluating results, manuscript writing. ZK performed the microcirculatory measurements, involved with technical procedures, analysis and interpretation of data, manuscript writing. FK performed hemorheological examinations, analyzing data. ET performed laboratory examinations. AM involved in microcirculatory data acquisition and data analysis. ZH and YCC performed histological examinations. MF involved in manuscript writing and critical revision. NN involved in planning and conducting experiment, evaluating results, manuscript writing. All authors read and approved the final manuscript.

COMPETING INTEREST

All authors declared no competing interests.

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