

RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Is the renal venous blood diluted (in terms of hemoglobin concentration) after renal circulation?

Cheng-Yi SHEN¹, Jun-An LI², Yong-Sheng FENG²,
Zhong TANG², Xiao-Si DAI³, Ren-Dong ZHANG³,
Zheng-Wei YANG^{3*}

Key Words

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¹Pathophysiological Research Laboratory, ²Department of Clinical Laboratory Sciences of The Affiliated Hospital, and ³Morphometric Research Laboratory, North Sichuan Medical College, Nanchong, Sichuan CHINA

* **Corresponding author**
North Sichuan Medical College
234 Fujiang Road
Nanchong, Sichuan
637007 - China
Tel: +86-817-2242778
Fax: +86-817-2242600
Email: zwyang@mail.nctele.com

SUMMARY

This study aimed at determining whether the renal arteriovenous reduction in hemoglobin concentration (i.e. lower concentration in the renal venous blood than in the renal arterial blood), which was previously observed with blood sampling through a ventral laparotomy in dogs and rabbits but should not be expected due to formation of urine out of the blood plasma in the kidney, could be reversed by a different blood sampling procedure. Blood was obtained, through a dorsal retroperitoneal incision without opening the abdominal cavity, from the renal vein first and then the renal artery in rabbits, and erythrocytes related parameters were measured with an automatic hematology analyzer. The renal arteriovenous reduction was markedly and consistently reversed, with the red blood cell counts, hemoglobin concentration and hematocrit being 13% ~ 26% higher in the venous blood. In conclusion, the previously observed renal arteriovenous reduction in erythrocytes related parameters might be an operational “artifact” resulting from changes, which remain to be clarified, in the renal blood flow, glomerular filtration or tubular absorption during the operations.

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Renal sirkülasyondan sonra renal venöz kan (hemoglobinin konsantrasyonu bakımından) dilüe olur mu?

ÖZET

Bu çalışma, daha önce köpek ve tavşanlarda ventral laparotomi yoluyla kan örnekleme ile gösterilen hemoglobinin yoğunluğunda renal arteriyovenöz azalmanın (örneğin renal venöz kanda renal arteriyel kandan daha düşük yoğunluk) ancak bu durumun böbrekte kan plazmasının dışarı çıkması sonucu meydana gelen sidikten dolayı oluştuğu beklenilmemeli, değişik örnekleme yöntemleriyle geri döndürülüp döndürülemeyeceğini belirlemeyi amaçlamıştır. Kan, karın boşluğunu açmadan dorsal retroperitoneal ensiyon yoluyla önce renal venadan daha sonra renal arterden elde edildi ve otomatik hematoloji analizatörü ile eritrositle ilişkili parametreler ölçüldü. Alyuvar hücre sayısı, hemoglobinin yoğunluğu ve hematokrit değerlerindeki renal arteriyovenöz azalma önemli derecede ve sürekli olarak venöz kandan %13-26 oranında daha yüksek bulundu. Sonuç olarak, daha önce bildirilen eritrosit ile ilgili parametrelerde gözlenen ve değişimle sonuçlanan renal arteriyovenöz azalmalar işlemsel “artifakt” olabilir ki renal kan akımı, glomerular filtrasyon veya tubuler absorpsiyonun uygulanan işlemler sırasında göz önünde bulundurulması gerekmektedir.

INTRODUCTION

A renal arteriovenous increase in erythrocytes-related parameters such as hemoglobin concentration, i.e. higher concentration in the renal venous blood than in the renal arterial blood, should be expected due to formation of urine out of the blood plasma in the kidney. However, it was a renal arteriovenous reduction in hemoglobin concentration (i.e. lower concentration in the venous blood) that was demonstrated by measuring the blood of the renal artery and vein drawn from catheters implanted through a ventral laparotomy in 7-15 dogs.¹⁻² In fact, as was reviewed,¹ a renal arteriovenous reduction in hemoglobin concentration was repeatedly observed in the dog or rat in 3 previous studies in the 1930s and 1940s, although a renal arteriovenous increase (~4%) in hematocrit was also reported in another study of 6 dogs (without description on how the renal blood was drawn).³ Obtaining the renal venous and then arterial blood by puncturing the blood vessels through a ventral laparotomy in 38 rabbits, we also observed such a reduction of about 5% on average and the venous value was smaller than the corresponding arterial value in 2/3 of animals.⁴ With administration of mannitol to increase excretion of urine, the reduction diminished but was not completely eliminated in 7 dogs.⁵ This brought us to serious questions: Could the renal venous blood be really diluted, in terms of hemoglobin concentration, after renal circulation? If so, could the erythrocytes have been destroyed in the kidney? Since laparotomy and pulling of visceral organs for exposure of the blood vessels might affect the renal blood pressure and circulation, this study was undertaken to determine whether the renal arteriovenous reduction would be reversed by obtaining the renal arterial and venous blood through a dorsal retroperitoneal incision without opening the abdominal cavity.

MATERIALS AND METHODS

As we previously described,⁴ post-pubertal New Zealand white rabbits were used, anesthesia induced by sodium pentobarbital, erythrocytes related parameters measured 2-3 times from each arterial or venous blood sample using an automatic hematology analyzer, data presented as mean \pm SD, and paired *t*-tests performed for direct comparison between the arterial and the corresponding (ipsilateral) venous values.

Blood samples were obtained from the renal artery and vein after exposure of the blood vessels through a dorsal retroperitoneal incision (~6 cm in length) without opening the abdominal cavity. The operation was carried out by the same experienced

hand (CYS) and took about 5-10 minutes. Four protocols were used to obtain blood samples and explanation is given in the Discussion section for the use of these protocols. No fluid was given to the animals during the experiments. All animals used in protocols 1-3 were euthanized immediately after experiment by injection of air into the marginal ear vein. The Research Section of North Sichuan Medical College gave ethical approval for the study.

Protocol 1

Animals: 6 male and 10 female, bodyweights 1.93 ± 0.17 (1.71 ~ 2.29) kg. On exposure of the left renal blood vessels, draw blood from the renal artery first using a needle and syringe, and then from the renal vein while pressing the artery to prevent it from bleeding.

Protocol 2

Animals: 3 male and 5 female, bodyweights 2.17 ± 0.12 (2.06 ~ 2.30) kg. Half an hour after exposure of the renal blood vessels and insertion of a catheter into the ureter to monitor urine excretion, blood was drawn from the following 4 blood vessels in order: (i) left or right (alternately chosen) marginal ear vein (by puncturing the vessel) and (ii) the ipsilateral central ear artery (by cutting the vessel), (iii) the left renal vein (using a needle and syringe) and (iv) the left renal artery (by cutting the vessel).

Protocol 3

Animals: 5 male and 6 female, bodyweights 2.15 ± 0.41 (1.60 ~ 2.80) kg. Immediately (6 animals) or half an hour (5 animals) after exposure of the renal blood vessels, blood was drawn from the marginal ear vein, central ear artery, renal vein and renal artery, respectively, as described in Protocol 2.

Protocol 4

Animals: 2 male and 6 female, bodyweights 2.02 ± 0.24 (1.70 ~ 2.45) kg. Blood was drawn, without anesthesia, from the central and marginal ear blood vessels in both ears. In one (left or right, alternately chosen) ear the central ear artery was pressed with the index finger and thumb to stop its blood flow for 20 seconds before blood sampling. Before or after (alternately chosen) the blood sampling in the ear, blood sampling was performed in the other ear without stopping the arterial blood flow.

RESULTS

Blood sampling with experimental Protocol 1 induced a 17-23% renal arteriovenous reduction in erythrocytes related parameters (Table 1). With

Protocol 2, a total of 0.90 ± 0.36 ml of urine was collected half an hour after the operational exposure and there was no significant difference between the speeds of urination (time intervals between two drops of urine) at 10, 20 and 30 minutes after operation (one-way repeated measures analysis of variance); the arteriovenous reduction in erythrocytes related parameters was markedly and consistently reversed, with a 16-26% renal arteriovenous increase being achieved (Table 2).

Table 1. Results obtained with Protocol 1 (mean \pm SD, n = 16)

Çizelge 1. Protokol 1 ile elde edilen sonuçlar (mean \pm SD, n = 16)

	Renal Artery	Renal Vein	A:V	n(A>V)/n
RBC	6.59 ± 0.52^a	5.65 ± 0.90	1.186 ± 0.147	16/16
Hb	14.0 ± 1.1^a	11.6 ± 1.7	1.225 ± 0.167	15/16
Hct	44.0 ± 2.3^a	38.1 ± 5.3	1.170 ± 0.142	14/16

A:V, ratio between the arterial and the corresponding venous values; n(A>V)/n, number of the arterial values bigger than the corresponding venous values / total number of arterial and venous values (pairs) measured. RBC, red blood cells (erythrocytes) ($10^{12}/l$); Hb, hemoglobin concentration (g/dl); Hct, hematocrit (%).
^a Significant difference was detected ($P < 0.05$, paired *t*-test) between the arterial and venous values.

Table 2. Results obtained with Protocol 2 (mean \pm SD, n = 8)

Çizelge 2. Protokol 2 ile elde edilen sonuçlar (mean \pm SD, n = 8)

	Renal Artery	Renal Vein	A:V	n(A>V)/n
RBC	5.11 ± 0.67^a	6.08 ± 0.25	0.843 ± 0.131	0/8
Hb	9.2 ± 1.3^a	12.5 ± 1.4	0.743 ± 0.156	0/8
Hct	34.0 ± 4.9^a	46.2 ± 4.6	0.747 ± 0.154	0/8
	Ear Artery	Ear Vein	A:V	n(A>V)/n
RBC	5.80 ± 0.57	5.85 ± 0.71	0.995 ± 0.048	4/8
Hb	10.6 ± 0.8	10.8 ± 1.2	0.984 ± 0.059	4/8
Hct	39.1 ± 2.4	39.4 ± 3.4	0.995 ± 0.049	4/8

See Table 1 for abbreviations and symbols.

A renal arteriovenous increase (14-17%) was reproduced with Protocol 3 (Tables 3 and 4). In the ear, however, arteriovenous difference in erythrocytes

related parameters was not detected (Tables 2, 3 and 5) except for blood sampling at half an hour after operation in Protocol 3 (Table 4), where an arteriovenous increase of $\sim 5\%$ was also observed.

Table 3. Results obtained immediately after operation in Protocol 3 (mean \pm SD, n = 6)

Çizelge 3. Protokol 3'teki operasyondan hemen sonra elde edilen sonuçlar (mean \pm SD, n = 6)

	Renal Artery	Renal Vein	A:V	n(A>V)/n
RBC	4.64 ± 0.33^a	5.67 ± 0.72	0.831 ± 0.143	1/6
Hb	10.2 ± 0.5^a	12.4 ± 1.7	0.833 ± 0.142	1/6
Hct	33.7 ± 1.5^a	41.4 ± 5.4	0.827 ± 0.137	1/6
	Ear Artery	Ear Vein	A:V	n(A>V)/n
RBC	5.19 ± 0.45	5.24 ± 0.25	0.990 ± 0.060	3/6
Hb	11.4 ± 0.7	11.3 ± 0.5	1.009 ± 0.041	3/6
Hct	37.6 ± 2.0	37.3 ± 1.5	1.009 ± 0.038	4/6

See Table 1 for abbreviations and symbols.

Table 4. Results obtained half an hour after operation in Protocol 3 (mean \pm SD, n = 5)

Çizelge 4. Protokol 3'teki operasyondan yarım saat sonra elde edilen sonuçlar (mean \pm SD, n = 5)

	Renal Artery	Renal Vein	A:V	n(A>V)/n
RBC	5.14 ± 0.22^a	5.98 ± 0.49	0.865 ± 0.092	0/5
Hb	10.9 ± 0.6^a	12.7 ± 0.9	0.864 ± 0.098	0/5
Hct	35.8 ± 1.9^a	41.7 ± 2.9	0.864 ± 0.094	0/5
	Ear Artery	Ear Vein	A:V	n(A>V)/n
RBC	5.68 ± 0.47^a	5.99 ± 0.36	0.948 ± 0.035	0/5
Hb	12.1 ± 0.8^a	12.7 ± 0.6	0.949 ± 0.026	0/5
Hct	39.5 ± 3.1^a	41.6 ± 2.3	0.949 ± 0.035	0/5

See Table 1 for abbreviations and symbols.

Table 5. Results obtained with Protocol 4 (mean \pm SD, n = 8)**Çizelge 5.** Protokol 4'te elde edilen sonuçlar (mean \pm SD, n = 8)

	Ear Artery *	Ear Vein	A:V	n(A>V)/n
RBC	5.88 \pm 0.38	5.80 \pm 0.44	1.014 \pm 0.038	6/8
Hb	11.5 \pm 0.8	11.4 \pm 0.9	1.007 \pm 0.037	6/8
Hct	37.8 \pm 2.1	37.4 \pm 2.5	1.013 \pm 0.039	6/8
	Ear Artery **	Ear Vein	A:V	n(A>V)/n
RBC	5.76 \pm 0.42	5.69 \pm 0.53	1.015 \pm 0.056	5/8
Hb	11.3 \pm 0.9	11.2 \pm 1.2	1.016 \pm 0.049	5/8
Hct	37.1 \pm 2.9	36.4 \pm 3.8	1.021 \pm 0.056	5/8

Blood was obtained *after the central ear arterial blood flow was blocked for 20 seconds or **without blocking the arterial blood flow. See Table 1 for other abbreviations and symbols.

DISCUSSION

This study aimed to determine whether the puzzling results of renal arteriovenous reduction in hemoglobin concentration or erythrocytes related parameters, as previously observed with blood sampling through a ventral laparotomy,^{1-2,4-5} could be reversed by blood sampling through a dorsal retroperitoneal incision without opening the abdominal cavity. Obtaining the renal venous blood after blocking the renal arterial blood flow (experimental Protocol 1) induced an even larger and more consistent arteriovenous reduction (Table 1). Speculating that this reduction might be related to blocking of the arterial blood flow or the operation-induced stress, we adopted Protocol 2: obtaining the venous blood first and then the arterial blood at half an hour after operation without blocking the arterial blood flow. As a result, the arteriovenous reduction was markedly and consistently reversed. To reproduce this result and to determine whether it was the procedure of blocking the arterial blood or the immediate blood sampling after operation in Protocol 1 that induced the reduction, we proceeded further to Protocol 3. As demonstrated the reduction was also reversed with either the immediate blood sampling after operation or blood sampling at half an hour after operation. If it was the blocking of the renal arterial blood flow that induced the reduction, would it be also true in the ear blood vessels? So we went on to Protocol 4. Unlike the renal blood vessels, no

arteriovenous difference was detected for the ear blood vessels.

In conclusion, the puzzling renal arteriovenous reduction in erythrocytes related parameters previously observed with blood sampling through a ventral laparotomy^{1-2,4-5} could be reversed by blood sampling through a dorsal retroperitoneal incision without opening the abdominal cavity, and the puzzle, which arose from previous studies and the Protocol 1 of the present study and seemed to be ignored for decades, might be artifacts resulting from operation-induced changes. However the changes in different blood sampling procedures remain to be clarified in future studies. It seems that the changes during the operations were more specific to the kidney in view of the "normal" scenario in the ear arteriovenous differences shown in the current study.

It is well known that (i) renal blood flow to the kidney is as large as about a quarter of the cardiac output, (ii) the blood flow and the glomerular filtration rate remain relatively constant as arterial blood pressure changes between 90 and 180 mm Hg, (iii) about 15% to 20% of the plasma that enters the glomerulus is filtered, and (iv) less than 1% of the filtered water and NaCl and variable amounts of the other solutes are excreted in the urine due to the reabsorption (and secretion) of the renal tubules under normal conditions in the human.⁶ The monitoring of urine production in Protocol 2 indicated continual renal function during the operations. The most striking result demonstrated in the current study was that the blocking of the renal arterial blood flow (Protocol 1), although for a very short period of time, led to a more consistent and marked renal arteriovenous reduction in erythrocytes-related parameters (Table 1) in comparison with our previous result.⁴ This suggested that the renal function changed instantly in response to the procedure, with relatively more plasma immediately flowing out of the renal vein, probably due to fewer erythrocytes inflow and/or more plasma outflow or tubular reabsorption. This was what might have happened, to some degree, during laparotomy and pulling of visceral organs for exposure of renal blood vessels in previous studies. No matter what changes occurred in the kidney in terms of blood circulation, glomerular filtration or tubular reabsorption during the operations, it now seems sure that the renal arteriovenous difference in erythrocytes-related parameters is sensitive to the procedure of renal blood sampling, and measurement of renal blood sampled through a dorsal retroperitoneal incision appears to be more ideal to reflect normal renal physiology ■

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