Original Paper

Perfusion Computer Tomography Assessment of the Effect of Angiotensin II On Blood Flow Distribution in Rabbits with Intrarenal VX2 Tumors

Ying-Jin Xu^a Song-Hong Wu^b Huai-Jun Liu^a Pei Niu^d Wen-Zeng Shen^dYing-Jie Xu^e Xiao-Ping Yin^c Tian-Le Zhang^c Xue-Zhao Li^d Fei Guo^dXiao-Long Niu^d Jin-Song Zhu^d

^aDepartment of Radiology, The Second Hospital of Hebei Medical University, Shijiazhuang, ^bNuclear Medicine, Affiliated Hospital of Hebei University, Baoding, ^cDepartment of Radiology, Affiliated Hospital of Hebei University, Baoding, ^dCollege of Medicine, Hebei University, Baoding, ^eDepartment of Radiology, Wangdu County Hospital, Wangdu, China

Key Words

Kidney • Rabbit • VX2 tumor • Computed tomography perfusion imaging • Hemodynamics • Angiotensin II

Abstract

Background/Aims: Unlike other organs, which only have one set of capillary network, the renal microvasculature consists of two sets of capillary network series connected by efferent arterioles. Angiotensin II constricts the efferent glomerular artery. Hence, renal tumor blood flow (BF) distribution may be different from tumors in other organs. This study aims to investigate the effects of angiotensin II on the hemodynamics of intrarenal VX2 tumors using perfusion computed tomography (CT). **Methods:** Twenty-four male New Zealand white rabbits were randomly divided into three groups: groups A (blank controls), group B (negative controls), and group C (angiotensin II-treated animals). Group B and C were established to the model of intrarenal VX2 tumors. Furthermore, perfusion CT of the kidney was performed in each group. Prior to perfusion CT scan in group C, the mean arterial blood was elevated to 150-160 mmHg by angiotensin II. The BF, blood volume (BV), mean transit time (MTT), capillary permeability-surface area product (PS), and relative permeability-surface area product (RPS) of tumors and renal tissues were calculated. **Results:** Compared with normal renal cortex tissues in group A, the BF, BV and PS values of tumors in group B were significantly lower, MTT was prolonged and RPS increased. Compared with group B, only the RPS of these tumors increased from $83.23 \pm 29.17\%$ to $120.94 \pm 31.84\%$ by angiotensin II infusion. Angiotensin II significantly increased the RPS value of the renal cortex distant from the tumor (CDT) and the right renal cortex (RRC). **Conclusions:** Perfusion CT can accurately observe the influence of angiotensin II on normal and tumor BF in kidneys. This clarifies the effect of angiotensin II on intrarenal tumor hemodynamics.

Y.-J. Xu and S.-H. Wu contributed equally to this work.

© 2018 The Author(s)

Published by S. Karger AG, Basel

Huai-Jun Liu

Department of Radiology, The Second Hospital of Hebei Medical University
215 Heping West Road, Shijiazhuang 050005 (China)
Tel. 86 13323111923 Fax. +86 311 85917290 E-Mail liuhj_hj@21cn.com (HJL)

Introduction

There are clear differences between tumor vessels and normal blood vasculature [1-3]. Normal microvasculature has a complete structure, including an endothelial cell lining, the presence of mural cells (smooth-muscle cells and pericytes) around endothelial cells, and the basement membrane enveloping both types of cells [4]. The blood vessels of tumors are dynamic, defective and leaky [5-10]. Furthermore, tumor blood vessels are irregular in size, shape and branching pattern. Abnormalities are present in all components of the vessel wall. Tumor blood vessels manifest large endothelial cell-cell gap openings that permit the leakage of macromolecules into the interstitial space of tumor tissues [6, 7]. The basement membrane is also often thickened abnormally or absent, further enhancing the aberrant permeability of tumor blood vessels [8]. Mural cells that normally cover and stabilize the outer vessel wall can also be missing or detached, leading to a more immature vessel structure. The absence of these contractile support cells may also further increase vessel permeability and weaken control over blood flow (BF) [9, 10].

Angiotensin has a direct stimulant action on the smooth muscle of the vascular system [11]. In the absence of these normal contractile support cells in tumor vessels, angiotensin II can selectively enhance the vascular permeability and BF of tumor tissues without increasing blood flow in normal tissues, and improve the diagnosis and treatment of tumors through the selective enhancement of drug delivery to the tumor tissue [12-17]. Selective arterial infusion [12-16] or the intravenous administration [17] of angiotensin II has been investigated as a method to increase perfusion in both human [12-14] and animal experiments [15-17] in various tumors, including cancers of the liver, kidney and gallbladder.

The fundamental principle of perfusion computed tomography (CT) is based on temporal changes in tissue attenuation. After the intravenous injection of contrast material, changes in tissue attenuation indirectly reflect tissue vascularity and vascular physiology. Perfusion CT is an evolving noninvasive technique that allows for the measurement of microvascular tumor circulation and tumor angiogenesis. *In vivo* tumor perfusion characteristics can be evaluated by analyzing the dynamics of tissue enhancement after intravenous contrast administration [18, 19]. CT perfusion imaging can predict the early response to treatment and survival of malignant tumor patients [20, 21].

The microvasculature of each organ is specialized in concert with the unique functions of each organ. The microvasculature of every organ has a hierarchical organization of arterioles, capillaries and venules, which have their own specialized structural and functional properties [5]. The microvasculature of the kidney consists of afferent arterioles, glomerular capillaries, efferent arterioles, capillary networks around the renal tubular, and venules [11, 22, 23]. Angiotensin II constricts the efferent glomerular artery rather than the afferent glomerular artery [11, 22, 23]. Therefore, it could be hypothesized that angiotensin II can influence normal renal tissues, and the renal tumor BF distribution may be different from tumors in other organs, which has its unique imaging findings. Perfusion CT can be used to understand this vasoactive effect. The purpose of the present study was to investigate the effects of the intravenous administration of angiotensin II on renal vascularity in normal rabbit renal tissues and rabbit intrarenal VX2 tumors by perfusion CT.

Materials and Methods

Experimental animal model

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Affiliated Hospital of Hebei University (Permit Number: 2014-10). Two rabbits bearing VX2 tumors in the left femoribis internus muscle were provided by the Experimental Center of Hebei University Health Science Center (Baoding, China). Twenty-four male New Zealand white rabbits (two months old; supplied by Xinglong Laboratory Animal Farm, Beijing, China; No. 11805800000637), weighing approximately 2.9-3.2 kg, were divided into three groups using the random number table. All rabbits were anesthetized with

a slow intravenous injection of 25% urethane, at 4 ml/kg (Shanpu, Shanghai, China). These rabbits were fixed on a bed stand on the right lateral decubitus, and the left kidney was exposed by performing a left paravertebral incision. An approximately 1.0-cm incision was established using a surgical blade. In group A (blank controls, $n=8$), solitary autologous perirenal adipose tissue were implanted into the left kidney incision. In group B (negative controls, $n=8$) and group C (angiotensin II-treated animals, $n=8$), solitary VX2 tumor tissue masses of 1-2 mm³ were implanted into the left kidney incision. Then, the left kidney incision was closed by applying pressure using a sterile gelatin sponge, and rapidly spraying and coating with medical adhesive (Compont, Beijing, China), in order to prevent the biogum adhesive from entering the adjacent organs and tumor tissue overflow. After spraying saline in the wound, each layer of the dorsum was sutured. The procedure was performed under aseptic surgical conditions, and the animals were returned to their cage and fed with standard rabbit food and water *ad libitum*. The day of tumor inoculation was considered as day zero. The tumors were allowed to grow for 2-3 weeks, and the tumor growth was monitored by ultrasonography (Vevo 2100 Imaging System, MS201 Probe, Fujifilm VisualSonics, Toronto, Canada) every 4-5 days after implantation. Solid tumors of 1.5-2.0 cm in diameter were used in group B and group C.

Preparation prior to CT examination

A 22-gauge closed intravenous catheter (BD Intima II, Suzhou, China) was placed in a marginal ear vein to sedate the animals with urethane before being fixed on a bed stand in the supine position. The cervical region of the rabbit was shaved and disinfected with povidone iodine. Through cervical median incision, the left common carotid artery was isolated. Then, mean arterial blood pressure (MABP) was measured *via* a catheter inserted into the left common carotid artery, and 100 IU of heparin per kilogram of body weight was administered. The pressure in the catheter was continuously recorded using a pressure transducer (TM1412016; Taimeng, Chengdu, China), and the output was sent to the biological function experimental system (BL-420S; Taimeng, Chengdu, China). A Foley catheter was advanced into the bladder for urine drainage and urinalysis.

Blood pressure was elevated by continuous infusion of angiotensin II (Solarbio, Beijing, China) into the marginal ear vein in group C. Angiotensin II was dissolved in normal saline at a concentration of 5 µg/ml, and was infused using a peristaltic pump at 1-2 µg/kg/min (HL-2; Huxi, Shanghai, China). Changes in blood pressure were measured using the biological function experimental system.

Perfusion CT examination

At the time of imaging, the biological function the experimental system, peristaltic pump and the entire rabbit were lined into the CT scanner (Discovery CT750 HD; GE Medical Systems, Milwaukee, WI, USA). The rabbits were fixed in the supine head-first position with an abdominal compression bandage, in order to reduce artifacts caused by unnecessary respiratory movement. Tubing for the contrast material, normal saline and the peristaltic pump were attached to the ear vein catheter. First, a nonenhanced abdominal CT examination was performed to locate the renal tumor for perfusion imaging in groups A, B and C. Subsequently, perfusion CT scans were initiated for rabbits in groups A and B using a volume helical shuttle mode of acquisition (120 kV, 150 mAs; scan field of view [SFOV], large body; display field of view [DFOV], 160 mm; matrix, 512×512 pixels; detector coverage, 40.0 mm; thickness, 5.0 mm; pitch, 1.375:1.000; rotation time, 0.4 seconds; 31 passes; total exposure time, 50.44 seconds), and 5.0 mL of contrast material (Ioversol, 320 mg I/ml; Hengrui Medicine, Jiangsu, China) was injected using a high-pressure syringe (ACIST, Eden Prairie, USA) at a rate of 1.0 ml/sec. The contrast material injection was followed by 3.0 ml of saline solution at the same flow rate. Prior to the perfusion CT scan, rabbits in group C were infused with angiotensin II using a peristaltic pump. MABP was elevated to 150-160 mmHg and maintained for 5-10 minutes, in order to perform perfusion CT scans using the same previous scanning parameters.

Image post-processing

The perfusion images were reconstructed from the raw projection data onto a 512×512 pixels matrix with a section thickness of 1.25 mm before being transferred to the GE ADW4.5 workstation. The CT perfusion software (Kidney Protocol, Body Tumor Perfusion 4.0; GE Healthcare) was used for perfusion analysis. A region of interest (ROI) of 5 mm² was carefully demarcated to obtain the satisfactory time-density curves (TDC). These ROIs included the abdominal aorta, tumor tissue, renal cortex directly adjacent to the tumor (CAT), renal cortex distant from the tumor (CDT), and right renal cortex (RRC). The paravertebral

muscle (PVM) was also obtained at the same level of the tumor (Fig. 1). The abdominal aorta was used instead of the renal artery, in order to avoid artifacts caused by unnecessary respiratory movement. Pseudo-color functional maps were obtained and various perfusion parameters were measured in the ROI. Thus, the BF, blood volume (BV), mean transit time (MTT) and capillary permeability-surface area product (PS) of the tissue could be calculated. The relative permeability-surface area product (RPS) refers to the ratio of extravascular-intravascular fluid exchange capacity to the intravascular fluid volume. RPS was calculated using the following formula: $PS/BF \times 100\%$; where BF and PS represents the blood flow and capillary permeability-surface area product, respectively.

Pathological examination

After the CT scan, pathological anatomy was performed to observe the presence of pleural effusion, abdominal effusion, and the metastasis of the lung, abdominal and pelvic organs. The kidney was taken and hematoxylin and eosin (H&E) staining was performed. Then, the relationship between tumor tissues and the surrounding renal tissue, renal sinus and renal capsule was observed.

Statistical analysis

All data were expressed as mean $\pm$ standard deviation (SD). All statistical analyses were carried out using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyze the statistical differences of the measured values of various perfusion parameters of the tumor, CAT, CDT and PVM among the different groups. The LSD-t method was used to adjust for multiple comparisons. Two-tailed student's *t*-test was used to compare perfusion parameters between the cortex adjacent to the tumor and the cortex distant from the tumor in groups B and C. Statistical significance was assigned at the 5% level.

Results

Inclusion vs exclusion criteria of intrarenal VX2 tumors

Inclusion criteria: (1) The maximum diameter of tumor is 1.5-2.0 cm by ultrasonography or perfusion CT observation, without obvious necrotic areas. The calcification area is no more than 40% of the tumor's maximum cross-section. (2) The main body of the tumor is within the outline of the kidney, which can slightly protrude from the outline of the kidney, and the renal capsule is intact. (3) The perirenal adipose space around the tumor can be observed by perfusion CT imaging, and there is no obvious thickening in the adjacent abdominal wall. (4) Urinalysis revealed no blood urine.

Exclusion criteria: (1) The maximum diameter of tumor is >2.0 cm by ultrasonography or perfusion CT, with obvious necrotic areas. The calcification area is $>40\%$ of the tumor's

Fig. 1. The axial perfusion CT image of a rabbit shows the placement of regions of interest (ROI). (1) With the aorta as an arterial input reference, the (2) tumor tissue, (3) the renal cortex immediately adjacent to the tumor (CAT), (4) the renal cortex distant from the tumor (CDT), and (5) the paravertebral muscle (PVM) are shown. Since the position of right kidney is significantly higher than that of the left kidney in rabbits, both kidneys cannot be shown on the same layer axial image. Hence, the ROIs of the right renal cortex (RRC) were not presented in this Fig. was no statistical significance. (C) Tumor PS were significantly lower than the normal renal cortex, and the PS value of tumor tissues, CAT, CDT, RRC and PVM had no obvious change by angiotensin II infusion. $P < 0.05$; $**P < 0.01$.

maximum cross-section. (2) The tumor tissue breaks through the renal capsule and perirenal adipose space around the tumor is discontinuous or disappears by perfusion CT imaging. The adjacent wall obviously thickened or formed a mass. (3) Pathological anatomy revealed pleural effusion, abdominal effusion, and the metastasis of lung, abdominal and pelvic organs. (4) Urinalysis revealed blood urine.

None of the rabbits revealed significant abdominal and surgical incision infection after the operation, and the incision was well-healed. All the tumors in groups B and C were eligible for inclusion criteria of the intrarenal VX2.

Changes in BF due to angiotensin II

Tumor BF (Fig. 2A) in groups B and C were significantly lower than the normal renal cortex BF in group A ($P < 0.001$ and $P < 0.001$, respectively; LSD-t). Tumor BF in group C (64.51 ± 21.01 ml/min/100 g) was lower than the tumor BF in group B (88.52 ± 24.25 ml/min/100 g), and the difference was not statistically significant ($P = 0.473$, LSD-t). CAT BF in group C was significantly lower than the normal renal cortex BF in group A ($P < 0.001$, LSD-t) and the CAT BF in group B ($P < 0.001$, LSD-t). The CDT BF in group C was significantly lower than the normal renal cortex BF in group A ($P < 0.001$, LSD-t) and the CDT BF in group B ($P = 0.010$, LSD-t). The RRC BF in group B was significantly greater than the RRC BF in groups A and C ($P = 0.009$ and $P < 0.001$, respectively; LSD-t). BF in the PVM was unaffected by the tumor and the infusion of angiotensin II ($P = 0.546$, ANOVA).

Changes in BV due to angiotensin II

The tumor BV (Fig. 2B) in groups B and C were significantly lower than the normal renal cortex BV in group A ($P = 0.001$ and $P < 0.001$, respectively; LSD-t). The BV of the tumor and CAT in group C (8.60 ± 8.60 ml/100 g and 20.01 ± 12.15 ml/100 g, respectively) were lower than the BF in group B (11.58 ± 6.53 ml/100 g and 29.15 ± 11.87 ml/100 g, respectively), and the difference was not statistically significant ($P = 0.516$ and $P = 0.135$, respectively, LSD-t). The BV in CAT, CDT, RRC and PVM were unaffected by the tumor and the infusion of angiotensin II ($P = 0.248$, $P = 0.930$, $P = 0.774$ and $P = 0.675$, respectively; ANOVA).

Fig. 2. Bar graphs of BF (A), BV (B), MTT (C) and PS (D). (A) Tumor BF was significantly lower than the normal renal cortex. Angiotensin II significantly decreased the BF value of CAT, CDT and RRC, while the BF value of tumor tissues and PVM had no obvious changes. (B) Tumor BV were significantly lower than the normal renal cortex, and the BV value of tumor tissues, CAT, CDT, RRC and PVM had no obvious change after angiotensin II infusion. (C) Tumor MTT was longer than the normal renal cortex, and angiotensin II prolonged the MTT value of CAT, CDT, RRC and PVM, but there was no statistical significance. (D) Tumor PS were significantly lower than the normal renal cortex, and the PS value of tumor tissues, CAT, CDT, RRC and PVM had no obvious change by angiotensin II infusion. * $P < 0.05$; ** $P < 0.01$.

Changes in MTT due to angiotensin II

The difference in MTT (Fig. 2C) among tumors, CAT, CDT, RRC and PVM was not statistically significant ($P = 0.190$, $P = 0.579$, $P = 0.171$, $P = 0.178$ and $P = 0.828$, respectively; ANOVA). The MMT of tumors in group B (9.23 ± 6.45 seconds) was longer than that in group A (4.37 ± 2.31 seconds), but the difference was not statistically significant ($P = 0.079$, LSD-t). The MMT of tumors in group B (9.23 ± 6.45 seconds) was longer than that in group A (4.37 ± 2.31 seconds), but the difference was not statistically significant ($P = 0.079$, LSD-t). Compared with group B, angiotensin II prolonged the MTT of CDT and RRC (group C vs. group B; 6.59 ± 4.78 seconds vs. 3.56 ± 1.55 seconds, and 6.39 ± 3.85 second vs. 3.68 ± 3.12 seconds, respectively; ANOVA), but the difference was not statistically significant ($P = 0.072$ and $P = 0.066$, respectively; LSD-t).

Changes in PS due to angiotensin II

Tumor PS (Fig. 2D) in groups B and C were significantly lower than the normal renal cortex PS in group A ($P = 0.041$ and $P = 0.036$, respectively; LSD-t), and the tumor PS was unaffected by the infusion of angiotensin II ($P = 0.951$, LSD-t). Furthermore, PS in CAT, CDT, RRC and PVM were unaffected by the tumor and the infusion of angiotensin II ($P = 0.846$, $P = 0.423$, $P = 0.683$ and $P = 0.633$, respectively; ANOVA).

Changes in RPS due to Angiotensin II

Tumor RPS (Fig. 3) in groups B and C were significantly greater than the normal renal cortex RPS in group A ($P < 0.001$ and $P < 0.001$, respectively; LSD-t). Furthermore, the tumor RPS increased from $83.23 \pm 29.17\%$ (group B) to $120.94 \pm 31.84\%$ (group C) by angiotensin II infusion ($P = 0.007$, LSD-t). Moreover, CAT RPS in group C was significantly higher than the normal renal cortex RPS in groups A and B ($P < 0.001$ and $P < 0.001$, respectively; LSD-t). Compared with group B, the RPS in CDT and RRC increased by angiotensin II infusion ($P = 0.019$ and $P = 0.003$, respectively; LSD-t). However, PVM RPS was unaffected by the tumor and the infusion of angiotensin II ($P = 0.477$, ANOVA).

Changes in perfusion parameters in CAT and CDT due to angiotensin II

There was no significant difference in perfusion parameters (BF, BV, MTT, PS and RPS) in group B between CAT and CDT ($P = 0.053$, $P = 0.776$, $P = 0.510$, $P = 0.166$ and $P = 0.065$, respectively; two-tailed student *t*-test) (Table 1). Furthermore, perfusion parameters in group C between CAT and CDT were unaffected by the infusion of angiotensin II ($P = 0.274$, $P = 0.225$, $P = 0.444$, $P = 0.395$ and $P = 0.183$, respectively; two-tailed student's *t*-test) (Table 1).

Table 1. Changes of perfusion parameters in CAT and CDT due to angiotensin II ($n = 8$). There is no data of group A in the table, because the group A is blank control group, without CAT and CDT

Group	parameters	CAT	CDT	t value	p
B	BF (ml/min/100 g)	465.83±88.55	555.59±80.95	2.116	0.053
	BV (ml/100 g)	29.15±11.87	30.92±12.52	0.290	0.776
	MTT (seconds)	4.04±1.28	3.56±1.55	0.675	0.510
	PS (ml/min/100 g)	101.31±1.91	102.30±0.16	1.461	0.166
	RPS (%)	22.51±4.70	18.73±2.52	2.005	0.065
C	BF (ml/min/100 g)	253.59±98.23	312.40±108.07	1.139	0.274
	BV (ml/100 g)	20.01±12.15	31.18±21.69	1.271	0.225
	MTT (seconds)	5.10±2.40	6.59±4.78	0.788	0.444
	PS (ml/min/100 g)	100.82±2.52	91.40±30.23	0.878	0.395
	RPS (%)	44.39±14.68	32.90±17.96	1.401	0.183

Fig. 3. Bar graphs of RPS. The RPS value of tumor tissues and CAT were significantly higher than the normal renal cortex, and angiotensin II significantly increased the RPS value of tumor tissues, CAT, CDT and RRC. However, the RPS value of PVM had no obvious changes. * $P < 0.05$; ** $P < 0.01$.

Fig. 4. The relation model between the tumor vascular bed and mother vascular bed of the renal cortex and other organs is shown. (A) The renal mother vascular bed consist of the glomerular capillary and peritubular capillary, which are two sets of the capillary network series connected by efferent arterioles. (B) The other organs and tissues only have one set of capillary network. (C) The tumor vascular bed and mother vascular bed are parallel circuits.

Fig. 5. The modeling diagram of changes in perfusion parameters of other organ tumors (a rabbit liver tumor, adapted from ref [16]) induced by angiotensin II administration is shown. (A) Resting condition; (B) angiotensin II administration; adapted from ref. [12, 25]. (A) In the resting condition, the values of PS in tumors were higher, the BV and MTT values were lower, but the BF values had no obvious changes, when compared with normal tissues. (B) After angiotensin II administration, the terminal arteriole of normal tissues contract, causing greater vascular resistance and simultaneously inducing greater perfusion pressure on the upper arterioles, resulting in the inflow of blood to the passive vascular network of the tumor. All the values of BF, BV and PS in tumors were obviously higher, while the MTT values were lower than that of tumors in the resting condition. Meanwhile, the values of BF and PS in normal tissues were higher, but the BV and MTT values were lower than that of normal tissues in the resting condition.

Discussion

The typical microvasculature of normal organs and tissues has a hierarchical organization of arterioles, capillaries and venules. The composition of the microcirculation is specialized in concert with the unique functions of each organ and tissue [5]. Two sets of capillary network series combined with efferent arterioles is a feature of the renal vascular bed (Fig. 4A). This structure is consistent with renal urinary function [11, 22-24]. Although the microvasculature of other organs and tissues also has its own specialized structure, these only have one set of capillary network (Fig. 4B). The tumor vascular bed and preexisting vascular bed (maternal vascular bed) are parallel circuits [12, 25, 26] (Fig. 4C).

As previously mentioned, tumor vascular abnormalities were present in morphology, structure and function, and there was a lack of autoregulation capability in the microcirculation of tumor tissues [9, 10, 27]. Furthermore, considerable studies have reported that normal tissues and organs have the ability to autoregulate BF. In particular, BF in the brain and kidney almost maintains stability within a certain range of blood pressure changes (80-160 mmHg) [22, 28]. A large number of clinical and experimental data has shown that angiotensin II can selectively enhance tumor vascular permeability and the BF of subcutaneous tissues, muscles, livers, and other tissues and organs [12-17, 26], such as the liver tumor shown in Fig. 5.

Fig. 6. The modeling diagram of changes in perfusion parameters of renal tumors induced by angiotensin II administration is shown. (A) Resting condition; (B) Angiotensin II administration (AT-II). (A) In the resting condition (normotension, group B), the RPS values of renal tumor tissues were significantly higher. The BF, BV and PS values were significantly lower than that in normal renal cortex tissues. (B) After angiotensin II administration (hypertension, group C), angiotensin II constricted the efferent arteriole more than the afferent arteriole. Then, angiotensin II simultaneously increased PP, GFR, FF and UV. The RPS value of normal renal cortex tissues (mother vascular bed) was significantly higher, the BF values were significantly lower, but the BV, PS and MTT values had no obvious changes, when compared with normal renal cortex tissues in group B. The RPS value of renal tumor tissues was significantly higher, while the BF, BV, PS and MTT values had no obvious changes, when compared with renal tumor tissues in group C. ↑↑, increase, $P < 0.01$; ↓, decrease, $P < 0.05$; ↓↓, decrease, $P < 0.01$.

Glomerular filtration barriers include mechanical barriers and charge barriers, and the glomerulus has a selective filtration function. This has orifices within the range of 80-130 nm in diameter [22, 29, 30]. Renal tumor tissues lose its normal glomerular and renal tubular morphology. There was a significant difference between the normal renal cortex and renal cell carcinoma in all perfusion parameters (BF, BV, MTT and PS) in humans [31]. Compared with both sides of the normal renal cortex, it was also found that the BF, BV and PS values of rabbit renal VX2 tumors were significantly lower, and the RPS of renal tumor tissues were higher than that of normal renal cortex tissues in group B (normotension) (Fig. 6A). These present results are consistent with the results of a literature [31]. Although angiotensin II did not change the capillary vascular permeability (PS) of intrarenal tumors and normal renal tissues, it improved the extravascular-intravascular fluid exchange capacity (RPS) of both tissues. Furthermore, the increase in normal renal tissue RPS caused the glomerular filtration rate (GFR), filtration fraction (FF) and urinary volume (UV) to simultaneously increase. The increase in tumor tissue RPS objectively reflects the abnormality of the tumor vasculature in morphology, structure and function (Fig. 6B). The result of the present study is different from the results reported by literatures [13, 15, 16].

The total area of the renal glomerular capillary was significantly larger than the total area of the tumor capillary. In terms of glomerular capillary permeability, the renal glomerular capillary network can be regarded as a large “tumor vascular bed”. The smaller the renal tumor vascular bed at the early stage, and bigger the “tumor vascular bed” (maternal vascular bed), which are connected in parallel (Fig. 6B). Angiotensin II constricts both afferent and efferent arterioles, but the constriction of efferent arterioles is greater than the constriction of afferent arterioles [11, 22, 23, 32, 33]. BF increased in the glomerular capillary, and BF and blood pressure decreased in peritubular capillaries [22]. After angiotensin II induced the high blood pressure, BF could enter into the tumor tissue, and enabled more BF to enter into the glomeruli (maternal vascular bed), which in turn reduced the BF of the intrarenal tumor. This is probably the reason why the BF, BV, PS and MTT of renal tumor tissues did not increase similar with the tumors in other organs, and why only the RPS of tumor tissues was significantly higher under angiotensin II-induced hypertension in the present study. In the maternal vascular bed, due to the constriction of the efferent arteriole, the MTT value was longer, and the BV and PS values did not exhibit any significant changes. Since perfusion pressure (PP), GFR, FF and UV were simultaneously increased by angiotensin II [23], the extravascular-intravascular liquid exchange ratio in the glomerular capillary increased, the BF value became significantly lower, and the RPS value became significantly higher. To our

knowledge, these findings have not been previously reported, in which perfusion CT was used to assess the hemodynamics changes of intrarenal tumors after angiotensin II-induced hypertension.

In the present, the discussion was limited to tumor tissues inside the kidney. As the tumor grew, it broke through the bondage of the renal fascia, and the extrarenal feeding artery joined the blood supply of the tumor tissue. This caused the tumor blood supply to multiply, instead of having only by renal artery. The effect of angiotensin II on the hemodynamics of tumors with multi-source feeding arteries will be discussed in future studies.

Conclusion

Perfusion CT can observe the influence of angiotensin II on normal renal tissues and intrarenal tumor BF distribution, to some extent, and the mechanism of angiotensin II in its effect on intrarenal tumor hemodynamics was elucidated. Angiotensin II induces more BF to enter the tumor tissue into the glomeruli, preventing the BF, BV, PS and MTT of renal tumor tissues from increasing similar to the tumors in other organs. However, no significant changes were found. Angiotensin II increased RPS in intrarenal tumors. Hence, RPS can objectively reflect the extravascular-intravascular fluid exchange capacity of capillaries, compared with PS.

Acknowledgements

We are particularly grateful to all the people who have given us help on our article. The study was supported by a grant from the National Natural Science Foundation of China (No.51473044).

Disclosure statement

No conflict of interests exists.

References

- 1 Kyoko H, Nako M, Chisaho T, Yasuhiro H: Tumor angiogenesis-characteristics of tumor endothelial cells. *Int J Clin Oncol* 2016;21:206-212.
- 2 Konerding MA, Miodonski AJ, Lametschwandtner A: Microvascular corrosion casting in the study of tumor vascularity: a review. *Scanning Microsc* 1995;9:1233-1244.
- 3 Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM: Openings between defective endothelial cells explain tumor vessel leakiness. *Am J Pathol* 2000;156:1363-1380.
- 4 Pasqualini R, Arap W, McDonald DM: Probing the structural and molecular diversity of tumor vasculature. *Trends Mol Med* 2002;8:563-571.
- 5 McDonald DM, Baluk P: Imaging of angiogenesis in inflamed airways and tumors: newly formed blood vessels are not alike and may be wildly abnormal. *Chest* 2005;128:602S-608S.
- 6 Ho KS, Poon PC, Owen SC, Shoichet MS: Blood vessel hyperpermeability and pathophysiology in human tumour xenograft models of breast cancer a comparison of ectopic and orthotopic tumours. *BMC Cancer* 2012;12:579-589.
- 7 McDonald DM, Baluk P: Significance of blood vessel leakiness in cancer. *Cancer Res* 2002;62:5381-5385.
- 8 Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM: Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2003;163:1801-1815.
- 9 Paduch R: The role of lymphangiogenesis and angiogenesis in tumor metastasis. *Cell Oncol (Dordr)* 2016;39:397-410.

10. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM: Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2002;160:985-1000.
11. Elkin M, Meng CH: The effects of angiotensin on renal vascularity in dogs. *Am J Roentgenol Radium Ther Nucl Med* 1966;98:927-934.
12. Maeda H: Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. *Adv Drug Deliver Rev* 2015;91:3-6.
13. Kuroda C, Mihara N, Hosomi N, Inoue E, Fujita M, Ohigashi H, Ishikawa O, Nakaizumi A, Ishiguro S: Spiral CT during pancreatic pharmacangiography with angiotensin II in patients with Pancreatic Disease. *Acta Radiologica* 1998;39:138-143.
14. Nagamitsu A, Greish K, Maeda H: Elevating blood pressure as a strategy to increase tumor targeted delivery of macromolecular drug SMANCS: cases of advanced solid tumors. *Jpn J Clin Oncol* 2009;39:756-766.
15. Singh SP, Ravoori MK, Dixon KA, Han L, Gupta S, Uthamanthil R, Wright KC, Kundra V: Angiotensin II increases gene expression after selective intra-arterial adenovirus delivery in a rabbit model assessed using *in vivo* SSTR2-based reporter imaging. *EJNMMI Res* 2016;6:25-33.
16. Wright KC, Ravoori MK, Dixon KA, Han L, Singh SP, Liu P, Gupta S, Johnson VE, Kan Z, Kundra V: Perfusion CT assessment of tissue hemodynamics following hepatic arterial infusion of increasing doses of angiotensin II in a rabbit liver tumor model. *Radiology* 2011;260:718-726.
17. Suzuki M, Hori K, Abe I, Saito S, Sato H: A new approach to cancer chemotherapy: selective enhancement of tumor blood flow with angiotensin II. *J Natl Cancer Inst* 1981;67:663-669.
18. D'Onofrio M, Cingarlini S, Ortolani S, Crosara S, DE Robertis R, Vallerio P, Grego E, Ciaravino V, Ruzzenente A, Landoni L, Scarpa A, Bassi C, Tortora G: Perfusion CT changes in liver metastases from pancreatic neuroendocrine tumors during everolimus treatment. *Anticancer Res* 2017;37:1305-1311.
19. Ma AD, Zhang Y, Xue Z, Li K: Angiogenesis of hepatocellular carcinoma under multislice spiral CT plain scan and enhanced scan. *J Biol Regul Homeost Agents* 2015;29:895-903.
20. Lv WF, Han JK, Cheng DL, Zhou CZ, Ni M, Lu Dong: CT perfusion imaging can predict patients' survival and early response to transarterial chemo-lipiodol infusion for liver metastases from colorectal cancers. *Korean J Radiol* 2015;16:810-820.
21. Ippolito D, Querques G, Okolicsanyi S, Franzesi CT, Strazzabosco M, Sironi S: Diagnostic value of dynamic contrast-enhanced CT with perfusion imaging in the quantitative assessment of tumor response to sorafenib in patients with advanced hepatocellular carcinoma: A feasibility study. *Eur J Radiol* 2017;90:34-41.
22. Zhu DN, Wang TH: Physiology. 8th edition. People's Medical Publishing House; 2013.
23. Regoli D, Gauthier R: Site of action of angiotensin and other vasoconstrictors on the kidney. *Can J Physiol Pharm* 1971;49:608-612.
24. Ito S: Control of afferent and efferent arterial tone; in Anderson WP, Evans RG and Stevenson KM (eds): *The renal circulation*. Elsevier, 2000.
25. Hori K, Saito S, Takahashi H, Sato H, Maeda H, Sato Y: Tumor-selective blood flow decrease induced by an angiotensin converting enzyme inhibitor, temocapril hydrochloride. *Jpn J Cancer Res* 2000;91:261-269.
26. Hori K, Zhang QH, Saito S, Tanda S, Li HC, Suzuki M: Microvascular mechanisms of change in tumor blood flow due to angiotensin II, epinephrine, and methoxamine a functional morphometric study. *Cancer Res* 1993;53:5528-5534.
27. Santagiuliana R, Ferrari M, Schrefler BA: Simulation of angiogenesis in a multiphase tumor growth model. *Comput Method Appl M* 2016;304:197-216.
28. Johnson PC: Review of previous studies and current theories of autoregulation. *Circ Res* 1964;15:S2-9.
29. Lahdenkari AT, Lounatmaa K, Patrakka J, Holmberg C, Wartiovaara J, Kestilä M, Koskimies O, Jalanko H: Podocytes are firmly attached to glomerular basement membrane in kidneys with heavy proteinuria. *J Am Soc Nephrol* 2004;15:2611-2618.
30. Choi CH, Zuckerman JE, Webster P, Davis ME: Targeting kidney mesangium by nanoparticles of defined size. *NAS* 2011;108:6656-6661.
31. Chen C, Kang Q, Wei Q, Xu B, Ye H, Wang T, Lu Y, Lu J: Correlation between CT perfusion parameters and Fuhrman grade in pT1b renal cell carcinoma. *Abdom Radiol* 2017;42:1464-1471.
32. Ardaillou R: Angiotensin II receptor. *J Am Soc Nephrol* 1999;10:30-39.
33. Chow BS, Allen TJ: Angiotensin II type 2 receptor (AT2R) in renal and cardiovascular disease. *Clin Sci (Lond)* 2016;130:1307-1326.