In vivo microvascular clearance of albumin in renal and extrarenal tissues in puromycin aminonucleoside (PAN) induced nephrotic syndrome

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Abstract

Background. The nephrotic syndrome is characterized by generalized oedema considered to be due to the fall in serum albumin and to sodium retention. The aim of the present study was to investigate whether a generalized disturbance in vascular integrity contributes to the oedema formation.

Methods. We used the PAN-(puromycin aminonucleoside) nephritis model in order to induce the nephrotic syndrome in female Wistar rats. Eight rats were given PAN, 15 mg/100 g body weight, intraperitoneally 10 days prior to the study, whereas 21 rats served as controls. Albumin clearance to tissues was measured using a dual isotope technique. Repeated blood samples as well as samples from various muscles, kidney, liver, lung, heart, abdominal wall and from ascites fluid were taken to determine radioactivity and tissue dry-to-wet weights. Clearance of albumin ($C_{alb}$) from plasma to interstitium was calculated from the (linear) increment in ‘plasma equivalent tissue albumin space’ as a function of time, corrected for intravascular volume and oedema. The plasma and urine concentrations of albumin were determined in a parallel study by single radial diffusion using monospecific rabbit anti-rat antisem in seven PAN animals and 13 controls.

Results. A marked fall in dry-to-wet weight ratios together with pronounced proteinuria, oedema and ascites were found in the PAN animals. Haematocrit decreased from 45% (32–51) to 30% (28–38) and serum albumin from 22.0 g/l (16.3–25.2) to 4.94 g/l (3.20–6.72) in control and PAN animals, respectively. However, $C_{alb}$ apparently remained unchanged in the PAN animals in comparison to controls in most tissues examined. Thus, in these in vivo experiments there was no direct evidence of an increased extravasation of albumin in extrarenal tissues.

Conclusions. There was no strong support for the contention that a generalized disturbance of capillary integrity outside the renal vasculature would contribute to the oedema formation in the PAN nephrotic syndrome.

Introduction

The integrity of vascular endothelium is of paramount importance to maintain and regulate intravascular volume and to control fluid and macromolecular exchange between the intra- and extravascular fluid compartments. In glomerulonephritis the permselectivity of the glomerular capillaries is severely deranged. When advanced, heavy urinary protein losses ensue, leading to a fall in plasma albumin, and hence, in plasma oncotic pressure ($COP_p$). Traditionally, the pathogenesis of the generalized oedema seen in the nephrotic syndrome is thought to be due to the reduction of $COP_p$ causing an altered Starling fluid equilibrium and a redistribution of fluid from plasma to interstitium. According to this concept, intravascular volume is contracted, leading to the activation of the renin–angiotensin–aldosterone system and to vasopressin release, and to a secondary state of sodium retention. Peripheral oedema would then just be the logical response to the initially altered Starling forces, caused by the hypoalbuminaemia. This in turn causes secondary increases in the interstitial hydrostatic pressure ($P_i$) and a marked reduction in interstitial colloid osmotic pressure ($COP_i$) [1] tending to buffer the initial Starling force alterations and serving as ‘oedema safety factors’ [2].

However, there is also the possibility that there may be a generalized increase of vascular permeability in extrarenal tissues in the nephrotic syndrome, as proposed by Lange and Meltzer [3,4] and recently by Lewis et al. [5], thus contributing to the oedema formation. In other words, the processes responsible for the reduced glomerular permselectivity may also cause changes in the permeability of peripheral vessels such as those in the skin and muscle, the latter comprising the largest vascular bed in the body.

In the present study the PAN-induced nephritis...
model was employed to investigate whether transvascular clearance of albumin (Cl_{alb}) is increased or not in extrarenal tissues in the nephrotic syndrome. The PAN-model was used, since it is assumed to mimic the minimal-change nephrosis in man. Albumin extravasation into tissues was assessed in vivo using a dual albumin tracer technique to monitor intra- and extravascular ‘plasma equivalent’ albumin spaces [6,7]. In addition, whole-body plasma and blood volumes were assessed simultaneously.

**Methods**

Female Wistar rats (175–215 g) were used. Eight rats were given PAN, 15 mg/100 g body weight, intraperitoneally (i.p.) 10 days prior to the study [8], whereas 21 rats served as controls. The animals had been normally fed until the day of study. Anaesthesia was given i.p. using pentobarbital 30–50 mg/kg body weight. All the PAN-treated rats were connected to a ventilator as well as eight of the control rats for comparison. Temperature was kept constant with the aid of a heater. The tail artery was cannulated and connected to a polygraph (Grass Instruments Co, model 7B Quincy, MA, USA) for continuous blood pressure monitoring using PE 50 tubing. The left carotid artery was cannulated for collection of blood samples and the right jugular vein for a slow infusion of saline (10 ml/kg body weight/h) throughout the experiment. Proteinuria prior to the experiments was tested using dipsticks (Albustix®, Miles Inc., Ekhart, IN, USA). In the present study animals were investigated only if the dipstick was 4+, which usually occurred 8–10 days after the PAN-injection. In a parallel study [9] performed to specifically study glomerular permselectivity, 13 control animals and four rats were sacrificed after another 5–10 min by i.a. injection of undiluted plasma or urine was used and seven PAN-treated animals were used for measurement of a number of plasma and urine proteins, of which only albumin is discussed here.

[125I]HSA (Human serum albumin, Institut for Energetikene, Kjeller, Norway), used as extravascular tracer (tracer I), was given intra-arterially (i.a.) at time 0 and [131I]HSA, used as an intravascular reference (tracer II), i.a. at 60 min (or in some cases at 30 min or 15 min). The animals were sacrificed after another 5–10 min by i.a. injection of saturated KCl. Free iodine was reduced to 0.3–0.5% using Microcon 30 filters, as measured after trichloracetic acid precipitation. Blood samples, each 25 μl, were taken at 5, 10, 20, 30, 35, 40, 50, 55 and 60 min. Tissue samples from abdominal skin, m. gastrocnemius, m. tibialis anterior and m. biceps femoris of both legs, large bowel, small bowel, stomach, kidney, liver, lung, heart, abdominal wall and from ascites fluid were taken directly upon sacrifice.

Samples were weighed and then measured to obtain at least 10^4 counts in a gamma counter (Wallac 1480 Wizard®, Turku, Finland) and then dried at 65–70°C until constant weight (24–48 h). Appropriate corrections for spill-over, background activity and radioactive decay were performed.

To calculate transcapillary clearance of tracer albumin (Cl_{alb}), the plasma equivalent spaces for albumin tracer I and tracer II were each assessed as the amount of tracer present per gram tissue (CPM/g) divided by the corresponding average plasma tracer concentration (CPM/L). Extravascular albumin tissue space was obtained by subtracting the intravascular albumin space (tracer II) from the total (extravascular + intravascular) albumin space (tracer I) at time T. Cl_{alb} was obtained by dividing the extravascular space by time and correlated to 100 g of tissue. This could be done since there is normally a linear increment in the plasma equivalent tissue albumin space as a function of time during the early extravasation of tracer albumin to tissues [7,10].

The degree of oedema was calculated from dry-to-wet weight ratio of PAN-rat tissues (DW/WW) and those of control rats (DW/WW) from the formula:

\[ E = (DW/WW)_{PAN}/(DW/WW)_{C} \times 100 \]

The ‘oedema factor’, f_0 = 1 + E/100, was used to always standardize the extravascular albumin space to tissue dry weight (actually to the wet tissue weight before any oedema formation had occurred), by multiplication of all extravascular spaces by f_0.

Plasma volume (PV) was calculated by dividing the total mass of radioactivity administered (at time zero) by the c.p.m. per millilitre plasma as extrapolated to time zero using a monoeponential plot. Blood volume was calculated from central haematocrit and PV after correction (using factor 0.91) for total body haematocrit when assessed from central haematocrit [11].

The plasma and urine concentrations of albumin were determined by single radial diffusion [12] using monospecific rabbit anti-rat antiseraum (Kemila, Sollentuna, Sweden). Eight microlitres of undiluted plasma or urine was used and the plates were incubated in a humid chamber at 8°C for 3 days. Purified albumin (Sigma Chemical Co., St Louis, MO, USA) was used as standard.

Results are given as medians and ranges. StatView ANOVA with post-hoc testing was used for statistical calculations.

**Results**

The results are shown in Tables 1–3. In summary, Cl_{alb} was unchanged in the PAN-rats in comparison to controls. The results are shown in Tables 1–3. In summary, Cl_{alb} was unchanged in the PAN-rats in comparison to controls.

**Table 1.** Cl_{alb} in μl/min/100 g in ml/100 g given as medians and ranges

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PAN</th>
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<tbody>
<tr>
<td>Skin</td>
<td>3.5</td>
<td>4.4</td>
</tr>
<tr>
<td>M. tib ant</td>
<td>(3.3–26.7)</td>
<td>(2.7–9.0)</td>
</tr>
<tr>
<td>M. gastrocn.</td>
<td>5.6</td>
<td>6.7</td>
</tr>
<tr>
<td>(0.0–42.6)</td>
<td>(4.4–10.8)</td>
<td></td>
</tr>
<tr>
<td>M. biceps fem.</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>(–0.2–32.9)</td>
<td>(2.9–13.9)</td>
<td></td>
</tr>
<tr>
<td>M. abdominis</td>
<td>7.5</td>
<td>5.4</td>
</tr>
<tr>
<td>(0.8–64.7)</td>
<td>(3.0–9.1)</td>
<td></td>
</tr>
<tr>
<td>Large bowel</td>
<td>18.0</td>
<td>13.9</td>
</tr>
<tr>
<td>(10.3–76.8)</td>
<td>(4.5–22.5)</td>
<td></td>
</tr>
<tr>
<td>Small bowel</td>
<td>42.5</td>
<td>15.4</td>
</tr>
<tr>
<td>(18.9–80.5)</td>
<td>(2.0–22.4)</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>9.3</td>
<td>4.5</td>
</tr>
<tr>
<td>(2.8–26.5)</td>
<td>(4.5–20.4)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2.1</td>
<td>191.8</td>
</tr>
<tr>
<td>(–36.3–162.1)</td>
<td>(119.5–600.1)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>13.4</td>
<td>30.5</td>
</tr>
<tr>
<td>(–133.5–170.3)</td>
<td>(63.7–4.6)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>7.0</td>
<td>22.1</td>
</tr>
<tr>
<td>(–488.7–262.2)</td>
<td>(129.4–67.9)</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>31.6</td>
<td>67.6</td>
</tr>
<tr>
<td>(–23.6–116.4)</td>
<td>(23.7–92.9)</td>
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control rats in all muscles examined. In the viscera, only the small bowel showed a significant decrease in \( C_{lab} \). As expected, the kidneys showed a marked and highly significant increase in \( C_{lab} \). Apart from this only the heart showed a slight increase of \( C_{lab} \). Ventilated and non-ventilated animals did not differ significantly with respect to \( C_{lab} \), in any of the tissues investigated. Thus, data from both ventilated and non-ventilated control groups were pooled.

Dry-to-wet weight ratios are shown in Table 2. Oedema formation was prominent in most tissues except in limb muscles (Table 2), ranging from 2.6 to 40.8 ml/100 g body weight. Blood and plasma volumes (BV, PV, Table 3) increased from 5.9±0.2 to 7.3±0.4 ml/100 g body weight \((P=0.0003)\) and from 3.5±0.1 to 5.3±0.1 ml/100 g body weight \((P<0.0001)\), respectively. Haematocrit decreased from 45% \((32–51)\) to 42% \((31–49)\) in the controls to 30% \((28–38)\) in the PAN-rats \((P<0.0001)\). Ascites fluid was measured in four nephrotic animals and amounted to 19 ml \((7.4–20)\), whereas in normals i.p. fluid volume is usually 2–3 ml \([13]\).

Increased albuminuria, from 0.41 g/l \((0.16–1.21)\) in controls to 18.75 g/l \((8.90–21.47)\) in nephrotic animals, was followed by a marked decrease in serum albumin, from 22.0 g/l \((16.3–25.2)\) in controls to 4.94 g/l \((3.20–6.72)\) in PAN animals.

**Discussion**

The present study indicates that, despite the marked glomerular leakage of albumin in the PAN-induced nephrotic syndrome, \( C_{lab} \) in peripheral capillaries remains largely unaltered in the nephrotic syndrome. In other words, there is no evidence that the PAN-model induces substantial alterations in the permeability of microvascular beds other than those of the kidney, i.e. in glomeruli or peritubular capillaries. Moreover, hypoalbuminaemia *per se*, except when extreme, will not cause any increases in microvascular permeability \([14,15]\). Besides, serum factors other than albumin, are actually needed for the maintenance of a normal capillary permeability. Circulating orosomucoid, for example, is needed to maintain a normal charge selectivity of the microvascular barrier in skeletal muscle and glomeruli \([16,17]\). Although there was a sufficient reduction in serum orosomucoid \((115–14 \text{ mg/l})\) to theoretically induce increases in microvascular permeability, such alterations were not obvious from the present *in vivo* experiments.

Albumin is the main colloid responsible for plasma colloid osmotic pressure and the first line of defence against oedema formation. Marked hypoalbuminaemia and a drop in COP\(_{p}\) *per se* may thus cause marked oedema formation according to the classical Starling hypothesis. In a study of analbuminemic rats, however, no oedema was seen, possibly explained by a compensatory increase in other plasma proteins, such as orosomucoid \([18]\). Renkin and co-workers \([19]\) in a study of analbuminemic rats found only slight differences in \( C_{lab} \) versus normal controls, though there were significant differences in a few individual tissues examined. The \( C_{lab} \) values in that study and in an earlier study \([7]\) are very similar to ours, except for skin, where Renkin *et al.* \([19]\) found an increased \( C_{lab} \) in analbuminemic rats. Furthermore, in moderate hypoalbuminaemia there are compensatory reductions in COP\(_{p}\) \([1]\), and increases in interstitial hydrostatic pressure (\( P_i \)), acting as ‘safety factors’ against oedema formation.

One must bear in mind that the individual clearance values not only reflect capillary permeability (\( P \)) but also the capillary surface area (\( S \)) and the microvascular pressure (\( P_i \)) \([10,20]\). Concerning the microvascular surface area, PAN-nephrosis would hardly imply any changes in \( S \), at least not any reductions in this parameter. If anything, \( S \) would be increased due to the plasma volume expansion (see below) and due to the low haematocrit in nephrosis, in analogy with the
situation in uraemic patients, who have a low haematocrit [21]. Furthermore, there is no evidence for an altered capillary hydrostatic pressure in the nephrotic syndrome in general, at least not in man [5]. However, we cannot exclude that there is an increased capillary surface area and an increased capillary filtration coefficient [5] in our nephrotic animals, which is partly offset by a reduced driving force, i.e. a reduced capillary hydraulic pressure gradient for albumin, caused by the nephrotic oedema (see below).

The reason for the observed increase in plasma and blood volumes is not obvious, but is consistent with several other studies in humans [22–26]. One possible explanation is that renal sodium retention during nephrosis leads to such increases. In acute experiments a reduction of plasma proteins has been shown to cause a fall in blood volume only when the hypoprotinaemia is severe [27], but on the contrary, in the chronic nephrotic syndrome an increase in blood volume is often found [22,28]. Actually, Koomans et al. in their study of nephrotic patients [25] found a small elevation of blood volume in overhydrated nephrotic patients, despite large variations in extracellular fluid volume. It thus seems that the regulation of plasma volume has priority over the regulation of interstitial fluid volume in this situation [25,29]. The renal sodium retention in the PAN-nephrotic syndrome has been studied by Ichikawa et al. [30]. PAN-nephrosis was induced by selective infusion of PAN into one kidney in rat experiments, while the other kidney was left intact. This caused Na-retention only in the affected kidney, whereas Na excretion was normal in the control kidney.

Wraight [31] explained the preservation and even increase in plasma volume in hypoalbuminaemia as a consequence of a decreased protein capillary ‘permeability’ in combination with a decreased interstitial fluid protein concentration and (COP), implying an essentially maintained transcapillary osmotic gradient in hypoprotinaemia. This is in line with the present data, but the findings should rather be understood in the light of a heteroporous capillary membrane theory [20,32]. In such a model the Starling fluid equilibration is maintained across the microvascular walls due to the presence of a large number of protein selective small pores (of radius 4–5 nm) in the endothelium. Macromolecules, however, reach the interstitium through a very small number of large pores (radius 25 nm). Across large pores there is no Starling equilibrium, because of the low effective colloid osmotic pressure gradient prevailing across these pores normally. Thus, there is always fluid filtration occurring through large pores, which is solely governed by the hydrostatic pressure gradient between plasma and interstitium. In line with this theory, Rippe et al. [32] showed a clear-cut proportionality between COP, and Cl,th at zero (or low) transcapillary net filtration rates. Thus, if plasma COP is lowered while the P, is not markedly altered, then there will be an initially increased fluid filtration across the microvascular walls. This will lead to an increase in P, and a reduction in COP. These changes may, at least partly, buffer the initial increases in transcapillary fluid filtration. Most importantly, the pressure gradient across large pores is now reduced following the reduction in the transcapillary hydrostatic pressure gradient. Thus, the increases in tissue pressure following hypoproteinaemia should result in a (slightly) reduced macromolecular transport from blood to tissue.

One possible source of error in this study is that the role of the ‘lymphatic safety factor’, i.e. the increase in lymph flow in order to maintain the transcapillary oncotic pressure gradient, and thereby prevent oedema, is unknown. Hollander [33], in a study of different patients with oedema of various aetiology, concluded that albumin in accordance with the two-pore theory [20] is removed from the tissues mainly by lymphatics. Furthermore, lymphatic flow is generally increased in various types of oedema, including those in liver cirrhosis and in the nephrotic syndrome. Conhaim calculated a several-fold increase in lymph flow in a study on sheep with severe hypoproteinaemia [34]. In a more recent study by Paulson et al. [35] a significant increase in intestinal lymph flow was demonstrated in nephrotic rats. One could then argue that any increase in transcapillary albumin transport in nephrosis would be offset by an increased lymphatic drainage of tracer. However, since the interstitial equilibrium distribution volume for albumin is quite large, at least in muscle (4–5 ml/100 g) [11], and the transcapillary albumin clearance is low (0.005 ml/min), the initial drainage of tracer from tissue to lymph should be negligible during the present conditions. This is because the rate constant of solute removal from the interstitium will be determined by Cl,th divided by the interstitial albumin distribution volume, i.e. here 0.005/5 = 1 × 10−3 min−1. This holds if the interstitium functions as a well-mixed compartment. However, the situation may be different in tissues with a relatively small interstitial volume, and a high Cl,th such as in the lung [36] or intestine [37]. Furthermore, there may be preferential channels existing between the capillary barrier and the lymphatics, with which tracer albumin may rather rapidly equilibrate. In that case, there may have been removal of tracer from the tissues during the conditions of the present experiments. In a number of recent studies by Renkin and co-workers, this component was, however, considered to be small [38,39].

One previous study [5] has indicated the presence of a moderately elevated filtration coefficient (LpS, hydraulic conductivity) in the calf in patients with different types of the nephrotic syndrome. Since LpS is dependent of both permeability and surface area, it could not be determined whether either or both of these parameters had been affected. Possibly, as mentioned above, the capillary surface area in e.g. muscle would be slightly increased in the nephrotic syndrome. One cannot, however, rule out that at least part of the increment in LpS in nephrotic oedema may be a spurious one due to methodological errors inherent in the LpS determination. LpS is determined from the reasonably straight weight gain slope occurring some
time after venous pressure elevation. This linear phase will occur earlier and with a greater slope in oedematous conditions than in non-oedematous conditions. This is because in oedematous conditions increases in filtration rate are not markedly buffered by Starling force adjustments, i.e. increases in Pf and reductions in COPi occurring during e.g. LPS determinations, which however occurs during non-oedematous conditions [40]. Another possibility is that the pre-to-post-capillary resistance ratio may have been reduced during oedema formation in the nephrotic syndrome, leading to a higher degree of capillary pressure elevation in the nephrotic subjects compared to normals for any level of venous pressure elevation performed during a LPS measurement. This would lead to an overestimation of LPS in oedematous conditions.

In summary, the present study of transvascular albumin clearance during in vivo conditions could not confirm the notion of a generalised increase in transvascular albumin permeation in PAN-induced nephrotic syndrome in rats.

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