A New Method for Evaluating an Increased General Capillary Permeability in Patients

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KUROYANAGI, T. and KURA, K. A New Method for Evaluating an Increased General Capillary Permeability in Patients. Tohoku J. exp. Med., 1977, 122 (4), 331-336 — The difference between total plasma volume determined with a substance which escapes from vascular beds in the presence of an increase of general capillary permeability and that determined with a substance which is confined to blood even in the presence of an increased capillary permeability may reflect the degree of an increase of general capillary permeability. The total plasma volume was determined by simultaneous injections of $^{131}$I-HSA and $^{51}$Cr tagged red cells. The capillary permeability was evaluated by calculating the difference ($\Delta$TPV) between total plasma volume determined with $^{131}$I-HSA and that determined with $^{51}$Cr tagged red cell. $\Delta$TPV averaged $-4 \pm 20$ ml/m$^2$ in 20 normal controls. The reproducibility of TPV was good. $\Delta$TPV in patients with systemic lupus erythematosus, idiopathic thrombocytopenic purpura, chronic active hepatitis, liver cirrhosis and subacute bacterial endocarditis was larger than that of controls, averaging 204 ml/m$^2$, 178 ml/m$^2$, 82 ml/m$^2$, 131 ml/m$^2$ and 179 ml/m$^2$, respectively. The increase of $\Delta$TPV was considered to indicate the increase of capillary permeability in these patients. A permeability increasing factor was present in serum of patients with an elevated $\Delta$TPV. There was a significant correlation between $\Delta$TPV and the titer of serum capillary permeability increasing factor in these patients. ——— serum permeability increasing factor; systemic lupus erythematosus; idiopathic thrombocytopenic purpura; subacute bacterial endocarditis; chronic active hepatitis; liver cirrhosis

Until recently the Landis method has been used to estimate the general capillary permeability of patients in vivo. However, it seems likely that this method does not reflect the capillary permeability in a natural state because of the mechanical loading of venous stasis.

The modern methods to determine total blood and plasma volumes depend on the dilution principle (Wintrobe 1974). A substance is introduced into the circulation, and after an appropriate interval for mixing, the space in which it is distributed is calculated from the degree of dilution. If a substance is confined to plasma, the distribution space is equivalent to the plasma volume. However, if a substance escapes from the vascular beds on account of increased capillary permeability, the distribution space is larger than the true plasma volume (Strumia 1968). Therefore, the difference between the total plasma volume determined with

Received for publication, February 14, 1977.
a substance which escapes from the vascular beds in the presence of an increase in capillary permeability and that determined with a substance which is confined to vascular beds even in the presence of an increase of capillary permeability may reflect the degree of an increase of capillary permeability.

Based on the above concept, we have devised a new method to evaluate the capillary permeability quantitatively in patients with various diseases in vivo.

The purpose of this report is to describe our new method and the results obtained with this method.

**MATERIALS AND METHODS**

Twenty normal adults, 10 males and 10 females, were examined as controls, and 12 patients with systemic lupus erythematous, 7 with idiopathic thrombocytopenic purpura, 6 with chronic active hepatitis, 8 with liver cirrhosis without splenomegaly and 5 with subacute bacterial endocarditis were studied.

The total blood and plasma volumes were measured by \(^{131}\)I-HSA and \(^{51}\)Cr tagged red-cell methods (Brozovic et al. 1966; Dacie and Lewis 1975; ICSH 1973). Approximately 20 ml of blood were added to 3 ml of sterile acid citrate dextrose. After centrifuging at 1500 \(\times\) g for 5 min, the plasma was transferred to another sterile bottle. The buffy-coat layer was discarded and the red cells of the above 20 ml blood were labelled with 0.1 \(\mu\)Ci of \(\text{Na}_2\text{CrO}_4\) per Kg of body weight of recipients. The labelled red cells were washed twice in sterile physiologic saline, and 5 \(\mu\)Ci of \(^{131}\)I-human serum albumin (HSA) were added to the plasma, which were then remixed with the labelled red cells.

An accurately measured amount of the mixed blood was injected and the remainder was diluted to 1:100 in 0.4 g/liter ammonia for use as a standard. Blood samples were collected at 10, 20 and 30 min after the administration of the above labelled blood, and the radioactivity of a measured volume of each sample and a similar volume of the standard was determined.

The radioactivities of \(^{51}\)Cr and \(^{131}\)I were measured with a pulse-height analyzer of well type scintillation counter. The radioactivity due to \(^{51}\)Cr in the blood was obtained from the mean of the three samples and that due to \(^{131}\)I was obtained from the value extrapolated to zero time.

Total plasma volume (TPV) and total blood volume (TBV) were calculated in the following ways.

(i) **\(^{131}\)I-HSA method**

The total plasma volume was calculated from the formulae:

\[
\text{TBV} = \frac{\text{radioactivity of standard (cpm/ml)} \times \text{dilution of standard} \times \text{volume injected (ml)}}{\text{radioactivity of post injection sample (cpm/ml, corrected to zero time)}}
\]

and TPV = TBV \(\times\) (1 – hematocrit).

(ii) **\(^{51}\)Cr tagged red cells method**

The total red cell volume (RCV) was calculated from the formula:

\[
\text{RCV} = \frac{\text{radioactivity of standard (cpm/ml)} \times \text{dilution of standard} \times \text{volume injected (ml)}}{\text{radioactivity of post injection sample (cpm/ml)}} \times \text{hematocrit (Ht)}
\]

Total blood volume was calculated by multiplying the value for RCV by the reciprocal of whole body hematocrit (Ht \(\times\) 0.9) (Dacie and Lewis 1975). The plasma volume was calculated by subtracting RCV from TBV.

(iii) The differences between TBV and TPV determined by \(^{131}\)I-HSA method and those
determined by $^{51}$Cr tagged red cell method ($\Delta$TBV and $\Delta$TPV) were calculated by subtracting the latter from the former and expressed as ml per m$^2$ of body surface. It is considered that $\Delta$TBV and $\Delta$TPV reflect the degree of capillary permeability in patients.

The permeability increasing factor of serum (Movat 1971) was studied as follows: 0.1 ml of sera, which were 25, 50, 75, 100, 125 and 150 times diluted with physiological saline, was injected intradermally to rabbits which had received intravenous injection of 10 ml of Evans blue solution 1 hr before. Then, 1 hr later the minimum dilution of serum showing the diameter over 10 mm of dye accumulation area where diluted serum was injected intradermally was determined.

**RESULTS**

$\Delta$TBV and $\Delta$TPV in normal adults

$\Delta$TBV and $\Delta$TPV of 20 normal adults (10 males and 10 females) averaged $+3.7 \pm 35.3$ ml/m$^2$ and $-4.1 \pm 20.0$ ml/m$^2$, respectively (Fig. 1).

The second similar examinations were carried out on 5 normal subjects one week after the first examination. The differences of $\Delta$TBV and $\Delta$TPV between the first and second examination are shown in Table 1. They averaged $6.1 \pm 2.2$ ml/m$^2$ for $\Delta$TBV and $6.4 \pm 2.6$ ml/m$^2$ for $\Delta$TPV.
TABLE 1. Reproducibility of the method

<table>
<thead>
<tr>
<th></th>
<th>TBV (ml/m²)</th>
<th>TPV (ml/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>KT</td>
<td>−71.8</td>
<td>−61.5</td>
</tr>
<tr>
<td>KK</td>
<td>−18.6</td>
<td>−24.8</td>
</tr>
<tr>
<td>KH</td>
<td>−42.6</td>
<td>−38.8</td>
</tr>
<tr>
<td>SK</td>
<td>43.7</td>
<td>37.6</td>
</tr>
<tr>
<td>FK</td>
<td>36.3</td>
<td>40.0</td>
</tr>
<tr>
<td>AM</td>
<td>16.5</td>
<td>9.8</td>
</tr>
</tbody>
</table>

Mean  | − | − | 6.1±2.2 | − | − | 6.4±2.6

TABLE 2. ΔTBV and ΔTPV in patients with systemic lupus erythematosus, idiopathic thrombocytopenic purpura, chronic active hepatitis, liver cirrhosis and subacute bacterial endocarditis

<table>
<thead>
<tr>
<th></th>
<th>ΔTBV (ml/m²)</th>
<th>ΔTPV (ml/m²)</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>−3.7±35.3</td>
<td>−4.1±29.0</td>
</tr>
<tr>
<td>SLE</td>
<td>205.1±55.7</td>
<td>203.8±67.3</td>
</tr>
<tr>
<td>ITP</td>
<td>170.0±64.1</td>
<td>178.2±57.0</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>123.5±32.0</td>
<td>82.0±34.1</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>107.0±32.0</td>
<td>131.4±48.0</td>
</tr>
<tr>
<td>SBE</td>
<td>256.5±27.7</td>
<td>178.5±34.6</td>
</tr>
</tbody>
</table>

Notes: SLE, systemic lupus erythematosus; ITP, idiopathic thrombocytopenic purpura; SBE, subacute bacterial endocarditis.

ΔTBV and ΔTPV in patients with various diseases

ΔTBV and ΔTPV averaged 20.5±55.7 ml/m² and 203.8±67.3 ml/m², respectively, in 12 patients with systemic lupus erythematosus, 170.0±64.1 ml/m² and 178.2±57.0 ml/m² in 7 patients with idiopathic thrombocytopenic purpura, 123.5±32.0 ml/m² and 82.0±34.1 ml/m² in 6 patients with chronic active hepatitis, 107.0±32.0 ml/m² and 131.4±48.0 ml/m² in 12 patients with liver cirrhosis and 256.5±17.7 ml/m² and 178.5±34.6 ml/m² in 5 patients with subacute bacterial endocarditis.

ΔTBV and ΔTPV in patients with capillary permeability increasing factor

25-times diluted serum did not show any dye accumulation in 20 normal subjects. Therefore, serum which caused a dye accumulation even after 25-times dilution was considered to have a permeability increasing factor.

ΔTBV and ΔTPV averaged −18.5±40.3 ml/m² and 16.7±43.6 ml/m², respectively, in serum permeability increasing factor negative patients, and 172.4±76.9 ml/m² and 154.8±43.3 ml/m² in serum permeability increasing factor positive patients.

Correlation between ΔTBV and serum permeability factor activities

When ΔTBV was plotted on the abscissa and the minimum dilution showing dye accumulation diameter over 10 mm was plotted on the ordinate, a significant
TABLE 3. $\Delta TBV$ and $\Delta TPV$ in permeability increasing factor positive patients

<table>
<thead>
<tr>
<th></th>
<th>PF (−)</th>
<th>PF (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta TBV$ (ml/m²)</td>
<td>$-18.5\pm40.3$</td>
<td>$172.4\pm76.9$</td>
</tr>
<tr>
<td>$\Delta TPV$ (ml/m²)</td>
<td>$-16.7\pm43.6$</td>
<td>$154.8\pm43.3$</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between $\Delta TBV$ and serum permeability increasing factor activity.

correlation ($r=0.732$) between them was demonstrated as shown in Fig. 2.

**COMMENT**

Until recently, the Landis method has been employed to evaluate the capillary permeability of patients in vivo. This method is to measure the changes of hematocrit and serum protein due to plasma exudation after the loading of venous stasis. Therefore, it seems likely that this method does not reflect the capillary permeability in natural state.

When we determine the total plasma volume by $^{131}$I-HSA method in patients with an increased capillary permeability, the total plasma volume estimated should be larger than the actual total plasma volume because of leakage of $^{131}$I-HSA from vascular beds to extravascular space. However, if we use $^{51}$Cr tagged red cells to estimate the total blood volume in these patients, tagged red cells are confined to vascular beds. Therefore, the blood volume and total plasma volume determined by $^{51}$Cr tagged red cell method reflect the actual values in these patients with an increased capillary permeability.

Therefore, differences ($\Delta TBV$ and $\Delta TPV$) between TBV and TPV which are determined with $^{131}$I-HSA and $^{51}$Cr tagged red cell may reflect the degree of the
exudation of albumin from vascular beds due to an increased capillary permeability. \(\Delta TBV\) and \(\Delta TPV\) averaged \(3.7 \pm 35.3 \text{ ml/m}^2\) and \(-4.1 \pm 20.0 \text{ ml/m}^2\) respectively, in normal controls. The reproducibility of this method was good. \(\Delta TBV\) and \(\Delta TPV\) in patients with systemic lupus erythematosus, idiopathic thrombocytopenic purpura, chronic active hepatitis, liver cirrhosis and subacute bacterial endocarditis were larger than those in normal adults. These results indicate the presence of an increased capillary permeability in these patients.

A permeability increasing factor has been found in serum of patients with an increased \(\Delta TBV\) and \(\Delta TPV\). A significant correlation was demonstrated between activities of serum permeability factor and \(\Delta TBV\), suggesting that serum permeability increasing factor plays a significant role in the mechanism of an increase of capillary permeability in patients.

References


