Original Article

Effects of a Nucleoside Transporter Inhibitor, Dilazep, on Renal Microcirculation in Rats

Masahiko KAWABATA, Manabu HANEDA, Tao WANG, Michiru IMAI, and Toshikazu TAKABATAKE

Adenosine, one of the endogenous modulators in renal hemodynamics, has recently been shown to be a mediator of tubuloglomerular feedback (TGF). Dilazep augments endogenous adenosine actions by blocking its cellular uptake. Our purpose in the present study was to clarify the effects of dilazep on renal microcirculation and the TGF mechanism. Clearance and micropuncture experiments were performed in anesthetized rats. TGF responsiveness was assessed in superficial nephrons by measuring the changes of early proximal flow rate (EPFR) in response to loop perfusion at 0–40 nl/min with artificial tubular fluid (ATF). Under dilazep administration (0.3 mg/kg i.v.) systemic BP and GFR were decreased and renal plasma flow was unaltered; as a result, the filtration fraction tended to decrease (p = 0.076). Renal vascular resistance was reduced, but not to a significant degree. The reduction in EPFR by loop perfusion was similar between controls (47 ± 2%) and rats administered dilazep i.v. (44 ± 5%). Intraluminal application of dilazep in ATF suppressed TGF-mediated EPFR reduction by 46 ± 4%, 43 ± 7%, and 37 ± 3% at dilazep concentrations of 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/l, respectively. TGF suppression with 10⁻⁴ mol/l dilazep was reversed by co-perfusion of 10⁻⁵ mol/l DMPX, a selective adenosine A₂ receptor antagonist. DMPX alone did not affect TGF response. In conclusion, these results indicate that systemic dilazep dilates postglomerular arterioles and does not affect TGF, and thus reduces GFR. A pharmacological concentration of dilazep applied to single nephrons clearly attenuates TGF, indicating afferent arteriolar vasodilatation. Extracellular adenosine augmented by dilazep dilates glomerular vessels at both afferent and efferent sites, probably via the activation of A₂ receptors. (Hypertens Res 2002; 25: 615–621)

Key Words: adenosine, afferent arteriole, efferent arteriole, macula densa, tubuloglomerular feedback

Introduction

The intrinsic control of renal hemodynamics depends on the tubuloglomerular feedback (TGF) mechanism as well as on the myogenic mechanism (1). The former alters nephron blood flow and glomerular filtration by changing afferent arteriolar resistance in response to the sodium chloride concentration at the macula densa (2). The TGF mechanism plays an important role in the regulation of water, electrolytes and blood pressure. In young spontaneously hypertensive rats (SHR), TGF activity is augmented compared to that in age-matched Wistar Kyoto rats, suggesting that it may participate in the initiation and/or development of hypertension (3). Adenosine, one of the endogenous modulators of renal hemodynamics, has recently been shown to be a mediator between the receptor and the effector of TGF, i.e., the macula densa and afferent arterioles in the juxtaglomerular apparatus (JGA) (4).

Nucleoside transport inhibitors increase extracellular adenosine concentration by competitively blocking the carrier-mediated process of cellular adenosine uptake (5). Dilazep and dipyridamole, acting as nucleoside transport inhibitors, markedly potentiate the vasodilatory action of adenosine in the heart and also inhibit platelet aggregation. These two inhibitors have been reported to reduce proteinuria in nephrotic or diabetic animals (6–8) as well as in...
nephrotic patients (9) via mechanisms that involve preservation of the charge barrier of the glomerulus (6–8) and reduction in GFR (6, 9). Dipyridamole has been shown to decrease GFR in dogs (10) and humans (9, 11). This decrease in GFR, which in humans was accompanied by a fall in filtration fraction (FF) (9), seems to indicate a reduction in intraglomerular pressure via the vasodilatation of postglomerular efferent arterioles. While several reports have documented the renal hemodynamic effects of dilazep (6, 12, 13), the effects in renal microcirculation have not been investigated. Because these adenosine potentiators have the potential to influence the TGF mechanism, and thereby the regulation of water, electrolytes and blood pressure homeostasis, the present experiments were undertaken to evaluate the effects of dilazep on glomerular microcirculation and the TGF mechanism in anesthetized rats.

Materials and Methods

Animal Preparations

This study was performed in accordance with the Guide for Animal Experimentation of the Shimane Medical University. Experiments were carried out on male Sprague-Dawley rats maintained on a commercial chow diet. Rats were prepared as previously described (3, 14) under anesthesia induced with 110 mg/kg i.p. thiopental sodium (Ravonal, Tanabe, Tokyo, Japan). The rat was placed on a thermostatically controlled heated table to maintain rectal temperature at 37.5°C. A tracheostomy tube was inserted, and a PE-50 polyethylene tube (Clay Adams, Parsippany, USA) was placed in the external jugular vein for infusion of 10% polyfructosan (Inutest; Fresenius Kabi, Linz, Austria) in 0.9% saline at a rate of 4.5 ml/kg/h. The right femoral artery was cannulated with a PE-50 tube for monitoring arterial pressure and collecting blood samples. The left kidney was placed in a plastic cup, immobilized in agar, and its pelvis cannulated with a PE-10 tube to collect urine samples. A 25-gauge needle connected to a PE-50 tube was inserted into the left renal vein to collect renal venous blood. Clearance and micropuncture experiments were started 1 h after the preparation had been finished.

Whole Kidney Clearance Study

Two 30-min clearances were performed as a control period. Dilazep was then infused for three 30-min clearances. Two additional 30-min recovery clearances were performed by reinfusing saline vehicle instead of dilazep solution. Dilazep dissolved in 0.9% saline containing 10% polyfructosan was infused via the jugular vein at a rate of 0.1 mg/kg/h following the initial bolus dose of 0.1 mg/kg (low dose, n = 5 rats) or 0.3 mg/kg/h following the initial bolus dose of 0.3 mg/kg (high dose, n = 6 rats). Repeated blood samples were taken from the femoral artery and renal vein at the midpoint of each clearance period. The volume, approximately 600 μl per rat throughout all the experimental periods, was replaced by 0.9% saline.

Measurement of Early Proximal Flow Rate (EPFR) during Loop Perfusion

During the clearance study, TGF response was estimated by measuring the changes in EPFR, an index of single nephron GFR, while the loop of Henle was perfused from the end proximal segment with artificial tubular fluid (ATF) at a rate of 40 nl/min, as previously described (3, 14). Successive, 3-min collections of tubular fluid were made from the early proximal segment during microperfusion at 0 or 40 nl/min. Tubular fluid was collected spontaneously, proximal to a mobile, 4- to 5-tubular diameter block of Sudan Black-stained mineral oil in 10- to 12-μm pipettes. The volume of tubule fluid was determined in a constant bore-capillary (Microcaps 1.0 μl; Drummond, Broomall, USA). The feedback response was expressed as the change in EPFR when the loop perfusion rate was increased from 0 to 40 nl/min.

EPFR measurements were performed during both the control period and the experimental period of high-dose dilazep infusion. In another group of rats (n = 10), EPFR was measured during the microinfusion of a pharmacological concentration of dilazep (10⁻⁶–10⁻⁴ mol/l) and/or an A₁ selective adenosine receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX, RBI, Natic, USA, 10⁻⁵ mol/l) (15), dissolved in ATF.

Analytical Techniques and Calculations

The polyfructosan concentrations in plasma and urine were measured by the anthrone method. Plasma and urine sodium and potassium concentrations were measured by a flame photometer (Model 775; Hitachi, Tokyo, Japan). GFR was determined from polyfructosan clearance. Renal plasma flow (RPF) was calculated as GFR · A₀/(A₀ - Vₚ), where A₀ and Vₚ are the arterial and renal venous concentrations of polyfructosan, renal blood flow (RBF) as RPF/(1 - hematocrit), and renal vascular resistance (RVR) as mean blood pressure (BP)/RBF. Results are expressed as the mean ± SEM. Student’s paired or unpaired t-test, or analysis of variance (ANOVA) was used for comparison. When ANOVA revealed a significant difference, Fisher’s protected least significant difference test was applied to identify specific group differences. Values of p < 0.05 were considered to indicate statistical significance.

Results

Whole Kidney Clearance

Low-dose dilazep infusion (0.1 mg/kg + 0.1 mg/kg/h) did not influence any of the clearance parameters, including systemic BP (Table 1). However, high-dose dilazep infusion (0.3
Table 1. Effects of Intravenous Infusion of Low-Dose Dilazep on Whole Kidney Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dilazep</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>114 ± 5</td>
<td>114 ± 4</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>RVR (mmHg · ml⁻¹ · min⁻¹ · g KW⁻¹)</td>
<td>16.3 ± 1.5</td>
<td>16.8 ± 0.7</td>
<td>15.9 ± 0.9</td>
</tr>
<tr>
<td>RBF (ml · min⁻¹ · g KW⁻¹)</td>
<td>7.23 ± 0.50</td>
<td>6.81 ± 0.20</td>
<td>7.17 ± 0.35</td>
</tr>
<tr>
<td>RPF (ml · min⁻¹ · g KW⁻¹)</td>
<td>3.51 ± 0.28</td>
<td>3.34 ± 0.15</td>
<td>3.54 ± 0.24</td>
</tr>
<tr>
<td>GFR (ml · min⁻¹ · g KW⁻¹)</td>
<td>1.09 ± 0.09</td>
<td>1.07 ± 0.04</td>
<td>1.09 ± 0.08</td>
</tr>
<tr>
<td>FF</td>
<td>0.31 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>UV (µl · min⁻¹ · g KW⁻¹)</td>
<td>4.6 ± 1.1</td>
<td>4.0 ± 0.8</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>U₉V (nEq · min⁻¹ · g KW⁻¹)</td>
<td>198 ± 86</td>
<td>184 ± 90</td>
<td>81 ± 24</td>
</tr>
<tr>
<td>FE₉V (%)</td>
<td>0.107 ± 0.036</td>
<td>0.107 ± 0.050</td>
<td>0.047 ± 0.012</td>
</tr>
<tr>
<td>U₉V (nEq · min⁻¹ · g KW⁻¹)</td>
<td>1,504 ± 339</td>
<td>1,250 ± 229</td>
<td>1,021 ± 197</td>
</tr>
<tr>
<td>FE₉ (%)</td>
<td>296 ± 56</td>
<td>281 ± 53</td>
<td>227 ± 38</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>52 ± 1</td>
<td>51 ± 1</td>
<td>51 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n = 5 rats. Control, control period; Dilazep, experimental period of dilazep infusion (0.1 mg/kg + 0.1mg/kg/h i.v.); Recovery, recovery period after cessation of dilazep infusion; MBP, mean blood pressure; RVR, renal vascular resistance; RBF, renal blood flow; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine volume; U₉V, urinary excretion of sodium; FE₉, fractional excretion of sodium; U₉V, urinary excretion of potassium; FE₉, fractional excretion of potassium; Ht, hematocrit; KW, kidney weight.

Table 2. Effects of Intravenous Infusion of High-Dose Dilazep on Whole Kidney Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dilazep</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>112 ± 4</td>
<td>107 ± 2*</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>RVR (mmHg · ml⁻¹ · min⁻¹ · g KW⁻¹)</td>
<td>15.1 ± 1.0</td>
<td>12.9 ± 1.5</td>
<td>14.0 ± 1.8</td>
</tr>
<tr>
<td>RBF (ml · min⁻¹ · g KW⁻¹)</td>
<td>8.32 ± 0.69</td>
<td>8.34 ± 1.29</td>
<td>8.20 ± 0.85</td>
</tr>
<tr>
<td>RPF (ml · min⁻¹ · g KW⁻¹)</td>
<td>4.15 ± 0.35</td>
<td>4.52 ± 0.54</td>
<td>4.19 ± 0.48</td>
</tr>
<tr>
<td>GFR (ml · min⁻¹ · g KW⁻¹)</td>
<td>1.16 ± 0.05</td>
<td>0.95 ± 0.04*</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>FF</td>
<td>0.29 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>UV (µl · min⁻¹ · g KW⁻¹)</td>
<td>4.3 ± 0.5</td>
<td>3.2 ± 0.3*</td>
<td>3.1 ± 0.3*</td>
</tr>
<tr>
<td>U₉V (nEq · min⁻¹ · g KW⁻¹)</td>
<td>39 ± 9</td>
<td>55 ± 19</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>FE₉V (%)</td>
<td>0.022 ± 0.005</td>
<td>0.037 ± 0.012</td>
<td>0.023 ± 0.008</td>
</tr>
<tr>
<td>U₉V (nEq · min⁻¹ · g KW⁻¹)</td>
<td>1,271 ± 211</td>
<td>849 ± 141</td>
<td>785 ± 153*</td>
</tr>
<tr>
<td>FE₉ (%)</td>
<td>240 ± 39</td>
<td>211 ± 33</td>
<td>171 ± 31</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>50 ± 1</td>
<td>49 ± 1</td>
<td>49 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n = 6 rats. Dilazep, 0.3 mg/kg + 0.3 mg/kg/h i.v. Abbreviations are as in Table 1. * p < 0.05 vs. Control (ANOVA).

Table 1 and Table 2 show the effects of intravenous infusion of low-dose and high-dose dilazep on whole kidney function. Low-dose dilazep infusion lowered systemic BP by 5.3 ± 2.3 mmHg. GFR was significantly decreased and RPF was unaltered; as a result, FF tended to decrease during the experiment period (p = 0.076). These parameters showed a tendency to return to the control level after the cessation of dilazep infusion, except that urine volume remained significantly low during the recovery period. The reduction in RVR during the experiment period was not significant. Urinary excretion of potassium (U₉V) decreased after the cessation of dilazep infusion.

**EPFR Response to Loop Perfusion**

Figure 1 shows the EPFR measurements in the absence of distal flow and during loop perfusion at 40 nl/min. Loop perfusion significantly decreased EPFR from 29.9 ± 1.4 to 16.1 ± 1.2 nl/min during the control periods (n = 15), and from 29.2 ± 1.4 to 16.6 ± 1.9 nl/min during the experimental periods (n = 9). The reductions in EPFR by loop perfusion were not different between two periods, 46.9 ± 2.1% and 43.7 ± 4.9% of EPFR at no loop flow, respectively.

Figure 2 shows the EPFR values during intratubular perfusion of drugs. Dilazep in ATF suppressed EPFR reduction during loop perfusion; EPFR was reduced from 26.8 ± 1.2 nl/min to 14.3 ± 1.0 nl/min (46.3 ± 3.6%) at 10⁻⁶ mol/l dilazep (n = 12), from 38.6 ± 3.3 nl/min to 21.4 ± 2.6 nl/min (43.4 ± 6.8%) at 10⁻⁵ mol/l dilazep (n = 8), and from 26.3 ± 2.0 nl/min to 16.3 ± 1.0 nl/min (36.5 ± 3.2%) at 10⁻⁴ mol/l dilazep (n = 15). The EPFR reduction by the maximal concentration
of dilazep used in this study, $10^{-4}$ mol/l, was significantly smaller than that in the controls (46.9 ± 2.1%). The reduction by $10^{-4}$ mol/l dilazep was clearly reversed by co-perfusion of $10^{-5}$ mol/l DMPX with $10^{-4}$ mol/l dilazep into the loop of Henle; EPFR was reduced from 28.4 ± 1.2 nl/min to 15.6 ± 1.2 nl/min (45.4 ± 3.1%) ($n$ = 11). Perfusion of $10^{-5}$ mol/l DMPX alone in ATF reduced EPFR from 26.0 ± 1.4 nl/min to 14.6 ± 1.1 nl/min (43.9 ± 2.3%) ($n$ = 14). These reductions observed with dilazep and DMPX or DMPX alone were not significantly different from those in the control group. Figure 3 summarizes EPFR reductions during systemic infusion of dilazep and intratubular perfusion of dilazep and/or DMPX.

**Discussion**

In the present clearance studies, infusion of high-dose dilazep clearly decreased GFR and, because RPF did not increase significantly, FF tended to be low during the infusion. In glomerular microcirculation, such hemodynamic changes are caused by a decrease in the resistance of postglomerular efferent arterioles and/or the ultrafiltration coefficient of glomerulus ($K_f$). Insignificant decrease in RVR in the present study would appear to support the former mechanism. The hypotensive effect was significant but mild, and thus systemic BP remained above the lower range of renal perfusion pressure in renal autoregulation. These effects were clearly dependent on the dose of dilazep, since that low-dose dilazep infusion did not affect systemic BP or any of the other hemodynamic parameters.

To our knowledge, there have been only three reports documenting the effects of dilazep on whole-kidney function (6, 12, 13). In one of these studies, oral administration of dilazep decreased GFR and urinary albumin excretion in nephrotic rats (6). In another, intravenous infusion of dilazep did not change RBF in dogs, but did increase coronary blood flow and decrease systemic BP (12). However, when dilazep was infused directly into the renal arteries of dogs in order to minimize its systemic influences, RBF was increased, RVR was decreased, and GFR remained unaltered (13). These findings support the idea that renal vasodilatation is predominant in the efferent arterioles rather than in the afferent arterioles. Dilatation of the efferent arterioles, even without systemic BP reduction, is thought to decrease transcapillary hydrostatic pressure in the glomerulus and thereby to reduce GFR and proteinuria. Because the protective effects of drugs on the kidney may be related to a reduction of glomerular hypertension, dilazep may play a role in nephroprotection through its effects on the efferent arterioles, as we suggested in our previous studies using efferent arteriole-dilating calcium channel blockers (15, 16).

The hemodynamic changes induced by dilazep or dipyridamole mimic those induced by intrarenal infusion of adenosine (17). In addition, treatment with a non-selective antagonist of both $A_1$ and $A_2$ adenosine receptors, 3-isobutyl-1-methyl-xanthine, antagonizes the renal vasodilatation by dilazep (13). These findings strongly suggest that dilazep dilates the renal vasculature via an adenosine-mediated mechanism.

As previously shown by micropuncture experiments in our (18) and other (19, 20) laboratories, an intraluminal route of application delivers agents through tubular cells into the interstitium surrounding the vascular effector cells in JGA. When dilazep was perfused into the tubular lumen of single nephrons, the macula densa-mediated reduction in EPFR was clearly decreased at the highest concentration, $10^{-4}$ mol/l, indicating that TGF response was attenuated at the pharmacological concentration. Because the TGF response involves
the vasomotor response of the afferent arterioles in JGA, the blunted response of TGF suggests dilation of this arteriolar segment by microperfused dilazep. Furthermore, the reversal of TGF attenuation by co-administration of an A2-specific adenosine receptor antagonist, DMPX, supports the idea that the vasodilatory mechanism of dilazep is mediated by adenosine A2 receptor activation. This is in line with previous reports in which very high concentrations of agonists induced A2 receptor-mediated afferent vasodilatation (19, 21). Intraluminal perfusion of N-ethyl-carboxamide adenosine (NECA), a preferential A2 receptor agonist, decreased the magnitude of TGF response at the concentration of 10⁻⁴ mol/l in perfusate (19). In the hydronephrotic rat kidney, application of NECA to bath solution dilated both pre- and postglomerular vessels when the concentration reached 10⁻⁵ mol/l (21). Thus, our results suggest that intraluminal application of 10⁻⁴ mol/l dilazep activates A2 receptors located at the afferent arterioles of JGA, probably via the potentiation of interstitial adenosine, and in turn, attenuates TGF-induced afferent vasoconstriction.

In the present micropuncture experiments, systemic infusion of dilazep at the depressor dose did not affect the reduction in EPFR induced by loop perfusion. The plasma concentration of dilazep in our rats is estimated to be around several hundred nmol/l at 1 h after the start of intravenous high-dose infusion with an initial bolus dose (22). This is comparable with the concentration in human plasma at 1 h after oral administration of 100 mg dilazep. Even if dilazep easily diffuses to the interstitial space or urine because of its relatively low molecular weight (695.6), the concentration around JGA during the systemic infusion should be much less than that which would be achieved during intraluminal application of

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**Fig. 2.** Reduction in EPFR when increasing the rate of loop perfusion from 0 to 40 nl/min with ATF (control), ATF containing 10⁻⁴ mol/l dilazep (dilazep 10⁻⁴ mol/l), ATF containing both 10⁻⁴ mol/l dilazep and 10⁻⁵ mol/l DMPX (dilazep 10⁻⁴ mol/l + DMPX), and ATF containing 10⁻⁵ mol/l DMPX (DMPX). Data are the mean ± SEM.

**Fig. 3.** Effects of intravenous dilazep (upper panel) and intraluminal dilazep and/or DMPX (lower panel) on percent reduction in EPFR (-%ΔEPFR) when increasing the rate of loop perfusion with ATF from 0 to 40 nl/min. Data are the mean ± SEM. * p < 0.05 vs. control (ANOVA).
10^-4 mol/l dilazep. This seems to be the reason that systemic infusion of dilazep failed to affect TGF response in the present series.

Loop perfusion of DMPX alone did not affect TGF responsiveness. This finding suggests that the TGF mechanism was under little tonic influence of endogenous adenosine on A2 receptors in the current experimental settings. In marked contrast, antagonists selective for adenosine A1 receptors have previously been shown to abolish TGF response either through systemic, luminal, or peritubular application of the drugs (18, 20); moreover, A1 agonists have clearly been shown to constrict afferent arterioles isolated from rabbits (23) and to activate TGF response in vivo (19). When extracellular adenosine is potentiated by dilazep, it might influence afferent arteriolar tone and TGF via A1 receptors in JGA. Our results, however, indicate that the vasoconstriction induced by A1 activation, if any, is not dominant over the vasodilatation by A2 activation during dilazep application, because TGF response was not enhanced but attenuated. Vallon and Osswald (24) reported that in rats treated orally with dipyridamole for three days, TGF activity was enhanced, suggesting that afferent arteriolar tone was increased through vasoconstrictive A1 receptors. The discrepancy between their results and ours might be explained by differences in the experimental protocols, i.e., the acute or chronic treatment, routes of drug administration, and nucleoside transport inhibitors used. In addition, there is a clear difference between the affinities of A1 and A2 receptors to adenosine. While the affinity of the former is so high that the receptor is sensitive to nanomolar concentrations of adenosine, the affinity of the latter is in the micromolar range, and the adenosine concentration in renal interstitial fluids is estimated to be around 0.2 \mu mol/l (25). Thus, under the present experimental conditions, which included potentiation of adenosine by dilazep, A1 receptor function may have been masked or dominated by the function of A2 receptors.

In summary, intravenous dilazep (0.3 mg/kg + 0.3 mg/kg/h) decreased GFR and systemic BP, but did not affect TGF response in the present study. FF showed a tendency to decrease. Intraluminal dilazep attenuated TGF response at the highest concentration of 10^-4 mol/l, and this attenuation was reversed by co-perfusion of DMPX. Luminal perfusion of DMPX alone did not affect TGF response. These results suggest that low-concentration dilazep dilates the postglomerular efferent arterioles but does not influence afferent arteriolar tone. Dilazep at the pharmacological concentration attenuates TGF-induced afferent vasoconstriction. The mechanism of this attenuation could be the activation of vasodilatory A2 receptors in afferent arterioles. Extracellular adenosine augmented by dilazep dilates glomerular vessels at both afferent and efferent sites, probably via the activation of A2 receptors.

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