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# Selective COX-2 Inhibition Improves Endothelial Function in Coronary Artery Disease

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**Background**—There is an ongoing debate as to whether the gastrointestinal safety of COX-2 inhibition compared with nonsteroidal antiinflammatory drugs (NSAIDs) may come at the cost of increased cardiovascular events. In view of the large number of patients at cardiovascular risk requiring chronic analgesic therapy with COX-2 inhibitors for arthritic and other inflammatory conditions, the effects of selective COX-2 inhibition on clinically useful surrogates for cardiovascular disease, particularly endothelial function, need to be determined.

**Methods and Results**—Fourteen male patients (mean age,  $66 \pm 3$  years) with severe coronary artery disease (average of 2.6 vessels with stenosis  $>75\%$ ) undergoing stable background therapy with aspirin and statins were included. The patients received celecoxib (200 mg BID) or placebo for a duration of 2 weeks in a double-blind, placebo-controlled, crossover fashion. After each treatment period, flow-mediated dilation of the brachial artery, high-sensitivity C-reactive protein, oxidized LDL, and prostaglandins were measured. Celecoxib significantly improved endothelium-dependent vasodilation compared with placebo ( $3.3 \pm 0.4\%$  versus  $2.0 \pm 0.5\%$ ,  $P=0.026$ ), whereas endothelium-independent vasodilation, as assessed by nitroglycerin, remained unchanged ( $9.0 \pm 1.6\%$  versus  $9.5 \pm 1.3\%$ ,  $P=0.75$ ). High-sensitivity C-reactive protein was significantly lower after celecoxib ( $1.3 \pm 0.4$  mg/L) than after placebo ( $1.8 \pm 0.5$  mg/L,  $P=0.019$ ), as was oxidized LDL ( $43.6 \pm 2.4$  versus  $47.6 \pm 2.6$  U/L,  $P=0.028$ ), whereas prostaglandins did not change.

**Conclusions**—This is the first study to demonstrate that selective COX-2 inhibition improves endothelium-dependent vasodilation and reduces low-grade chronic inflammation and oxidative stress in coronary artery disease. Thus, selective COX-2 inhibition holds the potential to beneficially impact outcome in patients with cardiovascular disease. (*Circulation*. 2003;107:405-409.)

**Key Words:** coronary disease ■ arteriosclerosis ■ endothelium ■ inflammation ■ free radicals

Gastrointestinal toxicity of traditional nonsteroidal antiinflammatory drugs and aspirin is related to inhibition of COX-1, which synthesizes gastroprotective prostaglandins. The antiinflammatory and pain-relieving effects are largely derived from inhibition of inducible COX-2–dependent inflammatory pathways. This led to the development of selective COX-2 inhibitors, of which celecoxib, rofecoxib, and second-generation agents such as valdecoxib are presently available. In patients with arthritis, clinical trials have documented the efficacy of coxibs compared with nonsteroidal antiinflammatory drugs (NSAIDs), with a lower incidence of gastrointestinal side effects.<sup>1,2</sup>

The COX-2–selective inhibitors, however, block prostacyclin ( $\text{PGI}_2$ ) formation without inhibiting platelet-derived thromboxane  $\text{A}_2$  ( $\text{TXA}_2$ ),<sup>3,4</sup> thereby increasing platelet activation, adhesion, and aggregation with a resultant potential

susceptibility for thrombosis and ischemic events. Indeed, the VIGOR (Vioxx Gastrointestinal Outcomes Research) trial comparing the COX-2 inhibitor rofecoxib with the NSAID naproxen in patients with rheumatoid arthritis showed a 5-fold increase in atherothrombotic cardiovascular events associated with rofecoxib.<sup>1</sup> Subsequently, the potential risks of cardiovascular events for both rofecoxib and celecoxib were highlighted in one overview, which was then disputed by others.<sup>5–8</sup> The controversy continues, because preclinical studies yielded conflicting results as to whether selective COX-2 inhibition exerted beneficial or detrimental effects on cardiovascular events in atherosclerosis.<sup>4,9–13</sup> This is of great public interest in view of the large number of patients with arthritis, most of whom are elderly and have a relatively high incidence of comorbidity, including hypertension, diabetes,

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and atherosclerosis, thereby placing them at considerable cardiovascular risk.

Until evidence from large-scale clinical trials becomes available, the effects of selective COX-2 inhibition on clinically useful surrogates for cardiovascular disease, particularly endothelial function, need to be determined. Hence, the goal of the present study was to evaluate the effect of celecoxib, a selective COX-2 inhibitor, on endothelial function, measured by high-resolution ultrasound, in patients with severe coronary artery disease in a double-blind, placebo-controlled, crossover design. In addition, plasma levels of oxidized LDL (ox-LDL) and high-sensitivity C-reactive protein (hs-CRP), both of which have been implicated in atherogenesis and represent sensitive markers of oxidative stress and low-grade chronic inflammation, respectively, were studied.

## Methods

### Assessment of Endothelial Function

We assessed flow-mediated dilation (FMD) and glycerol trinitrate (GTN) (0.4 mg sublingual, Nitrolingual Spray, Pohl-Boskamp)–induced vasodilation of the brachial artery after each treatment period by a high-resolution ultrasound vessel wall tracking device with a 10-MHz linear array transducer (WTS-2, Pie Medical). GTN acts as an exogenous NO donor directly on vascular smooth muscle cells, therefore inducing endothelium-independent vasodilation, whereas reactive hyperemia reflects endogenous NO formation, resulting in endothelium-dependent vasodilation. FMD of the brachial artery was induced by release of a wrist cuff inflated to suprasystolic pressure for 5 minutes. After release, we recorded the arterial diameter every 15 seconds for 3 minutes. After GTN application, we recorded the diameter every 30 seconds for 6 minutes.

### Analysis of Chemical Markers

For measurement of ox-LDL, we used a direct sandwich technique in which 2 monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule (WAK-Chemie). High-sensitivity CRP was measured with a chemiluminescent enzyme-labeled immunometric assay (Immulate 2002 MDSU, DPC). Prostaglandin measurements, including prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), PGI<sub>2</sub>, and TXA<sub>2</sub>, were based on the competitive binding technique in which the sample competes with a fixed amount of the according prostaglandin for sites on a monoclonal antibody (R&D Systems, Minneapolis, Minn). PGI<sub>2</sub> was measured as 6-keto-prostaglandin-F<sub>1α</sub>, its immediate and stable metabolite, and TXA<sub>2</sub> was measured as thromboxane B<sub>2</sub>, its immediate and stable metabolite.

### Study Protocol

We included 14 male patients (age 46 to 77 years, mean 66 years) with severe coronary artery disease (average of 2.6 vessels affected with stenosis >75%) and stable angina without coronary revascularization procedures 3 months before randomization. All patients were undergoing stable cardiovascular therapy. Exclusion criteria were blood pressure >160/90 mm Hg, chronic heart failure >NYHA I, insulin-dependent diabetes mellitus, renal insufficiency, and use of long-acting nitrates or an NSAID other than aspirin. We recruited patients from our cardiology outpatient clinic, and each gave written informed consent. The patients received celecoxib (200 mg BID) or matching placebo for a duration of 2 weeks in a randomized, double-blind, placebo-controlled crossover fashion. After assessment of baseline characteristics at randomization, we recorded vital signs and drew blood samples. Patients did not take study drugs or received standard treatment on the day of examination to obtain results at trough levels. Concomitant medication remained unchanged throughout the study course. The local ethics committee of the University Hospital of Zürich approved the study protocol.

**TABLE 1. Baseline Characteristics**

| Characteristics                              | Baseline (n=13) |
|----------------------------------------------|-----------------|
| Age, y                                       | 66±3            |
| Cardiovascular risk factors                  |                 |
| Arterial hypertension, %                     | 64              |
| Diabetes mellitus, %                         | 17              |
| Smokers, %                                   | 43              |
| Family history, positive, %                  | 50              |
| Body mass index, kg/m <sup>2</sup>           | 26.3±1.7        |
| Medication, %                                |                 |
| Aspirin                                      | 100             |
| Statins                                      | 93              |
| β-Blockers                                   | 57              |
| Calcium antagonist                           | 29              |
| ACE inhibitors/angiotensin receptor blockers | 50              |

### Statistical Analysis

Results are presented as mean±SEM. Measurements of FMD- and GTN-induced vasodilation represent the maximal increase in brachial diastolic arterial diameter and are expressed as percent change from baseline. For differences in parameters under placebo and celecoxib, we used two-tailed paired Student's *t* test (StatView 3.4, Abacus Concepts). We accepted statistical significance at *P*<0.05.

## Results

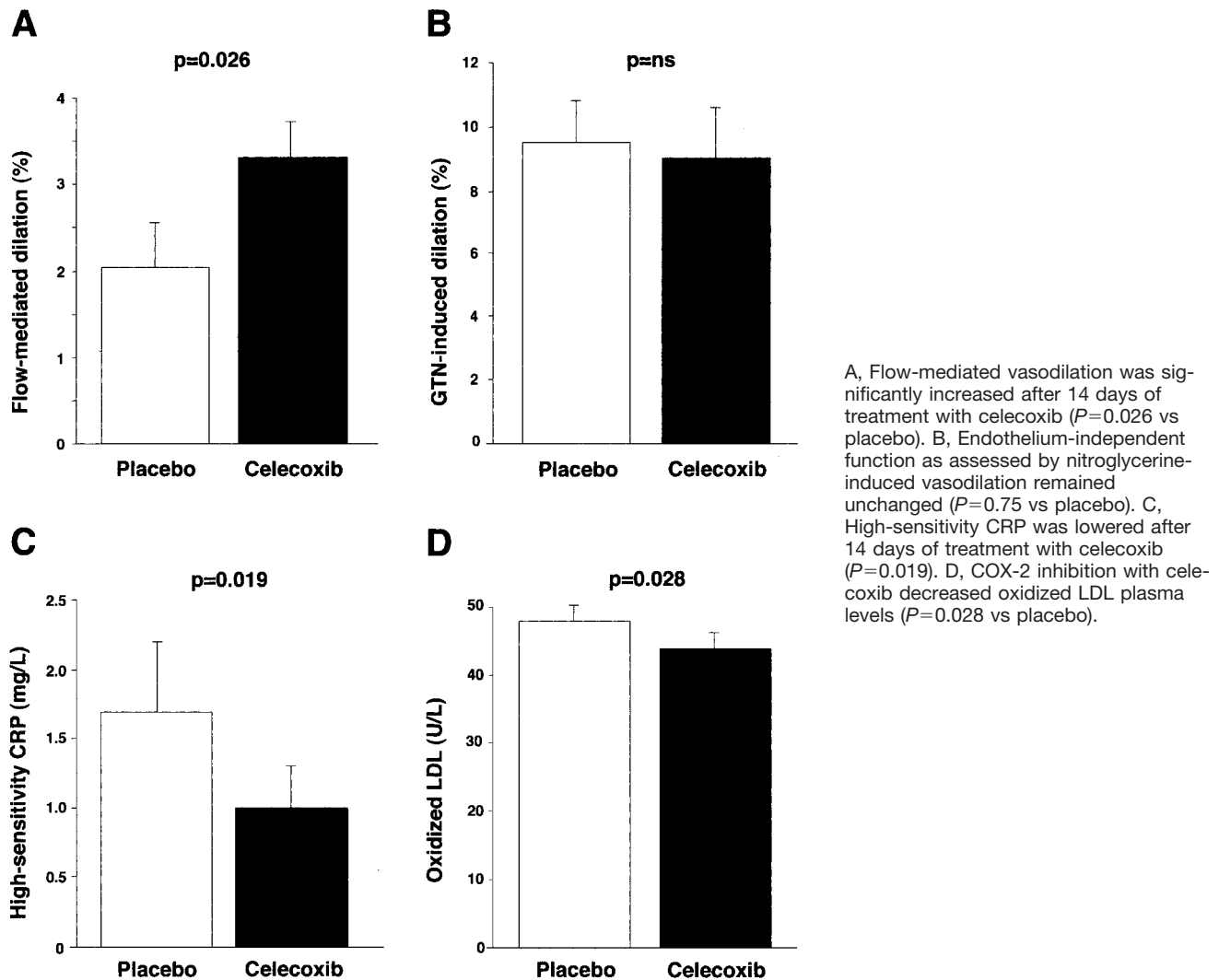
A total of 14 patients were included; 13 completed the study. One patient who was taking celecoxib at the time of onset of symptoms was excluded because of vertigo-like symptoms, which completely resolved after discontinuation of the study drug. Baseline demographic and clinical characteristics of the patients are presented in Table 1.

### Effects of Selective COX-2 Inhibition on Vascular Function and Chemical Markers

Flow-mediated vasodilation was significantly increased after celecoxib (3.3±0.4%) compared with placebo (2.0±0.5%), *P*=0.026 (Figure, A). GTN-induced vasodilation remained unchanged (celecoxib 9.0±1.6% versus placebo 9.5±1.3%; *P*=0.75) (Figure, B), as did average baseline diameter (celecoxib 4235±147 versus placebo 4394±116 μm; *P*=0.38). No difference could be found regarding the treatment sequence (placebo and then celecoxib or vice versa). hs-CRP and ox-LDL were significantly lowered by celecoxib compared with placebo (1.3±0.4 versus 1.8±0.5 mg/L, *P*=0.019, and 43.6±2.4 versus 47.6±2.6 U/L, *P*=0.028, respectively). Changes of PGE<sub>2</sub>, PGI<sub>2</sub>, and TXA<sub>2</sub> values after celecoxib or placebo did not reach statistical significance (156 versus 173 U/L, *P*=0.83; 909 versus 594 U/L, *P*=0.14; and 0.1 versus 0.1 U/L, *P*=0.16, respectively).

### Effect of Selective COX-2 Inhibition on Clinical Parameters

Heart rate and mean arterial blood pressure remained unaltered throughout the study (56 bpm after celecoxib versus 57 bpm after placebo and 97 versus 98 mm Hg, respectively). In addition, serum creatinine and sodium and potassium levels



did not change. For results of vascular, chemical, and clinical parameters, see Table 2.

### Discussion

This is the first study to demonstrate that a selective COX-2 inhibitor on top of standard therapy improves endothelial function and reduces markers of inflammation and oxidative stress in patients with coronary artery disease. Recognition that COX-2 is an inducible enzyme particularly associated with inflammation led to the development of selective COX-2 inhibitors that offer comparable efficacy and fewer unwanted side effects attributable to COX-1 inhibition, gastric ulceration in particular.<sup>1,2</sup> Gastrointestinal safety of selective COX-2 inhibitors, however, may come at the cost of increased cardiovascular events, as suggested by the results of the VIGOR trial.<sup>5</sup> Cardiovascular safety of coxibs was additionally challenged by studies in mice deficient in the PGI<sub>2</sub> or TXA<sub>2</sub> receptor.<sup>10</sup> PGI<sub>2</sub> receptor-deficient animals showed enhanced injury-induced vascular proliferation and platelet activation that was abolished in mice deficient of both PGI<sub>2</sub> and TXA<sub>2</sub> receptor, suggesting that PGI<sub>2</sub> inhibition with relatively unopposed platelet TXA<sub>2</sub> generation may lead to

increased thrombotic risk. Because increased PGI<sub>2</sub> biosynthesis seen in atherosclerosis seems to reflect the activity of both COX isoforms,<sup>14</sup> the selective reduction of PGI<sub>2</sub> that potentially may increase the risk of atherosclerosis remains a concern regarding COX-2 inhibitors. Although PGI<sub>2</sub> potently inhibits aggregation, its role in vivo remains still elusive, because endogenous plasma levels are presumably below the threshold for a systemic effect.<sup>15,16</sup> It is worth noting that in the present study, there was no additional decrease in PGI<sub>2</sub> formation and no increase in TXA<sub>2</sub> generation while the patients were taking celecoxib in addition to aspirin background therapy.

Because of concerns about cardiovascular safety of COX-2 inhibitors, present guidelines recommend that the large subgroup of patients with arthritis and additional cardiovascular risk factors should also receive aspirin when prescribed a COX-2 inhibitor.<sup>17</sup> Once TXA<sub>2</sub>-mediated aggregation is sufficiently blocked with aspirin, however, the results of the present study indicate that pleiotropic effects of COX-2 inhibition on the vascular wall come into play and indeed even may be beneficial in atherosclerotic vascular disease.

COX-2 is the isoform responsible for the enhanced production of the prostaglandins that mediate inflammation and

**TABLE 2. Results**

|                                          | Celecoxib<br>(n=13) | Placebo<br>(n=13) | P     |
|------------------------------------------|---------------------|-------------------|-------|
| <b>Vital signs</b>                       |                     |                   |       |
| Mean arterial pressure, mm Hg            | 97±4                | 98±3              | NS    |
| Heart rate, bpm                          | 56±2                | 57±2              | NS    |
| <b>Laboratory</b>                        |                     |                   |       |
| Total cholesterol, mmol/L                | 4.7±0.3             | 4.7±0.3           | NS    |
| Glucose, mmol/L                          | 6.0±0.5             | 6.2±0.5           | NS    |
| Creatinine, μmol/L                       | 93±3                | 91±3              | NS    |
| hs-CRP, mg/L                             | 1.3±0.4             | 1.8±0.5           | 0.019 |
| ox-LDL, U/L                              | 43.6±2.4            | 47.6±2.6          | 0.028 |
| PGI <sub>2</sub> , pg/mL                 | 156±54              | 173±55            | NS    |
| PGE <sub>2</sub> , pg/mL                 | 909±155             | 594±85            | NS    |
| TXA <sub>2</sub> , pg/mL                 | 0.1±0.01            | 0.1±0.01          | NS    |
| <b>Endothelial function measurements</b> |                     |                   |       |
| Arterial diameter, μm                    | 4235±147            | 4394±116          | NS    |
| Flow-mediated vasodilation, %            | 3.3±0.4             | 2.0±0.5           | 0.026 |
| Nitroglycerin-induced vasodilation, %    | 9.0±1.6             | 9.5±1.3           | NS    |

is the target enzyme for the antiinflammatory activity of NSAIDs.<sup>18</sup> Furthermore, COX-2 expression is induced by proinflammatory mediators, particularly by cytokines and reactive oxygen species,<sup>19</sup> and its expression has been found in animal models of atherosclerosis as well as in human atherosclerotic tissue.<sup>11,12,19</sup> Because atherosclerosis is increasingly recognized as a chronic inflammatory disease,<sup>20,21</sup> the impact of selective COX-2 inhibition on clinically useful surrogates, particularly endothelial function, needs to be determined.

The healthy endothelium maintains vascular homeostasis via production of paracrine factors, NO in particular.<sup>22</sup> NO is the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and reactivity. NO bioavailability is decreased in atherosclerotic vascular disease, thus promoting vasoconstriction, leukocyte adhesion, platelet aggregation, oxidation, and vascular inflammation.<sup>22</sup> Interestingly, decreased NO production has been implicated in the pathogenesis and clinical course of all known cardiovascular diseases and is associated with future risk of adverse cardiovascular events.<sup>23,24</sup> Thus, the findings of the present study that celecoxib improves NO-mediated endothelial function are of particular clinical interest, because they demonstrate for the first time that selective COX-2 inhibition holds the potential to beneficially impact atherosclerotic vascular disease by improving a well-established clinically useful surrogate for cardiovascular disease.

The observed beneficial effects of celecoxib on endothelial function may in part be explained by the reduction of hs-CRP, an exquisite marker of low-grade chronic inflammation and a strong independent predictor of future cardiovascular events.<sup>25</sup> CRP-mRNA is increased in the atherosclerotic vasculature<sup>26</sup> and reflects the vulnerability of the atheromatous lesion and the likelihood of a plaque to rupture.<sup>27</sup> Very recent data demonstrated the ability of CRP to directly quench the production of endothelial NO synthase through posttranscriptional effects on endothelial NO synthase

mRNA stability, leading to diminished NO bioavailability, which in turn impairs endothelial function.<sup>28</sup> This is in line with findings that a systemic inflammatory response leads to an impairment of endothelial function in both resistance and conduit vessels.<sup>29</sup> Furthermore, blunted systemic endothelial vasoreactivity was found to be related to elevated plasma levels of CRP in patients with coronary artery disease.<sup>30</sup>

Noteworthy is the growing evidence for a common pathway mediating vascular dysfunction, inflammatory gene induction, and oxidative stress.<sup>31</sup> An increasing body of evidence suggests that oxidative stress accounts in large parts for endothelial dysfunction.<sup>32</sup> Indeed, the present study demonstrates that COX-2 inhibition with celecoxib reduces formation of reactive oxygen species, as indicated by less formation of ox-LDL, which is a hallmark in early atherogenesis.<sup>21</sup> ox-LDL is not only an index of lipid peroxidation but also causes endothelial dysfunction by itself through the impairment of the signal transduction between endothelial cell-surface receptors and NO production, inhibition of NO synthase activity, and inactivation of NO released from endothelial cells.<sup>22,33</sup> The link between increased oxidative stress and reduced bioavailability of NO has been well established.<sup>32</sup> Interestingly, there is evidence that COX-2 may be a source of oxygen radicals itself,<sup>34</sup> and therefore inhibition of the enzyme activity may reduce oxidative stress. Furthermore, endothelial dysfunction in patients with coronary artery disease or coronary risk factors is reversed by antioxidative agents, such as vitamin C.<sup>35</sup> Hence, the findings of the present study suggest that increased oxidative stress may be an important mechanism for impaired endothelial function in patients with atherosclerosis or cardiovascular risk factors and that COX-2 inhibition interferes with this process. The endothelium may indeed provide a link between COX-2 and vessel wall inflammation, because disruption of the endothelial cell barrier between circulating blood and vascular smooth muscle cells and monocyte/macrophage sequestration at these sites with elaboration of inflammatory cytokines and the transmigration and uncontrolled proliferation of vascular smooth muscle cells typify the initial phase of the atherosclerotic process. The observed decrease in oxidative stress and vascular inflammation would thus be expected to reduce mononuclear cell infiltration, improve NO availability, reduce progression of atherosclerosis, and enhance plaque stability, possibly resulting in a net decrease in atherothrombotic events. This is supported by a recent preliminary study showing that the selective COX-2 inhibitor meloxicam in combination with aspirin reduces adverse outcomes in patients after acute coronary syndrome without ST-segment elevation.<sup>36</sup> In addition, the results of the NUT-2 trial suggest a positive additive effect of low-dose aspirin and COX-2 inhibition on clinical outcomes, which is in line with the findings of the present study that COX-2 inhibition has an additional benefit on top of aspirin on endothelial function in patients with coronary artery disease. It is of note that low-dose aspirin, at least in acute studies, improves endothelial function in patients with hypercholesterolemia<sup>37</sup> and atherosclerosis,<sup>38</sup> indicating that inhibition of platelet-vessel wall interaction by low-dose aspirin may have contributed to



the beneficial effects on endothelial function observed in the present study.

Of importance, the improvement of endothelial function and the reduction of hs-CRP and ox-LDL in the present study were observed on top of background therapy with aspirin and statins in all patients and with ACE inhibitors in 50% of patients. Because the reduction of vascular inflammation and oxidative stress have been well documented to contribute to the beneficial prognostic effects of statins and ACE inhibitors,<sup>20,39</sup> the results of our study suggest that COX-2 inhibition with celecoxib holds the potential as an add-on therapy to presently established standard pharmacotherapy in patients with atherosclerotic vascular disease. Notwithstanding that this study provides first evidence that COX-2 inhibition improves a well-established clinical surrogate of cardiovascular disease, the definitive answer as to the net effect of selective COX-2 inhibition on cardiovascular events can only be provided by well-designed large-scale clinical trials.

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