The Guanosine-Adenosine Interaction Exists In Vivo

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ABSTRACT

In cultured renal cells and isolated perfused kidneys, extracellular guanosine augments extracellular adenosine and inosine (the major renal metabolite of adenosine) levels by altering the extracellular disposition of these purines. The present study addressed whether this “guanosine-adenosine mechanism” exists in vivo. In rats (n = 15), intravenous infusions of adenosine (1 μmol/kg per minute) decreased mean arterial blood pressure (MABP) from 114 ± 8 to 83 ± 5 mm Hg, heart rate (HR) from 368 ± 11 to 323 ± 9 beats/min, and renal blood flow (RBF) from 6.2 ± 0.5 to 5.3 ± 0.6 ml/min. In rats (n = 15) pretreated with intravenous guanosine (10 μmol/kg per minute), intravenous adenosine (1 μmol/kg per minute) decreased MABP (from 109 ± 4 to 58 ± 5 mm Hg), HR (from 401 ± 10 to 264 ± 20 beats/min), and RBF (from 6.2 ± 0.7 to 1.7 ± 0.3). Two-factor analysis of variance (2F-ANOVA) revealed a significant interaction (P < 0.0001) between guanosine and adenosine for MABP, HR, and RBF. In control rats, the urinary excretion rate of endogenous inosine was 211 ± 103 ng/30 minutes (n = 9); however, in rats treated with intravenous guanosine (10 μmol/kg per minute), the excretion rate of inosine was 1995 ± 300 ng/30 minutes (n = 12; P < 0.0001 versus controls). Because adenosine inhibits inflammatory cytokine production, we also examined the effects of intravenous guanosine on endotoxemia-induced increases in tumor necrosis factor-α (TNF-α). In control rats (n = 7), lipopolysaccharide (LPS; Escherichia coli 026:B6 endotoxin; 30 mg/kg) increased plasma TNF-α from 164 ± 56 to 4082 ± 730 pg/ml, whereas in rats pretreated with intravenous guanosine (10 μmol/kg per minute; n = 6), LPS increased plasma TNF-α from 121 ± 45 to 1821 ± 413 pg/ml (2F-ANOVA interaction effect, P = 0.0022). We conclude that the guanosine-adenosine mechanism exists in vivo and that guanosine may be a useful therapeutic for reducing inflammation.

Introduction

Because extracellular adenosine stimulates cell-surface adenosine receptors (A1, A2A, A2B, and A3) and thereby modulates cell function (Fredholm et al., 2011; Grenz et al., 2011), it is critical to elucidate the mechanisms regulating adenosine levels in the extracellular compartment of various organ systems. In this regard, adenosine influences many aspects of renal function (Valton et al., 2006), and therefore it is particularly important to understand the determinants of extracellular adenosine levels in the kidney. Our recent in vitro (cell culture) investigations (Jackson et al., 2013; Jackson and Gillespie, 2013) demonstrate that in many renal cell types, including pregglomerular vascular smooth muscle cells, glomerular mesangial cells, and kidney epithelial cells, extracellular guanosine slows the removal of adenosine from the extracellular environment. We call this interaction the guanosine-adenosine mechanism and propose that this is an indirect mechanism by which extracellular guanosine can contribute to cell signaling without specific cell-surface G protein–coupled guanosine receptors, which may or may not exist (Traversa et al., 2003; Thauerer et al., 2012).

Our cell culture studies, however, left unanswered the question as to whether the guanosine-adenosine mechanism occurs in intact organs such as the kidney. To address this question, we recently performed experiments in isolated, perfused mouse kidneys (Jackson et al., 2014). These experiments show that the levels of guanosine and adenosine in the renal venous perfusate and in kidney tissue are highly correlated, that guanosine reduces the renal clearance of exogenous adenosine, and that inhibition of purine nucleoside phosphorylase (PNPase; an enzyme that metabolizes guanosine to guanine) increases the release of both guanosine and adenosine by the kidneys. These findings further support the conclusion that in the kidney guanosine modulates adenosine levels.

Although evidence for the guanosine-adenosine mechanism is accumulating, a critically important question is whether guanosine enhances the effects of adenosine on the cardiovascular and renal systems in vivo, as opposed to this mechanism being an artifact of in vitro experiments or merely a biochemical curiosity without functional significance. Therefore, one goal of the present study was to determine whether the guanosine-adenosine interaction can be observed in vivo.

Adenosine is an anti-inflammatory agent that inhibits both the innate and adaptive arms of the immune system (Hasko

ABBREVIATIONS: 2F-ANOVA, two-factor analysis of variance; GFR, glomerular filtration rate; HR, heart rate; LPS, lipopolysaccharide; MABP, mean arterial blood pressure; MFB, mesenteric blood flow; MVR, mesenteric vascular resistance; PE, polyethylene; PNPase, purine nucleoside phosphorylase; RBF, renal blood flow; RVR, renal vascular resistance; TNF-α, tumor necrosis factor-α; UrV, urine volume.
et al., 2007; Ohta and Sitkovsky, 2009; Colgan et al., 2013; Longhi et al., 2013; Poth et al., 2013). Because the present results indicate that guanosine can enhance adenosine signaling in vivo, a second objective of the present study was to test whether exogenous guanosine has potential as a pharmacologic agent for the management of inflammatory states.

Materials and Methods

Chemicals. Adenosine, guanosine, and lipopolysaccharide (LPS; Escherichia coli 026:B6 endotoxin) were from Sigma-Aldrich (St. Louis, MO).

Animals. This study used male Sprague-Dawley rats (Charles River, Wilmington, MA) that were between 15 and 20 weeks of age. The Institutional Animal Care and Use Committee approved all procedures. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Guanosine-Adenosine Interaction. Thirty rats were anesthetized (Inactin, 90 mg/kg i.p.; Sigma-Aldrich), and body temperature was monitored with a rectal probe and stabilized at 37°C using an isothermal pad and a heat lamp. A cannula [polyethylene (PE)-240] was inserted into the trachea to maintain a patent airway. The left femoral artery was cannulated (PE-50), and mean arterial blood pressure (MABP) and heart rate (HR) were monitored using a digital blood pressure analyzer (Micro-Med, Inc., Louisville, KY). Next, two PE-50 cannulas (cannula A and cannula B) were placed in a jugular vein, and an infusion of 0.9% saline was initiated into each cannula at 25 μl/min. After inserting PE-10 tubing into the left ureter for urine collection, noncannulating, transit-time flow probes (Transonic Systems, Inc, Ithaca, NY) were positioned on both the left renal artery (1 mm) and mesenteric artery (2 mm). These were connected to a two-channel transit-time flowmeter (model T-206; Transonic Systems, Inc.) for recording of renal blood flow (RBF) and mesenteric blood flow (MBF).

After a 90-minute stabilization period, in the control group (n = 15), saline infusions were continued via both cannula A and cannula B. However, in the guanosine group (n = 15), guanosine was infused via cannula A at 10 μmol/kg per minute. After 15 minutes, a 30-minute urine collection was initiated while measuring MABP, HR, RBF, and MBF. Next, in both groups, cannula B was used to infuse adenosine at 0.3 μmol/kg per minute. After 15 minutes, another 30-minute urine collection was initiated while measuring MABP, HR, RBF, and MBF. This procedure was continued as the dose of adenosine was increased to 1 and then 3 μmol/kg per minute. Renal vascular resistance (RVR) and mesenteric vascular resistance (MVR) were calculated by dividing the MABP by the RBF or MBF, respectively. In nine control rats and 12 guanosine-treated rats, urine collected before the infusions of adenosine was analyzed for guanosine, adenosine, and inosine using high-performance liquid chromatography–tandem mass spectrometry as previously described (Jackson et al., 2009). It was not possible to compare the urine levels of these purines in the presence of adenosine because urine volumes were severely reduced in those rats receiving guanosine plus adenosine.

Guanosine on Endotoxin-Induced Cytokine Release. Rats were anesthetized and instrumented as described already herein, and an infusion of 0.9% saline (50 μl/min) was initiated via cannula A. During the first experimental period (period 1), urine was collected for 30 minutes and MABP, HR, RBF, and MBF were time-averaged during the 30-minute urine collection. Also 1 ml of blood (in heparin) was taken during the middle of the 30-minute urine collection. The midpoint blood sample was centrifuged, and the plasma was collected and stored for later analysis. Blood-volume loss was compensated by injecting 1 ml of 0.9% saline. In some animals, the saline infusion via cannula A was continued, whereas in other animals, guanosine (dissolved in 0.9% saline) was infused via cannula A at either 5 or 10 μmol/kg per minute. After 15 minutes, another 30-minute urine collection was performed with measurements of MABP, HR, RBF, and MBF and with another 1-ml midpoint blood sample (period 2). Next, in all three groups, an intravenous bolus of LPS (30 mg/kg) was administered via cannula B. After 60 minutes, yet another 30-minute urine collection was initiated (period 3), during which MABP, HR, RBF, and MBF were again measured, and another 1-ml midpoint blood sample was taken. Tumor necrosis factor-α (TNF-α) and IL-1β were measured in plasma using R&D Systems (Minneapolis, MN) kits [rat TNF-α Quantikine ELISA kit (catalog no. RTA00) and rat IL-1β/IL-1P Quantikine ELISA kit (catalog no. RLB00), respectively]. Creatinine was measured in urine and plasma using the QuantiChrom Creatinine Assay kit (catalog no. DICT-500; Bioassay Systems, Hayward, CA). As previously demonstrated, hemodynamic parameters were stable over the time frame of the present study (Begany et al., 1996; Tofovic et al., 2001).

Statistics. Data are presented as mean ± S.E.M. Data for MABP, HR, RBF, RVR, MBF, MVR, urine volume, creatinine clearance (glomerular filtration rate [GFR]), and plasma TNF-α and IL-1β were analyzed using a repeated-measures two-factor analysis of variance (2F-ANOVA), and Fisher’s least significant difference tests were applied for post hoc analyses. Comparisons of purine excretions between control and guanosine-treated groups were performed with unpaired, twotailed Student’s t test. Survival analysis of control versus guanosine-treated rats was performed with the Kaplan-Meier method and log-rank analysis.
groups was analyzed using the Gehan-Breslow-Wilcoxon test. A value of $P < 0.05$ was considered statistically significant.

**Results**

In control rats not pretreated with guanosine, intravenous infusions of adenosine at 0.3 and 1 $\mu$mol/kg per minute caused a dose-related decrease in both MABP (Fig. 1A) and HR (Fig. 1B). Pretreatment of rats with an intravenous infusion of guanosine (10 $\mu$mol/kg per minute) did not significantly alter basal MABP ($114 \pm 4$ versus $109 \pm 4$ mm Hg in the control and guanosine-pretreated groups, respectively). However, basal HR was slightly but statistically significantly elevated in the guanosine-pretreated group ($369 \pm 11$ versus $401 \pm 10$ beats/min in the control and guanosine-pretreated groups, respectively). As illustrated in Fig. 1, A and B, respectively, adenosine-induced decreases in MABP and HR were markedly augmented in guanosine-pretreated rats, and these effects were highly statistically significant ($P < 0.0001$; 2F-ANOVA).

Basal RBF and basal RVR were similar in control versus guanosine-pretreated rats [for RBF, $6.2 \pm 0.5$ versus $6.2 \pm 0.7$ ml/min in the control and guanosine-pretreated groups, respectively]. In control rats, intravenous adenosine had only a slight effect on RBF (from $6.2 \pm 0.5$ to $5.3 \pm 0.6$, basal versus 1 $\mu$mol/kg per minute of adenosine, respectively) (Fig. 2A) and no effect on RVR (from $20.4 \pm 2.2$ to $20.4 \pm 3.3$, basal versus 1 $\mu$mol/kg per minute of adenosine, respectively) (Fig. 2B). In contrast, in the guanosine-pretreated group, adenosine profoundly decreased RBF (from $6.2 \pm 0.7$ to $1.7 \pm 0.3$, basal versus 1 $\mu$mol/kg per minute of adenosine, respectively) (Fig. 2A) and profoundly increased RVR (from $19.7 \pm 1.9$ to $53.4 \pm 12.9$, basal versus 1 $\mu$mol/kg per minute of adenosine, respectively) (Fig. 2B). The interaction between guanosine and adenosine on RBF and RVR was highly statistically significant by 2F-ANOVA ($P < 0.0001$ and $P = 0.0033$, respectively).

We also observed a statistically significant interaction between guanosine and adenosine on MBF (Fig. 3A) and MVR levels (Fig. 3B) ($P < 0.0001$ and $P = 0.0033$, respectively). In this regard, at 0.3 $\mu$mol/kg per minute, adenosine increased MBF and decreased MVR in guanosine-pretreated rats, but it had no effect on MBF or MVR in control rats. At 1 $\mu$mol/kg per minute, in guanosine-pretreated rats, adenosine decreased
MBF and returned MVR back to baseline. This biphasic response to adenosine in guanosine-pretreated rats was likely due to the fact that at 1 μmol/kg per minute, adenosine profoundly reduced MABP (to 58 ± 5 mm Hg), which likely triggered a baroreceptor-mediated reflex increase in sympathetic tone to the mesentery. This biphasic response to adenosine was not observed in the control rats not receiving guanosine. Notably, even in the absence of adenosine, guanosine significantly reduced basal MVR.

The guanosine-adenosine interaction was also observed with respect to urine excretion rate [urine volume (UrV)] (Fig. 4A), and this interaction was highly statistically significantly (P = 0.0002). Basal UrV was similar in control versus guanosine-pretreated rats (0.18 ± 0.03 versus 0.20 ± 0.04 ml/30 minutes in the control and guanosine-pretreated groups, respectively). In control rats, an infusion of adenosine at 0.3 μmol/kg per minute increased UrV to 0.43 ± 0.10 ml/30 minutes; in contrast, in guanosine-treated rats, the same dose of adenosine decreased UrV to 0.08 ± 0.04 ml/30 minutes. At a dose of 1 μmol/kg per minute, adenosine further decreased UrV in the guanosine-treated rats (to 0.02 ± 0.01 ml/30 minutes), whereas in the control rats, UrV returned to basal values (0.18 ± 0.05).

Our initial intention was to collect hemodynamic data in control versus guanosine-pretreated rats with an even higher dose of adenosine (i.e., 3 μmol/kg per minute). However, we discovered that although control rats could readily tolerate this dose of adenosine, within a few minutes after starting the infusion, many of the guanosine-pretreated rats rapidly died of shock levels of arterial blood pressure, and the ones that did not die were hemodynamically severely compromised. As shown in Fig. 4B, the mortality rate in guanosine-pretreated rats within 45 minutes of starting the higher infusion rate was 53% (P = 0.0012 compared with controls), with most expiring within a few minutes of initiating the higher dose of adenosine.
high-performance liquid chromatography–tandem mass spectrometry. It was not possible to compare the urine levels of these purines in the presence of adenosine because urine volumes were too low in the rats receiving guanosine plus adenosine. Intravenous infusions of guanosine vastly increased the renal excretion rate of guanosine (from 325 ± 22 to 47,597 ± 6748 ng/30 minutes; P < 0.0001; Fig. 5A). Although guanosine did not increase the urinary excretion rate of endogenous adenosine (Fig. 5B), guanosine profoundly increased the urinary excretion rate of the major endogenous metabolite of adenosine, namely, inosine (from 210 ± 103 to 1995 ± 300 ng/30 minutes; P < 0.0001; Fig. 5C).

Because adenosine is anti-inflammatory and guanosine increases adenosine, we examined whether guanosine alters the effects of LPS (a proinflammatory endotoxin). In the absence of guanosine, LPS increased plasma levels of the proinflammatory cytokine TNF-α from 164 ± 56 to 4082 ± 730 pg/ml (Fig. 6A). However, in animals pretreated with guanosine, this response was attenuated (guanosine at 5 μmol/kg per minute: from 318 ± 54 to 1550 ± 223 pg/ml; guanosine at 10 μmol/kg per minute: from 121 ± 45 to 1821 ± 413 pg/ml). The interaction between guanosine and LPS on plasma TNF-α was highly statistically significant by 2F-ANOVA (P = 0.0022; Fig. 6A). In the absence of guanosine, LPS increased plasma levels of the proinflammatory cytokine IL-1β from 65 ± 42 to 1173 ± 412 pg/ml (Fig. 6B). However, in animals pretreated with guanosine, this response tended to be attenuated (guanosine at 5 μmol/kg per minute: from 238 ± 52 to 931 ± 241 pg/ml; guanosine at 10 μmol/kg per minute: from less than detection limit to 493 ± 168 pg/ml). Although the interaction between guanosine and LPS on plasma IL-1β did not achieve statistical significance by 2F-ANOVA (Fig. 6B), the level of IL-1β in LPS-treated animals pretreated with guanosine at 10 μmol/kg per minute was significantly (P < 0.05) lower than the level in LPS-treated animals not pretreated with guanosine.

Guanosine not only reduced the cytokine response to LPS but also attenuated LPS-induced reductions in RBF (guanosine-LPS interaction on RBF by 2F-ANOVA, P = 0.0183; Fig. 7A). Also, LPS tended to decrease UrV in rats not treated with guanosine, yet in rats pretreated with guanosine, LPS either did not affect UrV (guanosine at 5 μmol/kg per minute) or increased UrV (guanosine at 10 μmol/kg per minute) (guanosine-LPS interaction on UrV by 2F-ANOVA, P = 0.0328; Fig. 7B). In the absence of guanosine, LPS tended to reduce HR, but in the presence of guanosine, LPS tended to increase HR. Although the guanosine-LPS interaction on HR was only near significant (2F-ANOVA, P = 0.0836; Fig. 8A), HR after LPS was significantly higher in guanosine (5 μmol/kg per minute)-pretreated rats compared with rats not pretreated with

Fig. 6. Bar graphs summarize the effects of LPS (30 mg/kg) on plasma levels of (A) TNF-α and (B) IL-1β in rats pretreated with intravenous infusions of guanosine at 0 (saline only), 5, or 10 μmol/kg per minute. Values represent means and S.E.M. *Significant difference (P < 0.05) between period 2 and period 3 within the respective group. †Significant difference (P < 0.05) versus period 3 of control (0 μmol/kg per minute of guanosine) group. <DL, less than detection limit.

Fig. 7. Bar graphs summarize the effects of LPS (30 mg/kg) on (A) renal blood flow and (B) urine volume in rats pretreated with intravenous infusions of guanosine at 0 (saline only), 5, or 10 μmol/kg per minute. Values represent means and S.E.M. *Significant difference (P < 0.05) between period 2 and period 3 within the respective group. †Denotes significant difference (P < 0.05) versus period 3 of control (0 μmol/kg per minute of guanosine) group.
guanosine. Although guanosine did not significantly attenuate LPS-induced reductions in GFR, guanosine did not worsen LPS-induced decreases in GFR (Fig. 8B). LPS had little effect on MABP (Fig. 9A) or MBF (Fig. 9B); importantly, guanosine did not cause LPS to induce hypotension or mesenteric ischemia (Fig. 9, A and B).

**Discussion**

The results of this study support the conclusion that the guanosine-adenosine mechanism is operative in vivo. This conclusion is based on the findings that infusions of guanosine remarkably increase the ability of adenosine to affect MABP, HR, RBF, RVR, MBF, MVR, and UrV. Also, guanosine infusions increase by approximately 10-fold the amount of endogenous inosine (the main metabolite of adenosine) excreted in the urine. The guanosine-adenosine interaction is so robust that a dose of adenosine that is nontoxic to normal rats kills within a few minutes over 50% of rats pretreated with a dose of guanosine that per se has little effect on the cardiovascular system or kidneys. These findings, particularly when coupled with our in vitro findings in cultured cells (Jackson et al., 2013; Jackson and Gillespie, 2013) and isolated, perfused kidneys (Jackson et al., 2014), leave little doubt that the guanosine-adenosine mechanism is operative and robust.

The guanosine-adenosine mechanism could explain previous protective effects reported for guanosine. There is now an overwhelming body of evidence in support of the conclusion that adenosine (Okusa et al., 1999, 2000, 2001; Lee and Emala, 2000, 2002; Okusa, 2002; Day et al., 2003, 2005; Lee et al., 2004a,b, 2007; Grenz et al., 2007a,b, 2008, 2011, 2012; Kim et al., 2009; Haskó et al., 2011) and inosine (Maggio et al., 1980; Marberger et al., 1980; Fitzpatrick et al., 1981; Rothwell et al., 1981; Mathur and Ramsey, 1983) protect the kidneys from acute injury. Less well known is the fact that guanosine is also renoprotective (Kelly et al., 2001). Likewise, adenosine is neuroprotective (for reviews, see Stone, 2002; Chen et al., 2007; Stone et al., 2007) and apparently inosine is as well (Shen et al., 2005). Importantly, there is a growing body of evidence that guanosine, like adenosine and inosine, is neuroprotective [for review of supporting evidence see Introduction in article by Dal-Cim and coworkers (Dal-Cim et al., 2013)]. The mechanism by which guanosine affords renoprotection and neuroprotection is unknown. The facts that adenosine and guanosine are both renoprotective and neuroprotective, that the existence of cell-surface guanosine receptors is questionable, and that guanosine increases both the extracellular levels of adenosine and inosine and augments the physiologic effects of adenosine strongly support the conclusion that the guanosine-adenosine mechanism mediates the protective effects of guanosine.
The discussion herein underscores the possibility for therapeutic intervention with either guanosine per se or with drugs that modulate guanosine. In this regard, guanosine could be delivered either intravenously or intramuscularly (Shin et al., 2008) or via intraperitoneal administration (Giuliani et al., 2012). Importantly, the results of the present study demonstrate that guanosine per se has little effect on basal arterial blood pressure or renal blood flow and therefore likely would be safe. Yet by increasing endogenous levels of extracellular adenosine and inosine in an event- and site-specific manner, guanosine could prove useful for the treatment of such disorders as tissue ischemia-reperfusion, sepsis, and inflammation. Indeed, our present findings indicate that intravenous guanosine attenuates endotoxin-induced increases in plasma levels of TNF-α (and possibly IL-1β) in rats. Consistent with the reduction in LPS-induced cytokine levels, guanosine preserves RBF and UrV in endotoxemia. We hypothesize that the ability of guanosine to protect against RBF and UrV changes induced by LPS is mediated by the anti-inflammatory effects of guanosine via adenosine. Importantly, guanosine does not destabilize MABP, HR, or GFR in endotoxemia, suggesting that this compound would be safe to use in sepsis. Because guanosine is metabolized by PNPase to guanine, inhibition of PNPase increases extracellular guanosine levels associated with cellular injury (Jackson et al., 2013). Therefore, inhibition of PNPase is another possible therapeutic approach for treating diseases in which elevated levels of extracellular guanosine, adenosine, and inosine might be advantageous. In normal, LPS-PNPase inhibitors likely would have few effects; in contrast, in damaged tissues (kidney injury or brain injury for example), PNPase inhibitors may be tissue protective.

Although the preceding discussion suggests that guanosine or PNPase inhibitors may be advantageous for the treatment of some disease states, it is conceivable that in other disorders one should focus on enhancing the metabolism of guanosine to prevent adverse effects of too much adenosine. The results of the present study show that guanosine can enhance the physiologic effects of adenosine so robustly that animals succumb to cardiovascular shock when higher doses of adenosine are infused. This finding suggests the possibility that guanosine could contribute to adverse outcomes following hemorraghic or cardiogenic shock.

The mechanism of the guanosine-adenosine interaction remains unknown. Our initial studies (Jackson et al., 2013; Jackson and Gillespie, 2013) rule out the participation of many possible candidate systems, including adenosine deaminase, adenosine kinase, equilibrative nucleoside transporters, concentrative nucleoside transporters, SLC19A1, SLC19A2, SLC19A3, and SLC22A2. Given the striking results of the present study, our future studies will focus both on the therapeutic applications of the guanosine-adenosine interaction and the mechanism underlying this interaction.

In summary, the present study demonstrates that extracellular guanosine can regulate extracellular adenosine and inosine levels in vivo and that this interaction results in profound increases in the cardiorenal effects of adenosine. These results are of significance because they reveal an important but overlooked physiologic mechanism and suggest novel pharmacologic approaches to modify the endogenous adenosine system safely and effectively to treat diseases. These findings justify the dedication of resources to determine the mechanism of the guanosine-adenosine interaction and to determine whether manipulating this interaction could be of therapeutic advantage.

**Authorship Contributions**

**Participated in research design:** Jackson.  
**Conducted experiments:** Mi.  
**Performed data analysis:** Jackson.  
**Wrote or contributed to the writing of the manuscript:** Jackson, Mi.

**References**


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