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Effect of Nevirapine Administration on Biliary Secretion/its Biochemical Composition in Albino Wistar Rats

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Abstract

Background: Nevirapine is an antiretroviral medication that prevents human immunodeficiency virus cells from multiplying in the blood. This study was undertaken to ascertain whether nevirapine administration affects bile secretion in albino Wistar rats.

Methods: Male and female albino Wistar rats (n=20, 50-125 g body weight) at the start of the experiment were used for the study. Rats in the control group (n=10) were administered normal saline (0.4 mg/kg body weight) + normal rodent chow, whereas the nevirapine group (n=10) were fed by gavage nevirapine (0.4 mg/kg body weight) two times daily (07:00 h and 18:00 h) in addition to normal rodent chow for 12 weeks. All animals were allowed free access to clean drinking water. Biliary secretion, cholesterol, bilirubin, conjugated and unconjugated bilirubin levels, and bile electrolytes were measured.

Results: Biliary secretion in the nevirapine-treated group was significantly lower ($p < 0.05$) than in the control group. Total cholesterol, total bilirubin and unconjugated bilirubin were significantly higher ($p < 0.001$) in the nevirapine-treated group when compared to control. Conjugated bilirubin was also increased in the nevirapine-treated group though not statistically different from the control. Electrolytes (sodium and chlorine) content of bile were significantly lower ($p < 0.01$ and $p < 0.001$) in the nevirapine-treated group when compared to control. However, (potassium and bicarbonate) content of bile were significantly higher ($p < 0.05$ and $p < 0.001$) in the nevirapine-treated group when compared to control.

Conclusion: Long term administration of nevirapine may lead to reduction in biliary secretion, increase bilirubin/ cholesterol levels and alter bile electrolytes' composition. This implies that NVP may provoke liver damage.

Keywords: Nevirapine; Biliary secretion; Cholesterol; Bilirubin; Conjugated/Unconjugated bilirubin; Bile electrolytes; Liver

Introduction

Nevirapine (NVP) is an antiretroviral (ARV) medication that prevents human immunodeficiency virus (HIV) cells from multiplying in the blood. Dramatic reductions in HIV-infected patients who have had access to highly active antiretroviral therapy (HAART) have been recorded [1]. Widespread use of HAART has led to dramatic reductions in morbidity and mortality among individuals infected with the HIV-1 [2,3]. NVP may cause severe or life threatening liver toxicity, usually emerging in the first six weeks of treatment [4,5]. When HIV disease is associated with a viral hepatitis, other pharmacological treatments are needed concurrently and if substance abuse is still present (including intake of alcohol, heroin and methadone), the risk of increased drug-drug interaction and end-organ toxicity is increased significantly especially because of the central role of liver tissue in drug metabolism [6,7]. NVP like other antiretroviral (ARV) agents have side effects and toxicities which affect the gastrointestinal system [8,9]. The human immunodeficiency virus type one (HIV-1), a causal organism of acquired immunodeficiency syndrome (AIDS), destroys immune system thereby allowing any opportunistic infections leading to death of the patient [10]. HIV-1 is known to be transmitted by transfer of blood or blood products, semen, vaginal fluid, pre-ejaculated fluid, breast milk and using intravenous drug containing injections. HIV-1 infects the T-lymphocytes helper cells containing CD+ receptor on the surface Cunningham et al. [11] or macrophages and dendrite cells and destroy them rapidly which lead to sharp decline in their counts. When the number of CD4+ T lymphocytes declines below a critical level (<200/ul), there is a complete collapse of cell-mediated immunity and the body becomes prone to opportunistic infections by any pathogen. The entry of HIV-1 into CD4+T cells or macrophages occurs via interaction between the glycoproteins (gp120) present on the viral envelope and the CD4 receptor present on the target cells. Certain specific co-receptors which are chemokine co-receptors such as CXCR4 (for T-lymphocytes) or CCR5 (for macrophages) are also needed for the internalization of virus into the cells after docking [12].

One of the many functions of the liver is to secrete bile, and bile plays important role in fat digestion and absorption Guyton and Hall [13]; the liver also serves as a means of excretion of several important waste products from the blood [13]. Bile secretion by the liver cells is normally stored in the gall bladder. Water, sodium, chloride and most other small electrolytes are continually absorbed through the gall bladder mucosa, and concentrating the bile constituents that contain the bile salts, cholesterol, lecithin and bilirubin [13]. Most of this gall bladder absorption is caused by active transport of sodium through the gall bladder epithelium, and followed by secondary absorption of chloride ions, water and most other diffusible constituents [13].

NVP is extensively bio-transformed via cytochrome p450 (oxidative) metabolism to several hydroxylated metabolites [14]. NVP is also an inducer of hepatic cytochrome p450 (CYP) metabolic enzymes 3A and 2B6 [15]. The non nucleoside reverse transcriptase inhibitors (NNRTIs) are the most likely class of ARV agents to cause acute hepatitis. The syndrome of lactic acidosis with hepatic steatosis related to the nucleoside analogue reverse transcriptase inhibitors may occur years after the introduction of combination ARV therapy [16]. Fatal hepatotoxicity including fulminant and cholestatic hepatitis, hepatic necrosis and hepatic failure has been reported in patients with NVP [17]. It is conceivable that NVP-associated hepatotoxicity may affect bile secretion and its biochemical composition. Hence, this study was instigated to investigate the effect of long term administration of NVP on biliary secretion and its biochemical composition using albino Wistar rats as a model.

Materials and Methods

Ethical approval

Permission was sought for, and obtained from the College Ethical Committee for the study. Nevirapine was obtained from Strides Arcolab Ltd., Bangalore, India

Experimental animals

Twenty albino Wistar rats (both sexes) with initial body weight of between (50-125 g) from the start of the experiment were used for this study. They were obtained from the disease free stock of the animal house, Department of Physiology, College of Medicine, University of Calabar, Nigeria and used for the study. They were kept in improvised plastic metabolic cages with wire net covers. The ethics for the use of experimental animals were strictly adhered to. They were maintained in the animal facility of Physiology Department, University of Calabar, at a temperature of $28 \pm 2^{\circ}C$ and 12 hours light/dark cycles.

Experimental protocol

Twenty albino Wistar rats used for this study were randomly assigned into two groups. Each rats used had a control and a NVP -treated group of ten rats each. They were used to determine the following: biliary secretion; biliary electrolytes (sodium, potassium, chloride, and bicarbonate ions); biliary bilirubin; and cholesterol from bile secretion. The control group was given 0.4 mg/kg body weight of normal saline, while the test group received oral administration of NVP (0.4 mg/kg body weight) for 2 weeks after which the dosage in each group was increased by giving twice daily (07:00 h and 18:00 h). The animals were all allowed free access to normal rat chow and clean drinking water. The feeding regimens lasted for 12 weeks (90 days) thereafter; the samples were collected for analyses.

Preparation of animals for collection of biliary secretion

Prior to the collection of bilary secretion, animals were fasted for 12 hours to get rid of any fecal matter in the stomach. This was followed by intraperitoneal injection of 25% urethane at a dose of 6 ml per kg body weight of the animal. The animals were then laid on the dissecting board, an incision was made on the neck to expose the trachea for tracheal cannulation, and this was to allow for clear airways. The stomach was opened along the linea alba to prevent bleeding. A laparotomy was performed and the liver lobes deflected antero-laterally to expose the common bile duct. The common bile duct was then cannulated with a portex cannula (internal diameter of 0.05 mm). The

quantity of bile collected after 3 hour was then measured and the flow rate calculated. The bile was then analyzed for electrolytes, bilirubin and cholesterol levels.

Biochemical assay

Sodium and potassium concentrations in bile were determined by flame photometry method using a corning flame photometer (M/N 41°C). The bile was sprayed into a non-luminous gas flame which became colored by the characteristic emission of metallic ions in the sample. The wavelength of the metal 598 nm and 767 nm for sodium and potassium respectively were selected by a light prism system and allowed to fall on a photosensitive detection system. The amount of light emitted is dependent on the concentration of metallic ion present by comparing the amount of light emitted from the sample with that from the standard solution. The amounts of sodium and potassium were determined following standard procedure. The chloride ion concentration was determined by ion selective electrode while the bicarbonate ion concentration was by the titration method using neutral red indicator. Bilirubin was determined using the van den Derg diazo reaction as described by Obembe et al. [18] following the manufacturer's instruction. This involved treatment of bile with diazotized sulphanilic acid and the red azobilirubin complex formed at an acid pH is estimated quantitatively in the spectrophotometer at 540 nm. The total serum cholesterol was estimated by the method Allain et al. [19] and later Obembe et al. [18] using a Dialab kit and absorbance was red at 546 nm. This involves the process whereby free cholesterol and cholesterol ester are released from lipoproteins by the action of surfactant. Then both existing free cholesterol and cholesterol released from its ester by cholesterol-ester hydrolase are oxidized by cholesterol oxidase. The resulting hydrogen peroxide reacts in the presence of peroxidase with the chromogen system 4-amine antipyrenes phenol to produce a red color compound.

Statistical analysis

All results are presented as mean ± standard error of mean. The data were analyzed using the Student's t-test (paired) and $p < 0.05$ was considered statistically significant.

Results

Biliary secretion

Figure 1, the rate of biliary secretion in the control and NVP -treated groups were (0.62 ± 0.04 liter/h) and (0.47 ± 0.04 liter/h). The biliary secretion in the NVP-treated group was significantly lower ($p < 0.05$) when compared to control.

Total cholesterol

Table 1, mean values for total cholesterol in control and NVP-treated groups was (0.78 ± 0.02 mmol/L) and 1.06 ± 0.05 mmol/L. Result shows significant increase ($p < 0.001$) in the NVP -treated rats as compared to control.

Total bilirubin

As shown in Table 1, mean values for total bilirubin in control and NVP-treated groups was (9.98 ± 0.75 µmol/L) and (13.96 ± 0.77 µmol/L). Result shows significant increase ($p < 0.001$) in the NVP-treated group as compared to control.

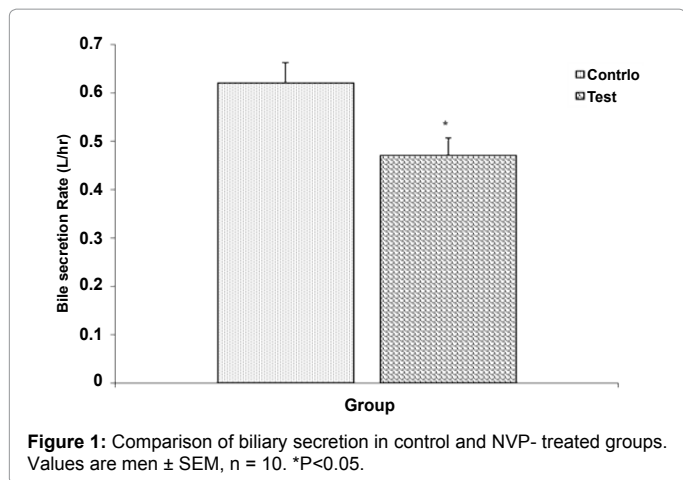**Figure 1:** Comparison of biliary secretion in control and NVP- treated groups. Values are men ± SEM, n = 10. *P<0.05.

	Control	NVP-treated
Na⁺	140.4 ± 0.49	138.2 ± 0.33**
Cl⁻	86.8 ± 0.68	85.2 ± 0.33***
K⁺⁺	4.56 ± 0.05	5.04 ± 0.05*
HCO₃⁻	19.6 ± 2.01	26.6 ± 0.27**
Total cholesterol	0.78 ± 0.02	1.06 ± 0.06***
Total bilirubin	9.98 ± 0.77	13.96 ± 0.75***
Conjugated bilirubin	4.86 ± 0.75	6.16 ± 0.61ᴺˢ
Unconjugated bilirubin	5.12 ± 0.38	7.8 ± 0.48***

*= P<0.05 vs control; ** = P<0.01 vs control; *** = P<0.001 vs control; NS = Not Significant vs control; Values are mean ± SEM, n = 10

Table 1: Summary of the effect of nevirapine administration on Bile Electrolyte Concentration; Total cholesterol mmol/L; Total bilirubin µmol/L; Conjugated bilirubin µmol/L and Unconjugated bilirubin µmol/L in albino Wistar rats.

Conjugated bilirubin

Mean values for conjugated bilirubin in control and NVP-treated groups was (4.86 ± 0.74 µmmol/L) and (6.16 ± 0.61 µmmol/L) ,Table 1. Result shows increase in conjugated bilirubin in the NVP-treated group (though not statistically significant) as compared to control.

Unconjugated bilirubin

Mean values for unconjugated bilirubin in control and NVP-treated groups was (5.12 ± 0.38 µmol/L) and (7.8 ± 0.48 µmol/L) Result shows significant increase (p<0.001) in the NVP -treated rats as compared to control (Table 1).

Bile composition

As shown in Table 1 above, mean values for serum sodium and chloride ions for control group was (140.4 ± 0.49 mmol/L) and (86.8 ± 0.05 mmol/L). Mean values for serum sodium and chloride ions for NVP-treated group were (138.2 ± 0.33 mmol/L) and (85.2 ± 0.33 mmol/L). Result show significant decreases (p<0.01 and p<0.001) in biliary electrolytes in the NVP-treated group as compared to control.

The mean values for serum potassium and bicarbonate ions for control group was (4.56 ± 0.68 mmol/L) and (19.6 ± 2.01 mmol/L). Mean values for serum potassium and bicarbonate ions for NVP-treated rats were (5.04 ± 0.33 mmol/L and (26.6 ± 0.27 mmol/L). Result show significant increases (p<0.05 and p<0.01) in biliary electrolytes in the NVP-treated group as compared to control.

Liver

Gross morphology of the liver from rats fed NVP showed diffused hepatocellular necrosis. These changes were observed in about 90% of the rats in this group. No such changes were observed in the liver of rats fed normal chow (control). Microscopic examination revealed disorganized cyto-architecture with sinusoidal and central vein endothelial desquamation in the NVP-treated group. On the other hand, microscopic examination in the control group revealed a normal healthy state of the liver with the portal tract intact, periportal hepatocytes arranged in plates and separated by sinusoids, within the portal tract were the portal vein, hepatic artery and bile duct (Figures 2b-3b).

Discussion

Effects of NVP administration given through oral gavage on biliary secretion and its biochemical composition in albino Wistar rats were studied. The results obtained showed that 12 weeks of NVP administration significantly decreased biliary secretions; caused insignificant increase in conjugated bilirubin but significant elevations in total cholesterol, total bilirubin, unconjugated bilirubin when compared to their controls respectively. There were significant decreases in biliary electrolytes (sodium and chloride) concentrations. Conversely, potassium and bicarbonate ions concentration were
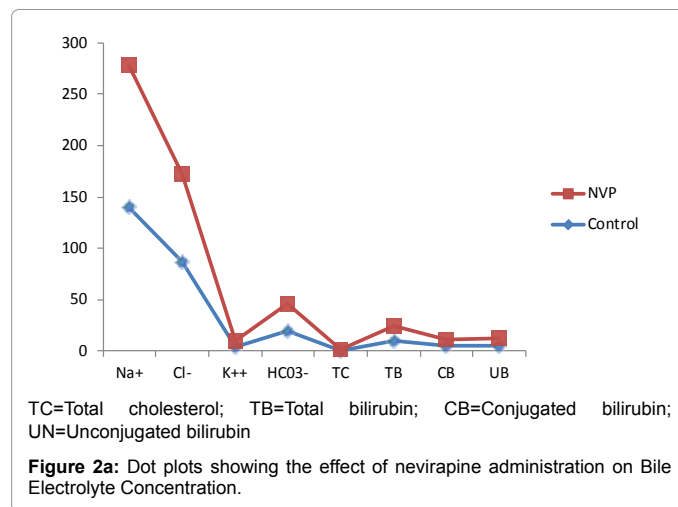
TC=Total cholesterol; TB=Total bilirubin; CB=Conjugated bilirubin; UN=Unconjugated bilirubin

Figure 2a: Dot plots showing the effect of nevirapine administration on Bile Electrolyte Concentration.
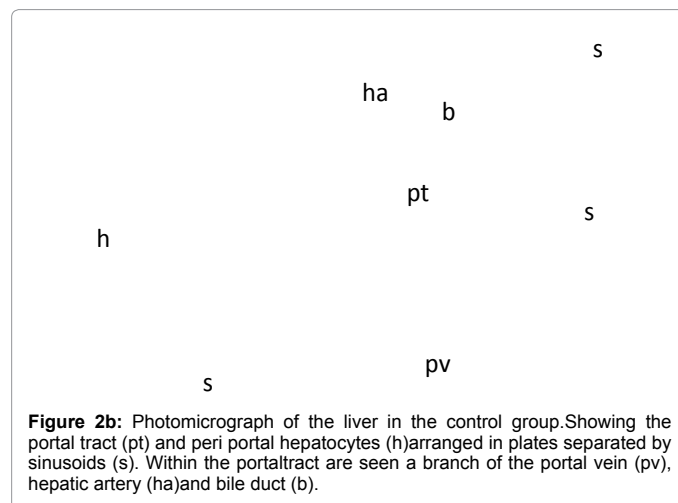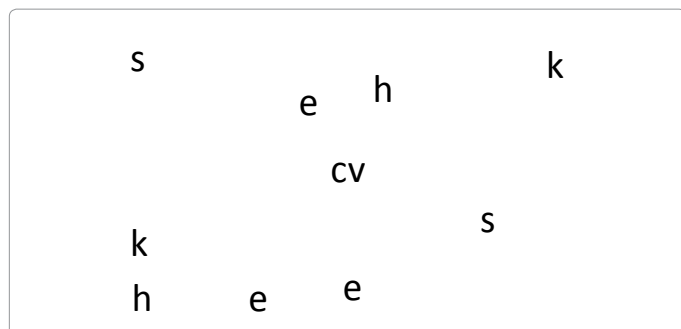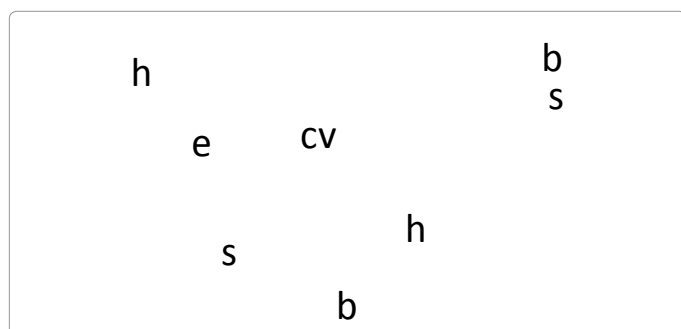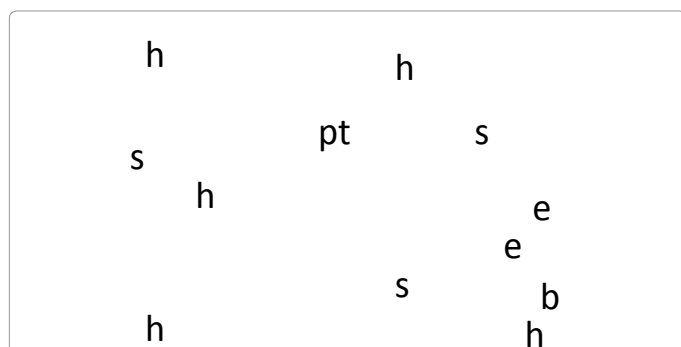

Figure 2b: Photomicrograph of the liver in the control group.Showing the portal tract (pt) and peri portal hepatocytes (h)arranged in plates separated by sinusoids (s). Within the portaltract are seen a branch of the portal vein (pv), hepatic artery (ha)and bile duct (b).

Figure 2c: Photomicrograph of the liver in the control group. Showing the hepatocytes arranged in plates radiating outwards from the central vein (cv), separated by sinusoids (s). Hepatocytes (h) are seen as polygonal shaped cells with large one or two centrally placed nucleus. Sinusoids are lined by flattened endothelial cells (e) continuous with that of the central vein. Resident macrophages (kuppfer cells, k) are seen as large irregularly shaped cells within the sinusoids.

Figure 3a: Photomicrograph of the liver of NVP treated group. Showing diffused hepatocellular (h) necrosis . The cells have undergone hydropic degenerative changes and show sub membrane blebs (b). There is also disorganized cyto architecture with sinusoidal and central vein endothelial desquamation (e). Inflammatory cells are however not seen.

Figure 3b: Photomicrograph of the liver of NVP treated group. Showing diffused hepatocellular necrosis (h). The cells have undergone hydropic degenerative changes with sub membrane blebs (b). There is also disorganized cyto architecture. Inflammatory cells are however not seen.

significantly increased in the NVP-treated animals when compared to their controls.

One of the functions of the liver is to secrete bile [13]. Bile serves two important functions; a role in fat digestion and absorption, and a means for excretion of several important waste products from the blood [13]. These include especially bilirubin, an end product of haemoglobin destruction, and excess of cholesterol [13]. Bilirubin is

a tetrapyrole created by the normal breakdown of heme [13]. Most bilirubin is produced during the breakdown of hemoglobin and other hemoproteins [20]. Accumulation of bilirubin or its conjugates in body tissues produces jaundice Muraca et al. [21], which is characterized by high plasma bilirubin levels and deposition of yellow bilirubin pigments in skin, sclera, mucous membranes, and other less visible tissues [22,23]. In the liver, uridine diphosphate (UDP)-glucuronyl transferase converts bilirubin to a mixture of monoglucuronides and diglucuronides referred to as conjugated bilirubin, which is then secreted into the bile by an ATP-dependent transporter [24]. This process is highly efficient under normal conditions, so plasma unconjugated bilirubin concentrations remain low [25].

Bile is normally stored in the gall bladder until needed in the duodenum. Active transport of sodium through the gallbladder epithelium is usually followed by secondary absorption of chloride ions, water and most other diffusible constituents [13]. Injury to the liver may alter this very important function of biliary secretion as well as affect its ability to conjugate bilirubin [13]. Result from the study showed decreased biliary secretion in the NVP-treated group of animals, this may be due to liver damage resulting in inability of the liver in this group of animals to secrete bile at normal rates compared to control. Also the decrease in bile secretion may be due to biliary obstruction. de Maat et al. [17] had reported fulminant and cholestatic hepatitis in patients with NVP. Deborah et al. [9] also reported biliary disorders (common bile duct strictures, sclerosing cholangitis, papillary stenosis, acalculous cholecystitis and vanishing bile duct syndrome) as associated with non-reverse transcriptase inhibitors administration.

Bile formation is sensitive to various hepatic insults, including high levels of inflammatory cytokines, such as may occur in patients with septic shock. High levels of conjugated bilirubin may secondarily elevate the level of unconjugated bilirubin [26]. From our study, even though the increase in conjugated bilirubin was not statistically different from the control group, it is important to point out that this slight difference is of biological interest as this could affect some of the physiological processes in the organs and tissues of the tested animals.

NVP is an inducer of hepatic cytochrome p450 (CYP) metabolic enzymes 3A and 2B6 Kumar et al. [15], and is extensively biotransformed to several hydroxylated metabolites [14]. From microscopic examination, the 12 weeks of NVP administration in the albino Wistar rats showed liver cells had undergone hydropic degenerative changes and sub-membrane blebs (Figures 2b-3b); this may point to chemical hepatotoxicity. The present result is in consonance with the work of Degott [27] who reported that liver damage is associated with alteration in bile secretion. Also in support, report by Akerlund et al. [28] who showed that there is always a compensatory increase in cholesterol synthesis when there is a disturbance in bile release and utilization due to liver damage. From the result of study, NVP-treated group of animals showed elevated levels of cholesterol, conjugated bilirubin (though not statistically different from its control group), unconjugated bilirubin. The exact mechanism of increase in conjugated and unconjugated bilirubin compared to control, despite apparent damage on the liver is uncertain. The generally high bilirubin level may be due to increased destruction of red cells. When red cells are destroyed, bilirubin is formed [29].

From the result of the present study, there was raised level of potassium and bicarbonate ions concentration. The reduction in sodium ion concentration in the NVP-treated group may be that the site for sodium absorption had been in an unhealthy state to absorb sodium

as observed with the duodenal hypertrophy and jejuna hyperplasia [8]. Another reason for the low level in sodium ion concentration might be because increased efflux of potassium ions normally cause increased influx of sodium ions to replace potassium in the blood resulting in hyponatriuemia [13]. The exact mechanism of this action is unclear. Further research on these mechanisms is however recommended.

In conclusion, long term administration of NVP may lead to reduction in biliary secretion, increase bilirubin and cholesterol levels alter bile electrolytes composition. This implies that NVP may provoke liver damage.

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