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Effects of vitamin C on liver and kidney enzymes and some biochemical parameters against paracetamol induced hepato-nephrotoxicity in rats.

Ola Ali Abdalally, Eda M. A. Alshailabiand Marfoua Saleh Ali.
e-mail: eda.muftah@omu.edu.ly

Zoology Department, Science Faculty, Omar AL-Mukhtar University, Libya.

Abstract

The current investigation aims to study the effects of VC against hepato-nephrotoxicity induced by PCM in rats. Forty male rats were divided into five groups with eight rats in each group. Group I (normal control): received distilled water orally, group II; received 500 mg/kg of VC, group III; received 500 mg/kg of PCM, group IV (protective group); received VC for 7 days, and then PCM for another 7 days, and group V treated with a combination of VC and PCM for 14 days. Results showed that a significant decline in percentage of change in body weights of treated rats with the PCM as compared to control rats while, animals that given VC with or before taken PCM showed a significant increase when compared with the PCM group. PCM caused significantly increased the liver and kidney enzymes (AST, ALP, ALT, TB, creatinine, urea, and uric acid) as well as cholesterol, and glucose levels when compared with normal rats. Whereas, animals in the protective group showed decreased liver and kidney enzymes and cholesterol, and glucose levels as compared to the PCM group. In conclusion, the results of this study demonstrate that VC was effective in reducing the hepato-nephrotoxicity caused by PCM in rats.

Keywords: *Hepato-nephrotoxicity, Enzymes, Paracetamol, Vitamin C, Rats.*

1. Introduction

The liver plays an important role in the biotransformation of drugs and toxins, in the fulfillment of many functions such as carbohydrate, fat, and protein metabolisms [1]. On the other hand, the kidney regulates many necessary functions for the body in all humans and animals. The general functions of the kidney are to regulate blood pressure, acid-base balance, electrolyte balance, and extracellular fluid volume, it also eliminates substances from the body including metabolic products, various toxins, and other foreign substances such as drugs, pesticides, and food additives [2]. So, these organs are the main target of drug-induced damage. For a long time, liver and kidney

diseases are considered a major public health problem around the world due to their potentiality to cause morbidity and mortality. Metabolic or drug/chemical-induced liver and kidney damage contribute to these diseases [3].

Paracetamol (PCM) is a usually and widely used analgesic and antipyretic drug [4, 5]. An acute paracetamol overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals and in severe cases to death [6, 7]. The paracetamol-glutathione conjugate that is formed in the liver is converted to mercapturic acid in the kidneys and excreted in urine. Besides, damage to the liver, paracetamol can also induce damage to the kidney medulla [8]. A number of studies have demonstrated that high dose PCM can increase the levels of reactive oxygen species (ROS), thus increasing cellular oxidative stress and causing liver and renal injury [9, 10].

Antioxidants are scavengers that prevent cell and tissue damage that could lead to cellular damage and disease [11]. Antioxidant agents have been used to prevent tissue damage in various clinical settings and experimental models and could help in preventing lipid peroxidation and hydrogen peroxide levels, resulting in reduced kidney and hepatic injury [12, 13] . Antioxidants such as vitamins have been shown to play very important roles in reducing the hepatotoxic effects of PCM [14]. VC is a cofactor for a number of metabolic enzymes and is an essential vitamin for humans. Under physiological conditions, it functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body [15]. VC is considered to be an important antioxidant in extracellular fluid, it also guards against aqueous radicals in the blood and protects plasma lipids from peroxidative damage caused by peroxyl radicals [16]. Furthermore, there are a few studies reporting the antioxidant power of VC against different chemicals-induced oxidative tissue injury [17, 18]. The current work was aimed to investigate the effects of VC on PCM-induced hepato-nephrotoxicity in rats.

2. Materials And Methods:

2.1. Experimental chemicals:

1-VC (ascorbic acid C6H8O6) (500 mg) was obtained from the pharmacy.

2- PCM (C8H9NO2) (acetaminophen or N-acetyl-p-amino-phenol) (500 mg) was obtained from the pharmacy.

2.2. Experimental animals:

Healthy male albino rats (*Rattus norvegicus*) with an average weight of 200-250 g were used in this study will be obtained from the Central Animal House, College of Veterinary, University of Omar Al-Mokhtar, El-Beida, Libya. All animals were allowed two weeks per-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in cages at room temperature. Rats were fed with laboratory diet and water ad libitum with fresh daily supplies.

2.3. Experimental design:

Forty male albino rats were randomized into five groups eight rats in each:

Group (1): Normal control group (NC), animals were given distilled water orally by gavage for 14 days.

Group (2): VC treated group (VC), animals were given VC (500 mg/kg/b.w) according to [17] orally by gavage for 14 days.

Group (3): PCM treated group (PCM), animals were given PCM (500 mg/kg/b.w.) according to [14] orally by gavage for 14 days.

Group (4): Protective group (PRO), animals were given VC (500 mg/kg/b.w.) for 7 days then given PCM (500 mg/kg/b.w.) orally by gavage for 7 days.

Group (5): VC and PCM treated group (VC+PCM), animals were given combination of VC (500 mg/kg/b.w.) and PCM (500 mg/kg/b.w.) orally by gavage for 14 days. All rats were received treatments six days a week [19]. At the end of the experimentation and 24 hours after the last dose, all animals were weighted and blood samples were collected from cutting the jugular vein for liver and kidney functions tests and another parameters.

2.4. Determination of the body weight:

Body weight was evaluated in all animals at the beginning (initial weight) and at the end of the experiment (final weight) by using a sensitive electronic balance. Percentage of change in the body weight was calculated according to [20] by using this formula:

$$\text{Percentage of change in the body weight} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

2.5. Serum biochemical analysis:

Other blood samples were collected and left to clot, then centrifuged at 3000 rpm for 10 minutes and stored at -80 ºC until biochemical analysis. Sera were used for the determination of biochemical analysis such as (aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin (TB)), were evaluated to determine the enzymatic activities of the liver and (creatinine, urea, and uric acid), were evaluated to determine the enzymatic activities of kidneys. Also, calcium, cholesterol, and glucose levels of the control group and the experimental groups were performed in the Al-Razi Laboratory for Medical Analysis, El-Beida City.

2.6. Statistical analysis:

Results were expressed as mean ± standard error (SE). The macroscopic and microscopic lesion scores and other parameters were analyzed using significance by one way ANOVA. Means were separated using Tukey's test at $P < 0.05$. The T test also using for compared between two means. All statistical procedures were performed with the Minitab statistical analysis package program (Minitab version 17).

3. Results And Discussion

3.1. Determination of body weights:

Averages of the percentage of change in body weights of rats belonging to the control and experimental groups are given in Table (1). Rats that given PCM for 14 days were showed, a significant decrease ($P < 0.05$) in the mean value of body weights (18.933 ± 0.193) compared to other groups. Whereas, animals that reserved VC for 7 days then given PCM for another 7 days (PRO) showed, a significant increase ($P < 0.05$) in the mean value of body weights (24.767 ±

0.279) when compared with the PCM group (18.933 ± 0.193). Besides, no remarkable changes in the mean value of body weights between VC + PCM, VC, and NG groups and between PRO, VC, and NG groups. These results have been supported by the findings of [21] who suggested that PCM causes inhibiting of body weight. These decreases in body weight gain may be due to dictate the impact of the drug on the overall growth and developmental metabolism of the animals. Therefore, the observed reduction in the body weight of the PCM-treated animals may imply possible impairment in growth-linked metabolic processes. Also, maybe due to PCM toxicity condition linked with hepatic and renal damage. Moreover, [22] said that drugs at high doses lead to undesirable side effects, such as the production of reactive oxygen species that lead to oxidative stress. Hence, these ROS can damage lipids, proteins, and DNA, thus altering the structure and function of the cell, tissue, organ, and system respectively. On other hand, animals that given VC with or before taken PCM showed a significant increase when compared with the PCM group. These findings might have been due to the role of VC as an antioxidant that prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues [23]. Also, VC enhanced cell viability, mitochondrial activity, an increase in cellular activity, and proliferation of cells [24]. In agreement with our results, Abd El Latif *et al.,* [25] stated that antioxidants can be a useful choice as could improve digestive enzymes and enhance growth performance and immunity due to their bioactive components involved in many physiological activities.

Table 1. Averages of percentage change in body weights in control and experimental groups (%).

Parameter	NG	VC	PCM	PRO	VC+PCM
% of body weights	23.917±0.407 A B	23.783±0.426 A B	18.933±0.193 C	24.767±0.279 A	22.850±0.46 B

Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B & C) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group. VC+PCM=Vitamin C + Paracetamol.

3.2.Serum biochemical analysis:

3.2.1. Determination of the enzymatic activities of liver:

3.2.1.1. Determination of the aspartate transaminase (AST):

The mean values of aspartate transaminase (AST) level of control and experimental groups were presented in Table (2). In the mean values of AST showed, a significant increase ($P < 0.05$) in the PCM group (238.6 ± 13.31) and VC + PCM group (250.2 ± 14.1) as compared to NG (99.2 ± 7.78). Besides, there was an increase significant between VC (168.8 ± 11.53) and PRO (163.6 ± 10.77) groups as compared with the control group, but it were decline than PCM and VC + PCM groups.

3.2.1.2. Determination of the alkaline phosphatase (ALP):

From the inspection of the data recorded in the Table (2), no significant effects were observed on alkaline phosphatase (ALP) level between normal control and vitamin C group, while a highly significant increase ($P < 0.05$) in the mean value of alkaline phosphatase level in paracetamol group (526.0 ± 23.42) when compared with the control group (172.50 ± 7.15). In addition, there was a significant increase ($P < 0.05$) in the mean value of ALP in PRO (314.2 ±15.86) and VC + PCM (229.00 ± 6.70) groups as compared to control, nevertheless it were decline when compared with PCM group.

3.2.1.3. Determination of the alanine aminotransferase (ALT):

Data recorded for alanine aminotransferase (ALT) were presented in Table (2). Statistically, no significant effects were observed on the mean value of ALT between the PRO group (38.2 ± 1.58), and VC + PCM group (46.7 ± 1.45) as compared with the PCM group (52.3 ± 1.8) which showed an increasing significance in the mean value of ALT when compared with control rats (39.8 ± 2.51).

3.2.1.4. Determination of the total bilirubin (TB):

On measuring total bilirubin (TB), the data were presented in Table (2). A non-significant change in total bilirubin was recorded for the vitamin C group (0.2 ± 0.03), and the protective group (0.5 ± 0.06) as compared to normal control group (0.2 ± 0.05). Whereas, animals that reserved of paracetamol only (PCM) and with vitamin C (VC + PCM) showed, a significant increase ($P < 0.05$) in the mean value of TB (1.0 ± 0.17 "PCM") and (1.1 ± 0.17 "VC + PCM") as compared with control (0.2 ± 0.05).

Results showed that PCM caused a significant increase ($P < 0.05$) in activities of liver enzymes (AST, ALP, ALT, and TB levels) as compared to normal control group. These findings come in agreement with [26, 27, 28] who found that administration of PCM caused a significant release of liver enzymes into circulation. This first goes to confirm that PCM toxicity can likely generate free radicals, hence, the elevated levels of ALT, AST and ALP. Mechanisms of paracetamol toxicity have been extensively documented, where the excessive formation of a highly reactive intermediate metabolite, N-acetyl-para-benzoquinone-imine (NAPQI), occurs when large doses of the drug are ingested. This is in line with the work of [14, 29] who said that the paracetamol toxic metabolite, NAPQI binds to the sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation causing leakage of the plasma membrane, and an increase in serum levels of liver enzymes. Activities of liver enzymes are enzymes specific to liver damage and are used routinely for the determination of liver damage, the observed increase in the serum liver enzymes activity is considered to be a significant indicator of PCM-induced acute liver damage as confirmed by [23]. Raised activities of these enzymes indicate cell damage which might have resulted from several mechanisms. Peters *et al.* [30] and Hafez *et al.* [31] reported that the increased levels of liver enzymes are generally a result of liver disease associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis, drugs, and obstructive jaundice. Previous studies have reported the serum liver enzymes are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity. When liver tissues are damaged, additional liver enzymes are released from the cytoplasm into the blood and raise the serum enzyme levels indicating cell necrosis and inflammatory reactions. As a result, the amount of liver enzymes in the blood is directly associated with the amount of tissue damage [1, 31, 28]. In addition, the observed hyperbilirubinemia may be due to excessive heme destruction and blockage of the biliary tract in PCM-treated rats. This obstruction might have resulted in mass inhibition of conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes [26]. This agrees with the earlier reports by [32, 33, 34], where PCM was reported to have caused alteration in serum concentrations of TB. Furthermore, the levels of ALT, AST, ALP, and TB was observed to decrease significantly ($P < 0.05$) in the experimental rats treated with VC for 7 days then given PCM for another 7 days (PRO group) when compared with the experimental rats treated with PCM for 14 days is in agreement with the results of [14, 26, 24]. Both animal and human studies have shown

VC to be a potent antioxidant that mediates its antioxidant effect by scavenging free ROS [14]. Moreover, vitamin C, as an antioxidant agent, may have inhibited the chain reactions of paracetamol-generated free radicals and scavenging free reactive oxygen species before reaching their hepatic tissue [17]. Thus, the results of the present study suggest that the hepatoprotective effect of VC is possibly due to its toxicity ameliorating effects, inhibition of free radicals generation, and/or free radical scavenging activity before they reached their hepatic targets as confirmed by [26].

Table 2. Average of mean values of AST, ALP, ALT, and TB levels in control and experimental groups.

Parameter	NG	VC	PCM	PRO	VC+PCM
AST (IU/L)	99.2 ± 7.78 C	168.8 ±11.53 B	238.6 ±13.31 A	163.6 ±10.77 B	250.2 ± 14.1 A
ALP (IU/L)	172.50 ± 7.15 D	138.2 ±12.97 D	526.0 ±23.42 A	229.00 ±6.70 C	314.2 ±15.86 B
ALT (IU/L)	39.8 ± 2.51 BC	46.3 ± 1.05 AB	52.3 ± 1.8 A	38.2 ± 1.58 C	46.7 ± 1.45 AB
TB (mg/dL)	0.2 ± 0.05 B	0.2 ± 0.03 B	1.0± 0.17 A	0.5 ± 0.06 B	1.1 ± 0.17 A

Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B, C, & D) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group. VC+PCM=Vitamin C + Paracetamol.

3.2.2. Determination of the enzymatic activities of kidney:

3.2.2.1. Determination of the creatinine level:

On detecting the creatinine level from the data were given in Table (3), it is denoted no remarkable changes between VC, PRO, and VC + PCM groups as compared to control animals. Conversely, animals that reserved for paracetamol only showed, a significant increase ($P < 0.05$) in the mean value of the creatinine level (1.5 ± 0.09) as compared with controls (0.8 ± 0.03).

3.2.2.2. Determination of the urea level:

The mean values of the urea level of control and experimental groups were presented in Table (3). Statistically, a significant increase ($P < 0.05$) occurred in the mean value of urea level in the PCM

group (44.7 ± 0.71) when compared with control groups (26.5 ± 1.23). In contrast, no significant changes in the mean value of urea level in PRO (24.8 ± 1.01), and VC + PCM (32.3 ± 1.41) as compared to control group (26.5 ± 1.23).

3.2.2.3. Determination of the uric acid level:

From results recorded in the Table (3), compared with the normal control rats (3.50 ± 0.09), a highly significant increase ($P < 0.05$) in the uric acid level was recognized in PCM treated group (7.00 ± 0.42). Nevertheless, a non-significant change in the mean value of uric acid level was recorded in groups (PRO "3.1 ± 0.15" and VC + PCM "4.3 ± 0.1") as compared to control group (3.50 ± 0.09).

These results were supported by [35, 36, 25] who submitted that PCM administration showed a significant increase in creatinine, urea, and uric acid levels between PCM treated animals and controls. Creatinine, urea, and uric acid are major catabolic products of muscle, protein, and purine metabolism, respectively, and their serum concentrations give clues to the functional capacity of the nephrons at the glomerular and tubular levels. These waste products (urea and creatinine) are passed into the blood stream for removal by the kidneys and their increased level in blood is a direct indication of renal dysfunction [21]. Urea is the major end product of protein catabolism and is primarily produced in the liver and secreted by the kidneys. It is the primary vehicle for removal of toxic ammonia from the body [37]. Serum urea and creatinine levels may be indicators of acute tubular necrosis caused by toxicity [38]. Yakubu *et al.,* [39] stated that the significant increase in serum urea concentration may be attributed to damage of the urea cycle leading to an increase in the production of the metabolic produce. Consequently, an increase in urea levels may be due to defects in urea synthesis that may result in ammonia intoxication. Moreover, [40] stated that the markedly high serum rates of urea and creatinine in rats were indicative of marked necrosis of kidney epithelium and dilatation of proximal tubules with interstitial inflammation, damage in the last part of nephron and collecting system. Also, the increased serum concentrations of creatinine, blood urea nitrogen, and uric acid may be indicative of renal injury and cell necrosis resulting from the formation of NAPQI in excess of GSH detoxification ability. This is consistent with previous studies, where paracetamol administration proved toxic to renal tubular cells [21].

On the other hand, the results in this study showed a significant decrease in PRO and VC + PCM group in the mean values of creatinine, urea, and uric acid when compared with PCM group. This is accompanied with [41] who revealed normal renal cortical cells, indicating that such a dose of VC can significantly protect the kidney from the nephrotoxic effect of PCM. Besides that, [21] suggests that antioxidant plant was able to prevent or extenuate the deleterious influence of PCM. This observation also indicates that antioxidant plant at the investigated doses could preserve renal functions and delay the progression of renal pathological conditions.

Table 3. Average of mean values of creatinine, urea and uric acid levels in control and experimental groups.

Parameter	NG	VC	PCM	PRO	VC+PCM
Creatinine (mg/dL)	0.8± 0.03 B	0.7± 0.07 B	1.5± 0.09 A	0.7± 0.03 B	0.8± 0.04 B
Urea (mg/dL)	26.5± 1.23 CD	34.8± 1.9 B	44.7± 0.71 A	24.8± 1.01 D	32.3± 1.41 BC
Uric acid (mg/dL)	3.50± 0.09 BC	3.00± 0.27 C	7.00± 0.42 A	3.1± 0.15 C	4.3± 0.1 B

Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B, C & D) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group. VC+PCM=Vitamin C + Paracetamol.

3.2.3. Determination of the calcium level:

The mean values of the calcium level of control and experimental groups were obtainable in Table (4). Statistically, a significant increase ($P < 0.05$) occurred in the mean value of the calcium level in paracetamol group (10.00 ± 0.4) when compared with VC + PCM group (9.2 ± 0.13). Although, no a significant changes between the mean value of PRO group (9.5 ± 0.12), and VC + PCM group (9.2 ± 0.13) when compared with NC group (9.4 ± 0.11).

3.2.4. Determination of the cholesterol level:

On noticing the cholesterol level from the data were assumed in Table (4), it is denoted that rats that reserved of paracetamol had a high significant increase ($P < 0.05$) (223.6 ± 15.4) as compared

to control rats (111.6 ± 3.84). Whereas, no significant effects were observed in PRO rats (116.6 ± 1.96), and VC + PCM rats (106 ± 1.38) as compared to control groups.

3.2.5. Determination of the glucose level:

The mean values of the glucose level of control and experimental groups were presented in Table (4), showed a no remarkable changes between the mean value of the glucose level in PRO group (31.7 ± 0.99), VC + PCM group (34.00 ± 1.59), and VC (30.00 ± 1.98) when compared with normal group (31.2 ± 2.37). While, a high significant increase ($P < 0.05$) in the mean value of the glucose level in PCM group (68.4 ± 4.04) as compared to control group (31.2 ± 2.37).

The results of the current study showed no significant difference was noted in the calcium level between PCM treated groups and control. However, the results showed a significant increase was observed in cholesterol and glucose levels between the PCM group and other groups. These results were supported by[42, 28, 36] who reported that a significant changes in the cholesterol level between PCM groups and control. In contrast, [28] found that the PCM caused a significant rise in glucose level in rats that given 300 mg/kg for 30 days when compared with control rats, this is in agreement with the findings. The increase in the cholesterol level by paracetamol may be indicative of toxicant-induced cholestasis secondary to hepatic cellular inflammation [35]. Moreover, [28] suggested that the changes in serum levels of cholesterol and glucose in rats administered a high dose of PCM compared to the control group could relate to hepatic dysfunction which led to disturbances in the levels of these parameters because the liver is the organ responsible for their metabolism. Ibrahim *et al.* [43] said that the possible explanation of the observed hyperlipidemia might reflect the deterioration of liver cells to metabolize lipids or lipid peroxidation. The increase in serum lipids may be attributed to the increased liver synthesis and/or diminished liver degradation; where reduced lipoprotein lipase activity plays a role in the lipids increment.

Although, from the examination of the data recorded in this study showed non-significant of calcium, cholesterol, and glucose levels between VC, PRO, and VC + PCM groups and normal control group. This is accompanied by [28] reported that the VC caused the significantly reduce stress-induced rise in serum cholesterol is believed to possess an anti-cholesterol effect. Also, [8]

stated that antioxidant plants lower blood cholesterol levels by inhibiting intestinal absorption of dietary cholesterol and reabsorption of bile acids.

In this work, treatment of rats with VC at a dose of 500 mg/kg restored the levels of glucose and cholesterol to normal levels. This might be linked to its considerable ability to protect hepatocytes from injury, thereby reserving its role in cholesterol and glucose levels modulation.

Table 4. Average of mean values of calcium, cholesterol and glucose level in control and experimental groups.

Parameter	NG	VC	PCM	PRO	VC+PCM
Calcium (mg/dL)	9.4 ± 0.11 AB	8.00 ± 0.09 C	10.00 ± 0.4 A	9.5 ± 0.12 AB	9.2 ± 0.13 B
Cholesterol (mg/ml)	111.6 ± 3.84 C	153.2 ± 4.05 B	223.6 ± 15.4 A	116.6 ± 1.96 C	106.0 ± 1.38 C
Glucose (m mol/L)	31.2 ± 2.37 B	30.00 ± 1.98 B	68.4 ± 4.04 A	31.7 ± 0.99 B	34.00 ± 1.59 B

Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B, & C) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group. VC+PCM=Vitamin C + Paracetamol

4. Conclusions

The present findings clearly demonstrate that paracetamol is capable of inducing biochemical changes in the liver and kidney tissues of the experimental rats. Besides, that VC has a protective effect against hepatotoxicity and nephrotoxicity PCM-induced in male rats.

5. Recommendations

The protective effect of VC was evident. In spite of the promising results of this study, further studies including different doses of VC are required in order to look for the dose able to restore the

liver and kidney to their exact normal appearance. Also, detailed studies concerning the therapeutic efficacy, safety, and stability of a combined fixed dose of VC and PCM are needed.

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