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ORIGINAL ARTICLE

Nephroprotective Potential of Ca and Mg against Cd and Pb in Rats

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ABSTRACT

Adverse environmental impacts from excessive heavy metals dispersed from mines and smelter sites include contamination of water, soil and phytotoxicity leading to potential risks to human health. One of the organs that are exposed to heavy metal toxicity is the kidney. In this study, concurrent administration of different combinations of Cd, Pb, Ca and Mg were carried out using a rat model to determine the nephrotoxicity of Cd and Pb, and also the nephroprotective potential of Ca and Mg against Cd and Pb. Studies indicate that the urinary excretion of Cd^{2+} and Pb^{2+} ions were increased as the concentrations of the combination of Ca and Mg were elevated thus: 0.0302±0.002, 0.0665±0.001, 0.0911±0.002, 0.2284±0.005, 0.0808±0.000 and 0.0246±0.001, 0.0095±0.002, 0.0881±0.0.003, 0.1037±0.003, 0.0940±0.000 respectively. The addition of either Cd or Pb salt alone without the addition of Ca and Mg salts show that the urinary excretion of Cd^{2+} ions decreased as compared to control irrespective of the concentration of Cd or Pb salt added, while the Ca^{2+} excretion increased as follow: 0.0302±0.002, 0.0163±0.002, 0.0144±0.000, 0.0122±0.002, 0.0131±0.000 and 8.0700±0.006, 14.6400±0.016, 10.3600±0.058, 16.1700±0.037, 21.8100±0.026 respectively. The biochemical and histopathological analyses show that Cd and Pb have nephrotoxicity, while Ca and Mg have nephroprotective potential against Cd and Pb in rats. This nephroprotection stems from the fact that the excretory pattern of the metals revealed that there exist mutual exclusivity between Ca/Cd, Ca/Pb, Mg/Cd, and Mg/Pb.

Keywords: *Nephrotoxicity, Nephroprotective potential, Heavy metals, Environmental pollution, Mutual exclusivity.*

INTRODUCTION

Mining and smelting operation are important causes of heavy metals contamination in the environment due to activities such as mineral excavation, ore transportation, smelting and refining, and disposal of the tailings and waste water around mines [1, 2]. Adverse environmental impacts from excessive heavy metals dispersed from mines and smelter sites include contamination of water and soil, phytotoxicity, soil erosion and potential risks to human health [3, 4].Cd and Pb in the environment have been a concern since the 1960s when a painful bone disease itai-itai was reported to have been caused by Cd pollution in Japan [5] and Pb contamination of soil and sediment, and petrol in England was first reported by Davis and Holmes [6]. In larger doses Cd and Pb can accumulate in the liver and kidneys, and can replace Ca and Mg in bones and other organs leading to diseases [3, 7]. From an historical perspective, water quality and health management paradigms have evolved over the past several decades as our understanding of water quality issues and its health effects on humans increase [8]. This environmental pollution by Cd and Pb has raised growing concerns about their effects on the health of the general population since renal toxicity is one of the major problems identified [9].

From general knowledge, the primary function of the kidney is the excretion of body wastes and harmful chemicals including heavy metals. Large amount of cardiac output (one fourth) circulate through the kidney, the greatest rate of blood flow for any organ. This predisposes the kidney to heavy metal toxicity as the biological half-life of Cd and Pb is in the order of decades [10]. Exposure to high levels of Cd and Pb may cause kidney damage leading to renal failure [10, 9].

In our previous work, Ca and Mg were found to have hepatoprotective potential against Cd and Pb induced hepatotoxicity [11]. This made us ask the question: Could Ca and Mg also have nephroprotective potential against Cd

and Pb induced nephrotoxicity in rats? This forms the bases for this present work.

MATERIALS AND METHODS

This study was conducted in 2007 to 2008 in the Department of Biochemistry laboratory of the University of Jos. The histopathological studies were done in the laboratory of Anatomy Department, University of Jos.

***Experimental Animals*:**

Seventy (70) adult male Wister rats weighing 336g on the average were obtained from the animal house of the University of Jos, Nigeria. Commercial Feed Produced by Grand Cereal and Oil Mill Limited, Jos, Nigeria, was used.

Chemicals:

Lead acetate and magnesium sulphate, both analar, were products of British Drug House (BDH), Poole, England. Cadmium chloride and calcium sulphate were products of May and Baker (M & B) Limited, Dagenham, England. Bovine Serum Albumin (BSA) was a product of Sigma Chemicals.

Experimental Design

The work was divided into three phases. Seventy rats were used in all. In the first phase, twenty- five (25) rats were divided into five groups of five rats per group in cages. Varying concentration of the combination of Cd (0.008, 0.013, 0.018, 0.023mg) and Pb (0.020, 0.040, 0.060, 0.080mg) respectively in that order, were given to the rats, each group taking a particular concentration of the combination of the metals. Group 1 was the control and was placed on tap water only. Group 2 was placed on the combination of 0.008mg of Cd and 0.02mg of Pb; group 3 on 0.013mg of Cd and 0.040mg of Pb; group 4 on 0.018mg of Cd and 0.060mg of Pb; while group 5 was place on 0.023mg of Cd and 0.080mg of Pb.

In the second phase, twenty-five rats were divided into five groups of five rats per group as in the first phase. The same concentrations of Cd and Pb used in phase 1 were used with the addition of varying concentrations of Ca and Mg of 0.054, 0.088, 0.122, and 0.154mg to groups 2, 3, 4.and 5. The first group was the control and no metal was added.

In the third phase, twenty rats were divided into four groups of five rats per group. The lowest and the highest concentrations of Cd and Pb used in the first two phases were used without the addition of Ca and Mg.

The salts of these metals were made into solutions and given to the rats *ad libitum*. Their feed was also mashed with the same solutions meant for each group. Each group was placed on its solution for 14 days [12].

Sample Collection and Preparation

Daily urine samples were collected and stored in plastic test tubes with corks. After the end the experiments, the rats were sacrificed. The liver was identified and fixed in 10% formal saline for histopathological studies.

Digestion of urine samples

The digestion mixture was prepared by mixing perchloric acid ($HCLO_4$) and Nitric acid (HNO) in the ratio of 6:1. The mixture was added to a specified volume of urine in a 25ml beaker and heated on a hot plate in a fume cupboard until the sample became almost dry. More of the digestion mixture was added continually until the sample turned whitish and dry. This means that all organic materials are burnt off. The whitish precipitate was then dissolved in deionised water to the volume of the urine used.

Methods used for the determination of the concentrations of Cd^{2+}, Pb^{2+}, Ca^{2+} and Mg^{2+} ions and the histopathological studies.

The urine samples were analyzed using Hitachi atomic absorption spectrophotometer while the histopathological examinations were done using the routine method for H & E staining protocol [13]. Digestion of urine samples were done by the wet digestion method.

Statistical Analysis:

The analysis of variance at 95% level of confidence was used to test for the significant differences in the urinary excretion of Cd, Pb, Ca, and Mg ions.

RESULTS

Table 1 show that when Ca and Mg were added concurrently with Cd and Pb, more of Cd^{2+} and Pb^{2+} ions were excreted in the urine. There was no significant difference ($p>0.5$) in the excretion pattern of Ca^{2+} ions between the control group and all the other groups until the highest concentrations when the excretion of Ca^{2+} and Mg^{2+} ions are high while the excretion of Cd^{2+} and Pb^{2+} ions decreased. More of Mg^{2+} ions were excreted than Ca^{2+} ions in all the groups.

Table 1: The Effect of Concurrent Administration of Cd, Pb, Ca and Mg on the Urinary Excretion of Cd^{2+}, Pb^{2+}, Ca^{2+} and Mg^{2+} ions in rats.

Group	Treatment	$[Cd^{2+}]$	$[Pb^{2+}]$	$[Ca^{2+}]$	$[Mg^{2+}]$
1.	No metal added	0.0302 0.002	0.0246 0.001	8.07 0.006	12.21 0.008
2.	Ca + Mg	0.0665 0.001	0.0095 0.002	7.91 0.008	20.20 0.021
3.	Ca + Mg	0.0911 0.002	0.0881 0.003	6.76 0.012	22.02 0.005
4.	Ca + Mg	0.2284 0.005	0.1037 0.003	9.58 0.016	12.85 0.005
5.	Ca + Mg	0.0808 0.000	0.0940 0.000	21.51 0.027	24.00 0.007

Table 2 shows that when Cd alone was administered at a low concentration, less of Cd^{2+} ions, more of Pb^{2+} and Ca^{2+} ions were excreted; there was no significant difference ($P>0.5$) in Mg^{2+} ion excretion as compared to control. Also, when lead alone was administered at a low concentration, there was decreased excretion of Cd^{2+} and Mg^{2+} ions but there was an increase in the excretion of Pb^{2+} and Ca^{2+} ions. When Cd alone was administered at a high concentration, there was a decrease in the excretion of Cd^{2+} and Mg^{2+} ions but there was an increase in the excretion of Pb^{2+} and Mg^{2+} ions. When Cd alone was administered at a high concentration, there was a decrease in the excretion of Cd^{2+} ions while there was increase in the excretion of Pb^{2+}, Ca^{2+} and Mg^{2+} ions. There was a sharp increase in the excretion of Mg^{2+} ion. When Cd alone was administered at a high concentration, there was a decrease in the excretion of Cd^{2+} ions while there was increase in the excretion of $Pb^{2+,}$, Ca^{2+} and Mg^{2+} ions. There was a sharp increase in the excretion of Mg^{2+} ion. When Pb alone was administered at high concentration, there was also a decrease in the excretion of Cd^{2+} ions and no significant difference ($P>0.5$) in the excretion of Pb^{2+} ions as compared to control. There was also increase in the excretion of Ca^{2+} and Mg^{2+}, but more Ca^{2+} ion was excreted.

Plates 2a, 3a, 4a, and 5a show that as the concentrations of the combination of Cd and Pb were elevated; there was a progressive damage with the worst damage in the highest concentrations as compared to the control (plate 1). Plates 2b, 3b, 4b and 5b show that the damage observed in the absence of Ca and Mg were ameliorated but not obliterated.

Table 2: The Effect of Administering Two Different Concentrations {High (H) and Low(L)} of Cd, or Pb on the Excretion of Cd^{2+} , Pb^{2+} ,Ca^{2+} and Mg^{2+} ions in rats.

Group	Treatment	$[Cd^{2+}]$	$[Pb^{2+}]$	$[Ca^{2+}]$	$[Mg^{2+}]$
1.	No metal added	0.0302 0.002	0.0246 0.001	8.07 0.006	12.21 0.008
2.	Cd (L)	0.0163 0.002	0.0400 0.010	14.64 0.016	12.53 0.009
3.	Pb (L)	0.0144 0.000	0.0350 0.007	10.36 0.058	3.59 0.001
4.	Cd (H)	0.0122 0.002	0.076 0.001	16.17 0.037	21.99 0.007
5.	Pb (H)	0.0131 0.000	0.0250 0.002	21.81 0.026	19.61 0.004

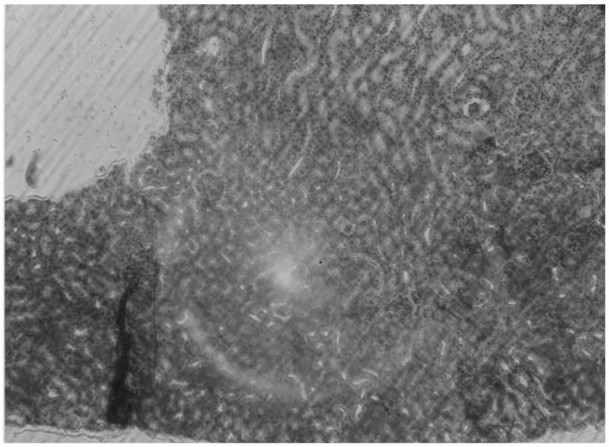

Plate 1b: L/S x 400
Representative Renal biopsy of the rats fed without the addition of Lead and Cadmium, showing normal and healthy section of the kidney.

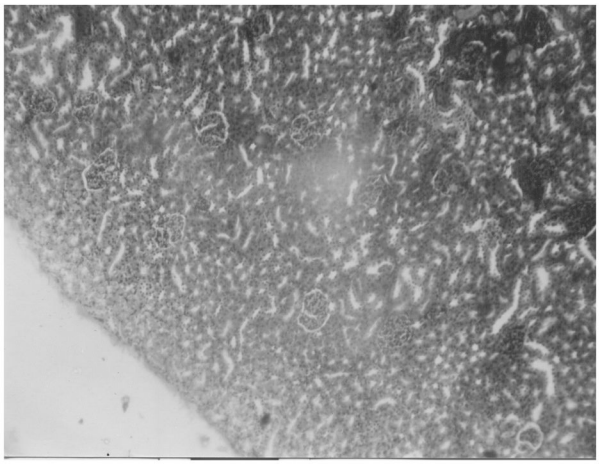

Plate 2a: L/S x 400
Representative Renal biopsy of the rat treated with 0.008mg and 0.020mg of cadmium and lead respectively in combination, showing relatively normal cells of the glomerulus and convoluted tubules.

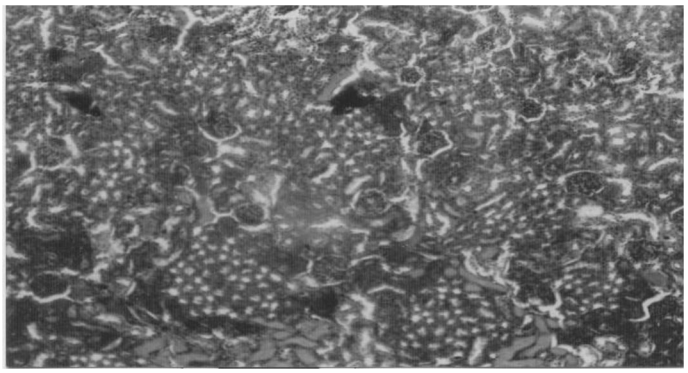

Plate 2b: L/S x 400
Representative renal biopsy of the rats treated with 0.008mg/L and 0.020mg/L of cadmium and lead respectively in combination with the addition of calcium and magnesium. It shows relatively normal cells of the glomerulus and convoluted tubules.

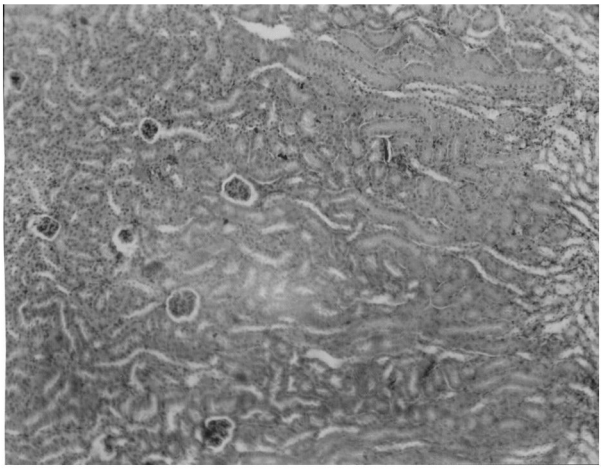

Plate 3a: L/S x 400

Representative renal biopsy of the rats treated with 0.013mg and 0.040mg of cadmium and lead respectively in combination, showing condensed glomerulus with renal tubular damage (arrows).

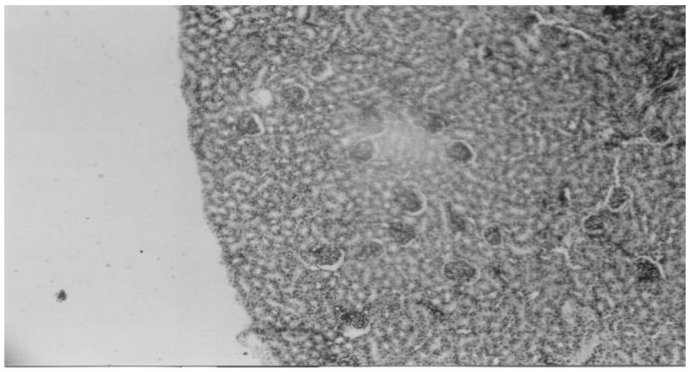

Plate 3b: L/S x 400

Representative renal biopsy of the rats treated with 0.013mg and 0.040mg of cadmium and lead respectively in combination with the addition of calcium and magnesium. It shows relatively normal cells of the glomerulus and convoluted tubules as compared to plate 3a.

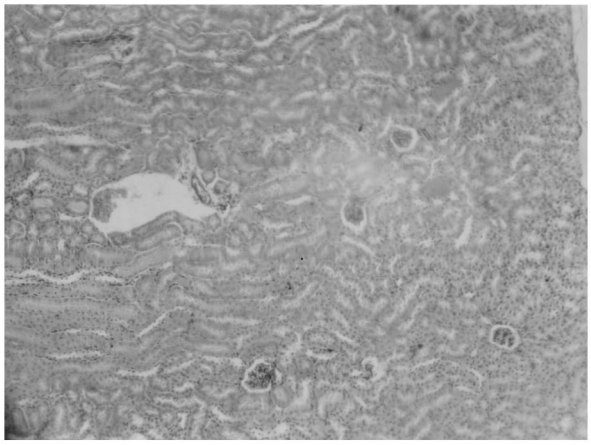

Plate 4a: L/S x 400

Representative renal biopsy of the rats treated with 0.018mg and 0.060mg of cadmium and Lead respectively in combination, showing condensed glomerulus with renal tubular damage (arrow)

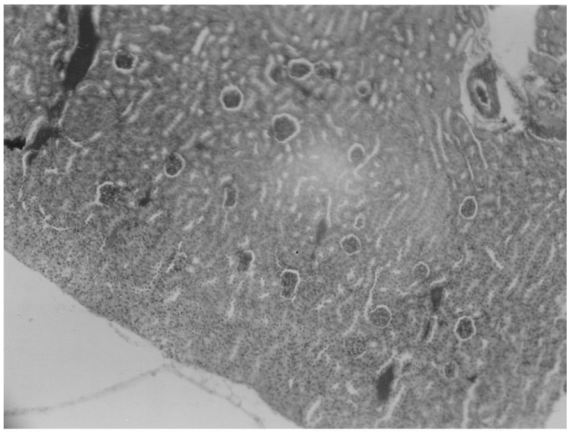

Plate 4b: L/S x 400

Representative renal biopsy of the rats treated with 0.018mg and 0.060mg of cadmium and lead respectively in combination with the addition of calcium and magnesium. It shows relatively normal cells of the glomerulus and convoluted tubules as compared to plate 4a.

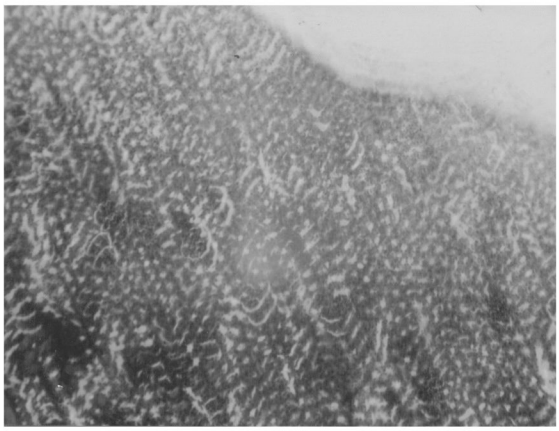

Plate 5a: L/S x 400

Representative Renal biopsy of the rats fed with Lead, Cadmium, showing a rapidly progressive and severe glomerular nephritis with strong renal tubular dysfunction (arrow).

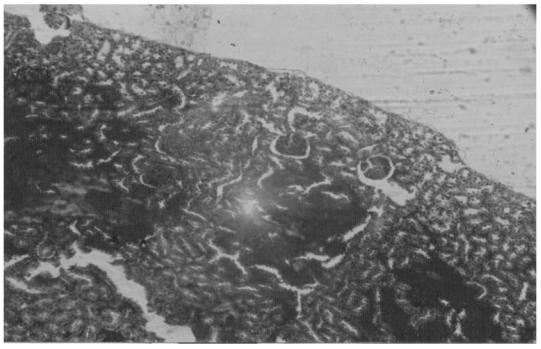

Plate 5b: L/S x 400

Representative Renal biopsy of the rats treated with 0.023mg and 0.080mg of cadmium and lead respectively in combination, showing a milder glomerular nephritis with some relatively normal kidney section as compared to plate 5a (arrow).

DISCUSSION

The observed trend in plates 2a, 3a, 4a and 5a respectively indicate that as the concentrations of the combination of Cd and Pb were elevated; there was a progressive damage with the greatest damage in group 5 which contain the highest concentrations of the combinations of the two metals. This is consistent with the fact that the higher the concentrations of the heavy metals the more damage is inflicted on the cell [11]. When the combination of calcium and magnesium was added, the nephrotoxicity was ameliorated but not obliterated as indicated on plates 2b, 3b, 4b, and 5b. This observation is consistent with the fact that when the availability of essential micronutrients is increased, the toxicity of toxic heavy metals is decreased [14, 11]. Increase synthesis of metllothionein (MT), a thiol-rich protein that sequesters Cd and prevent acute hepatotoxicity, will lead to chronic toxicity as cadmium-MT is excreted from the liver and absorbed by the kidney. Although MT offers mostly protective effects against Cd toxicity, it has been shown that it is indirectly involved in contributing to Cd's main toxic effect: renal failure [15].

The excretion pattern of Ca^{2+} and Mg^{2+} ions indicate that more Cd and Pb^{2} ions were excreted in the urine when Ca and Mg were added to the combination of Cd and Pb. This agrees with the observations by other scientists that more Pb^{2+} and Cd^{2+} ions are absorbed by people on a calcium-poor diet than those on a calcium – rich diet or magnesium-rich diet [16, 17].

There was no significant difference ($p>0.05$) in the excretion pattern of Ca^{2+} ions between the control group and the other groups that contained the combination of Cd, Pb, Ca and Mg until at the highest concentration of the metals, when the excretion of Ca^{2+} and Mg^{2+} ions were very high, while the excretion of Cd^{2+} and Pb^{2+} ions were decreased. This means that more Cd^{2+} and Pb^{2+} ions were retained in the cell. Some studies showed that an increase in Ca^{2+} ion urinary excretion is an indication of early manifestation of cadmium nephrotoxicity in Cd- MT injected rats [18, 19]. More Mg^{2+} ions were excreted than Ca^{2+} ions in all the groups signifying that calcium is a better competitor for binding sites on the divalent metal transporter 1 (DMT1), responsible for the transport of essential and nonessential divalent metal ions such as Ca^{2+}, Mg^{2+}, $Zn^{2+,}$ $Pb^{2+,}$ $Cd^{2+,}$ Fe, etc into cell [16].

It was also observed that when Cd or Pb alone was administered at low concentrations, less of Cd^{2+} ions, and more of Pb^{2+}, Ca^{2+} and Mg^{2+} ions were excreted (table 1). This explains the fact that Cd is very toxic even at low concentrations. Therefore, it can displace Ca, Mg and Pb in this mixture. There is strong evidence, for instance, pointing to the role for Ca channels in the uptake of Cd by some cells which showed that Ca channel blockers depressed Cd uptake in several, though not all, cell types [20] and other trans-membrane transporters [21]. This means that the movement of Cd through Ca channels and DMT1 occur in competitive interaction between the divalent metals [22]. At high concentration of Cd alone or Pb alone, much higher concentrations of Ca^{2+} and Mg^{2+} ions were excreted in the urine as compared to the control and at lower concentrations of Cd alone or Pb alone. This implies that more of Cd^{2+} ions are retained in the cell; Lower Cd^{2+} ions were excreted as compared to control and at lower concentrations of Cd alone or Pb alone. It was also observed that the addition of lead alone at high concentration led to more Ca and Mg ions being excreted leading to less excretion of lead in the urine. This means that Pb at high concentration can also compete with Ca and Mg for binding sites. On the other hand, when Cd alone was administered at high concentration, more Mg^{2+}, Ca^{2+} ions were excreted in the urine as compared to control.

CONCLUSION

Our results provide evidence that the ingestion of Cd and Pb may pose great risk to the kidney because the metals can interfere with essential nutrients of similar chemistry such as Ca and Mg. The results also explain the fact that there exists mutual exclusivity between these four metals as was reflected in the urinary excretion pattern. Also, Ca and Mg have nephroprotective potential against Cd and Pb nephrotoxicity but the protection is not as good as was in our previous work on hepatotoxicity. Therefore, people living in polluted areas may be advised to take foods rich in Ca and Mg so as to mitigate the nephrotoxicity that may occur as a result of ingesting foods or water (or both) containing high concentrations of Cd and Pb.

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