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Effect of copper aspirinate on contraction of isolated rabbit aortic strips[1]

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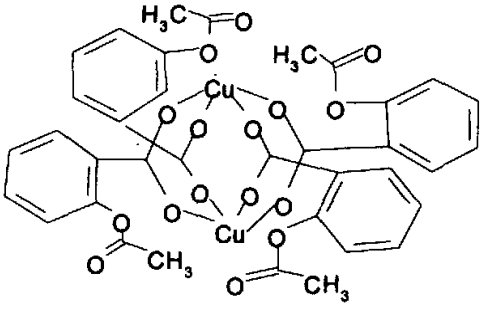
KEY WORDS copper aspirinate; aorta; vascular smooth muscle; vascular endothelium

ABSTRACT

AIM: To investigate the effect of copper aspirinate on contraction of vascular smooth muscle. **METHODS**: Isolated rabbit aortic strips, including intact endothelium strips and endothelial cell-denuded aortic strips, were suspended in modified Krebs solution to determine effects of copper aspirinate on the contraction induced by norepinephrine (NE), KCl, and $CaCl_2$, while $CuSO_4$, aspirin, and vehicle were used as controls. **RESULTS**: Copper aspirinate possessed antagonistic effect on contraction of rabbit aortic strips induced by NE with an IC_{50} value of 31 nmol/L, while $CuSO_4$ had much less antagonistic effect with an IC_{50} value of 0.29 μmol/L, and aspirin did not work in the same preparation. No effect of copper aspirinate were found on the contraction induced by KCl and $CaCl_2$. Effects on endothelial cell denuded aortic strips were similar to those in the normal aortic strips. **CONCLUSION**: Copper aspirinate possessed different effects from aspirin and $CuSO_4$ on vascular smooth muscles. It inhibited contraction induced by NE with an activity stronger than $CuSO_4$ at the same Cu^{2+} concentration, this action might be due to blockade of the receptor-operated Ca^{2+} channels, and it might not be linked to the endothelium.

INTRODUCTION

Aspirin is a traditional non-steroidal anti-inflammatory drug, but its principal ulcerogenicity has limited its wide application. Copper aspirinate, a copper salt of aspirin, was reported having more potential effects as an inhibitor of inflammation and platelet aggregation than aspirin[1,2], and was reported to reduce thromboxane A_2 (TXA_2) levels and elevate 6-keto-$PGF_{1\alpha}$ levels in plasma[3]. In the present investigation, we examined effects of copper aspirinate on the contraction of isolated rabbit aortic strips induced by norepinephrine (NE), KCl, and $CaCl_2$.



Structure of copper aspirinate

MATERIALS AND METHODS

Reagents Copper aspirinate (Cu 14.99 %, C 51.21 %, H 3.32 %; purity > 98 %) was synthesized by Kunming Institute of Precious Metals. It was dissolved in 0.9 % NaCl solution. $CuSO_4$ and aspirin were purchased from Sigma Co, USA, and dissolved in 0.9 % NaCl solution before use.

Animals New Zealand white rabbits of either sex, weighing 2.0 - 3.0 kg, were obtained from Animal Department, Yunnan Pharmacological Laboratories of Natural Products.

Preparation of isolated rabbit aortic strips Rabbits were killed by a heavy blow to the head. The descending thoracic aorta was cut into 3 mm × 30 mm spiral strips. Endothelial cell-denuded aortic strips were

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prepared by mechanical method with cotton pipe-cleaners. The denudation was confirmed by the absence of relaxation to direct application of ACh 1 μmol/L[4].

The strips were suspended in modified Krebs' solution 20 mL containing (mmol/L): NaCl 128, KCl 4.7, NaH_2PO_4 0.8, $CaCl_2$ 2.2, $MgCl_2$ 0.3, $NaHCO_3$ 13, glucose 5.6. The bath solution was continuously bubbled with O_2, maintaining a pH of 7.4±0.5 at 37 ℃ ± 0.5 ℃. The tension of the strips was recorded isometrically by electromechanical transducers connected to a recorder (model U-135 C, Shimadzu, Japan). The strips were loaded with an initial tension of 2 g for 2 h equilibration in the solution which was renewed every 20 min[5,6].

Contraction induced by NE and KCl Contraction peak values induced by NE 1.4 μmol/L or KCl 40 mmol/L were expressed as 100 % and inhibitory rates were determined in the presence of copper aspirinate and controls. Copper aspirinate and controls were added cumulatively to test IC_{50} values[7]. Also, NE and KCl were applied cumulatively to obtain cumulative concentration-response curve for copper aspirinate 31 nmol/L, $CuSO_4$ 62 nmol/L, with the same concentration of Cu^{2+} as copper aspirinate, and aspirin 0.12 μmol/L, with the same concentration of aspirin as copper aspirinate.

Contraction induced by $CaCl_2$ After 2 h equilibration in the modified Krebs solution, the aorta strips were equilibrated in modified Ca^{2+}-free Krebs solution for 30 min, and then in modified Ca^{2+}-free Krebs solution containing KCl 40 mmol/L for 20 min[7]. The tested drugs were added to examine effects on contraction induced by $CaCl_2$ and cumulative concentration-response curve were obtained by the same procedure.

Statistic methods IC_{50} was calculated by nonlinear regression analysis after logarithmic transformation of the concentrations applied. The data were analyzed by unpaired *t*-test.

RESULTS

Cumulative application of copper aspirinate resulted in a concentration-related inhibition of NE in isolated rabbit aortic strips(Fig 1). Copper aspirinate showed higher antagonistic effects on contractions induced by NE 1.4 μmol/L, with IC_{50} value and 95 % confidence intervals of 31 (19 - 49) nmol/L, while $CuSO_4$ showed less antagonistic effects with IC_{50} value and 95 % confidence intervals of 0.29 (0.23 - 0.36) μmol/L (Fig 2). Aspirin did not show any effects in the same preparation (Fig 1 - 3).

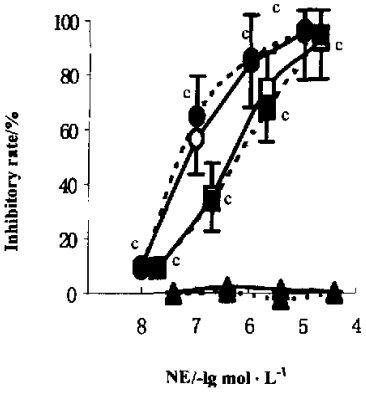

Fig 1. Effect of copper aspirinate (controls: $CuSO_4$, aspirin) on contraction induced by NE 1.4 μmol/L in rabbit aortic strips. Copper aspirinate (normal strips ○); copper aspirinate (endothelial cell denuded strips, ●); $CuSO_4$ (normal strips □); $CuSO_4$ (endothelial cell denuded strips, ■); aspirin (normal strips △); aspirin (endothelial cell denuded strips, ▲). $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* aspirin.

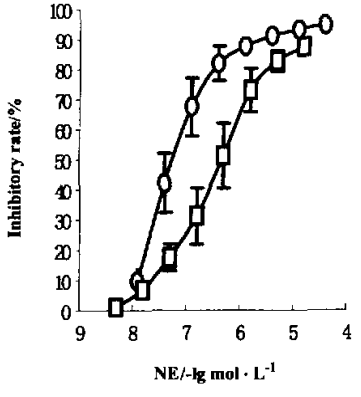

Fig 2. Cumulative concentration-response of copper aspirinate (control: $CuSO_4$) on contractions induced by NE 1.4 μmol/L. Copper aspirinate (○); $CuSO_4$ (□). $n = 6$. $\bar{x} \pm s$. IC_{50} values of copper aspirinate and $CuSO_4$ were 31 nmol/L and 0.29 μmol/L respectively.

Copper aspirinate, $CuSO_4$, and aspirin did not show any antagonistic effects on the contraction induced by KCl or induced by $CaCl_2$ under high concentration of KCl 40 mmol/L either (Fig 3).

In endothelial cell denuded aortic strips, similar effects of copper aspirinate and controls on contraction induced by NE, KCl, and $CaCl_2$ were observed (Fig 1,

3). There was no significant difference between the effects of copper aspirinate on normal strips and endothelial cell denuded aortic strips ($P > 0.05$).

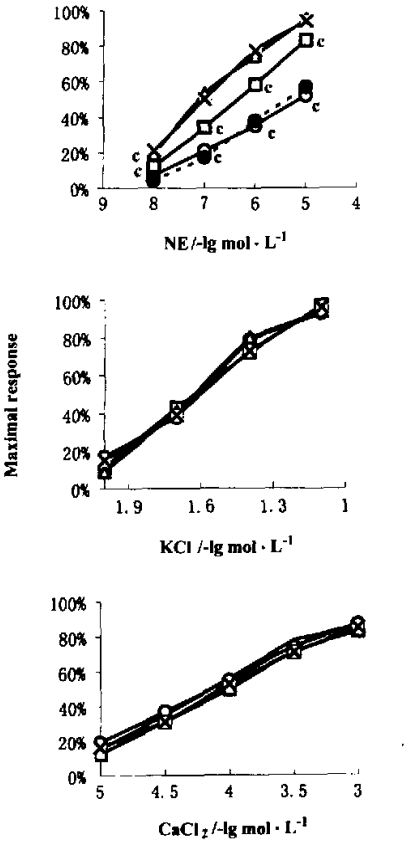

Fig 3. Cumulative concentration-response of rabbit aortic strips on contractions induced by NE, KCl, and $CaCl_2$ in the presense of copper aspirinate 31 nmol/L, $CuSO_4$ 62 nmol/L, and aspirin 0.12 μmol/L. Copper aspirinate (normal strips: ○; endothelial cell denuded strips: ●); $CuSO_4$ (strips □); aspirin (strips △); vehicle (⊗). $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* vehicle.

DISCUSSION

Although Cu^{2+} itself possessed antagonistic effects on contraction induced by NE and aspirin did not, copper aspirinate, with Cu^{2+} and aspirin, was confirmed to have stronger inhibition and it was not only attributed to Cu^{2+} but also to the complex itself. Therefore, copper aspirinate possessed different effects from aspirin and $CuSO_4$ on vascular smooth muscle. However, the drugs did not inhibit contraction induced by KCl and $CaCl_2$. In endothelial cells denuded aortic strips, effects of the drugs were almost the same as in intact endothelium strips.

It is suggested that copper aspirinate relax vascular smooth muscles through suppressing contraction induced by NE. The action was not due to the blockade of the "potential-dependent calcium channels" (PDC or "voltage-dependent Ca^{2+} channels") that could be opened by high concentration of KCl, but due to the blockade of the "receptor-operated calcium channels" (ROC) that could be opened by NE causing decrease of calcium influx and intracellular binding-calcium release[8,9]. This conclusion is in agreement with the previous reports that proved copper aspirinate lowered calcium influx and intracellularly bound calcium release in the platelets[3]. The study also suggests that the action might not be linked to the endothelium.

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阿司匹林铜对离体兔主动脉血管条收缩的影响[1]

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关键词 阿司匹林铜；主动脉；血管平滑肌；血管内皮

目的：观察阿司匹林铜对离体兔胸主动脉血管平滑肌的作用. **方法**：取兔胸主动脉条，观察阿司匹林铜对去甲肾上腺素(NE)、KCl、$CaCl_2$ 诱导收缩作用的影响. **结果**：证实阿司匹林铜和对照物硫酸铜拮抗 NE 诱导的兔胸主动脉条收缩，IC_{50} 分别为 31 nmol/L 和 0.29 μmol/L，而阿司匹林本身没有拮抗作用. 阿司匹林铜对 KCl、$CaCl_2$ 诱导的收缩没有影响. 在去内皮细胞兔胸主动脉条上，观察到相同的作用. **结论**：阿司匹林铜具有较强的拮抗 NE 诱导离体兔胸主动脉条收缩的作用，但不能拮抗 KCl、$CaCl_2$ 诱导的收缩，提示阿司匹林铜通过阻断受体调控钙通道，舒张血管平滑肌.

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