Effect of esophageal cancer- and stomach cancer-preventing vinegar on N-nitrosopropionline formation in the human body

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INTRODUCTION
N-nitroso-compound (NNC) exposure can induce formation of many kinds of tumors in the human body, and as such represents a potential environmental cause of some human cancers. Many of the precursors of NNC are abundant in the modern environment, such as nitrate, nitrite and amino acid compounds, all of which can synthesize corresponding NNCs, and the occurrence of stomach cancer has been associated with the ingestion of these precursors. Indeed, the gastric juice of patients with stomach cancer have been shown to have higher concentrations of both nitrates and compounds that can form nitroso compounds, compared to healthy individuals. So the NNCs in the human body may have important theoretical and practical significance in preventing cancers.

Vitamin C is well known for its efficacy in blocking NNC formation in the human body. Therefore, in this study, we tested whether esophageal cancer- and stomach cancer-preventing vinegar (OSCPV, China-produced patent vinegar [Patent No. ZL90102404], which is rich in vitamins) exerts a protective effect by blocking the synthesis of NPRO in the human body.

MATERIALS AND METHODS

Materials
Sodium nitrate AR was purchased from Xi’an Chemical Reagent Factory (Batch No. 83091), L-Pro chromatography depurant was purchased from Shanghai Biochemical Research Institute of the Chinese Academy of Sciences (Batch No. 8507169). NPRO and N-nitrosopipelic acid (NPIC) (experimental quality) were purchased from IARC. Nitroso-methylurea, and OSCPV were also obtained.

Methods
All the subjects underwent gastroscopy. During the 2 d prior to the start of the experiment and during the experimental period, the subjects’ food intake was restricted to rice and steamed buns. On the day when a urine sample was taken, the subjects were given a cooked wheat pastry for breakfast. The dietary rules strictly followed the requirements of the experiment. During the experiment, the subjects did not eat meat products or any other food that may contain NPRO, and they took no cigarettes, tea, beer, fruits, or medicines; only water was allowed for drinking.

The experiment course encompassed the following three stages. Stage A: On day 3 of the experiment, we tested the output of NPRO in the 24 h urine sample, which represented the background level. Stage B: At 7:00 a.m., the subjects drank 10 mL of water containing 300 mg NaNO2 and another 10 mL of water containing 500 mg L-Pro as well as 15 mL of OSCPV, after which the counting time for urine collection was initiated. Twenty minutes later, the subjects ate breakfast. Stage C: At 48 h after stage B (this timing was used to ensure that the subjects had been able to completely discharge the NPRO gained in stage B), urine was collected. All urine samples were collected into individual polythene pails containing 10 mL of a solution of 3.6 n sulfosol in 20% ammonia sulfonate (AS solution). Each sample was tested respectively to measure output of NPRO corresponding to each stage of the experiment. To determine whether the urine collection was complete, we also tested the 24 h output of creatinine.

We used the methods of Ohshima et al.21 for extracting and testing NPRO in the urine. The main procedure is as follows: 25 mL urine is added to 400 mg NPIC (the internal standard) and 2 mL AS is added, after which the sample is extracted a total of 3 times using mixing with 30 mL, 20 mL and 20 mL ethyl acetate respectively. The final extract is dehydrated with anhydrous sulfate and placed into a rotary evaporator (inside temperature of 60 °C). After evaporation,
the solute is mixed with 2 mL ether and dissolved in diazomethane methyl methyl ester for 5 min. The methods of preparing diazomethane methane and dissolving with methyl ester involved putting about 1 g nitroso urea (synthesized in-house) into a 250 mL flask and then adding 60 mL ether, 20 mL methanol and 10 mL 60% KOH solution, and shaking the flask. In order to dissolve with methyl ester, the ether solution (which is filled with diazomethane methane) was placed into a 2-mouth flask. The diazomethane methane was combined with mitogen into the solution of ether containing dissolved NPRO and NPIC. The methyl ester dissolution was terminated when the solution of ether turned yellow. The procedure was conducted in a hood. Next, the solution was condensed to 10 mL with nitrogen and 0.5 mL of GC-TEA was added to test the content of methyl ester of NPRO. The content of methyl ester of standard NPRO, which was taken through the same treatment, was used as a quantitative reference. The chromatographic column used in the next step was a 2 mm × 3 mm stainless steel column with filling material of 60-80 order chromosorb WAW-DMCS and 10% Carbowax 20 M spread. The carrier was argon and the speed of the flow was 20 mL/min. The temperature of the column was 185 ℃, of the evaporator was 257 ℃, of the TEA pyrolysis was 500 ℃, and of the interface was 200 ℃. Under these conditions, the NPRO and NPIC were able to be well dissociated, with no other interfering peak produced. The lowest detection limit was < 0.5 ppb, and the recovery rate was 85%.

RESULTS
In the experiment, we took the output of NPRO—the level of endogenous nitrosification—as an index. After the 76 patients took 55 mg L-Pro, the output of NPRO in the 24 h urine sample rose from 26.18 nmol to 66.84 nmol, for an increase of 2.5 times. In contrast, after taking OSCPV and L-Pro, the NPRO in urine was reduced, to a concentration lower than the background level, which shows that OSCPV can block the synthesis of NPRO in the human body completely and suggests OSCPV as an ideal blockage agent for prevention of cancer (Tables 1, 2).

CONCLUSION
OSCPV is an effective agent that can block the synthesis of NNC. It is effective in blocking the synthesis of NPRO, which can induce cancer in the human body, making this agent capable of preventing human cancers in an effective manner.

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