Cytotoxic and Antigenotoxic Activities of Phosvitin From Egg Yolk

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Sunhee Hoon, Postdoc;
Dong U. Ahn, Professor,
Department of Animal Science

Abstract

Egg yolk phosvitin is one of the most phosphorylated proteins in nature, and thus has a strong metal-binding ability. The objective of this study was to evaluate the cytotoxic and antigenotoxic activities of phosvitin *in vitro*. Using the MTT assay, the cytotoxicity of phosvitin was evaluated in human cancer cell lines of various tissue origins, including the cervix (HeLa), breast (MCF-7), stomach (AGS), lung (A549 and SK-MES-1), liver (HepG2), and larynx (Hep-2). The growth of all cancer cell lines was inhibited in a dose-dependent manner by phosvitin. Among the cancer cell lines tested, MCF-7 and SK-MES-1 were the least sensitive and HeLa, AGS, and HepG2 were the most sensitive to phosvitin. The IC_{50} values of phosvitin were 5.38, 11.57, 4.78, 6.98, 11.82, 3.93, and 9.97 mg/mL for HeLa, MCF-7, AGS, A549, SK-MES-1, HepG2, and Hep-2, respectively. The protective effects of phosvitin against DNA damage in human leukocytes indicated that phosvitin showed protective effects against the oxidative stress-induced DNA damages in human leukocytes. These results suggested that phosvitin has a high potential to be used as an anticancer agent for humans.

Introduction

DNA damage and oxidative stress play important roles in various illnesses and pathological conditions, including carcinogenesis and aging, in human. Cancer is the second leading cause of mortality worldwide, and scientific society and commercial sectors show very strong interests in discovering new anticancer agents from natural sources. In recent years, some scientific evidences indicated that certain bioactive proteins and peptides could have several beneficial effects on human health.

Phosvitin is a phosphoglycoprotein present in egg yolk and represents about 7% of yolk proteins. The molecular weight of phosvitin ranges from 35 to 40 kDa and is composed of 217 amino acids residues. More than 50% of the amino acid residues in phosvitin are serine and 90% of which are phosphorylated. This specific structure makes phosvitin a very strong metal (iron, calcium, magnesium, etc.) chelator.

Phosvitin is reported to have metal-chelating, antioxidant, emulsifying, and antimicrobial activities. It was found that phosvitin has a high iron-binding capacity, exhibited a strong antibacterial activity against a broad spectrum of bacteria, and has anti-tyrosinase and melanin biosynthesis activities. However, there has been little study on the anticancer activity of phosvitin from egg yolk, so far. The objective of this study was to investigate the antigenotoxic activity of phosvitin against normal human peripheral blood mononuclear cells (PBMCs) and cytotoxic activity of phosvitin against human cancer cell lines.

Materials and Methods

Phosvitin was prepared from chicken egg yolk. Human normal and cancer cell lines including HeLa and HepG2, MCF-7 (human breast adenocarcinoma), AGS, and A549 cell lines were used to evaluate the cytotoxic and antigenotoxic activities of phosvitin *in vitro*. *T*he cytotoxic effect of phosvtin was tested *in vitro* using the 3-(4,5-dimethylthizol-2-yl)-2,5-diphenylatetetrazolium bromide (MTT) assay. The concentration required for a 50% inhibition of cell viability (IC_{50}) was determined graphically. DNA Damage was determined by Alkaline Comet Assay.

Statistical Analysis: All cytotoxicity of phosvitin results were presented as mean ± standard error (SE), and statistical analysis was performed using the SPSS package for Windows. The IC_{50} value was calculated by Soft Max Pro version 6.3. The mean values were compared using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests ($p < 0.05$).

Results and Discussion

In vitro Cytotoxicity of Phosvitin: Different cells had a different sensitivity to the growth inhibition effect of phosvitin (Fig. 1). The results expressed as IC_{50} represent the effective concentration of phosvitin required for 50% cytotoxic activity under the experimental conditions (Table 1). In the present study, the cytotoxic effect of phosvitin on normal cells (MRC-5) was significantly different from that on human cancer cells. At all concentrations of phosvitin, there was no more than 20% of cytotoxic activity against MRC-5 cells.

Phosvitin showed a strong cytotoxic effect and the effect increased as the level of phosvitin increased. Phosvitin at 40 mg/mL displayed 94.63% cytotoxicity to HeLa cells (Fig. 1A) and the IC_{50} value of phosvitin to HeLa cells was 5.38 mg/mL (Table 1). Phosvitin showed a weak cytotoxic activity to MCF-7 cells at low concentration (10 mg/mL), but it suppressed the growth of MCF-7 cells in a dose-dependent manner (Fig. 1B). Phosvitin inhibited the growth of AGS cells by 40.84, 55.30, 62.03, 88.23, and 93.67% at concentrations at 2.5, 5, 10, 20, and 40 mg/mL, respectively. The IC_{50} value of phosvitin to AGS cells was

4.78 mg/mL (Fig. 1C) and that of A549 cells was 6.98 mg/mL (Fig. 1D). The SK-MES-1 cells were the least sensitive to phosvitin among the cancer cell lines tested. The IC_{50} value of phosvitin to SK-MES-1 cells was 11.825 mg/mL (Table 1), but increasing the concentration of phosvitin from 2.5 to 5 mg/mL resulted in no growth inhibition, as assessed by the MTT assay (Fig. 1E). Phosvitin showed IC_{50} value of 3.93 mg/mL to HepG2 cells and > 70% cytotoxicity was observed at 10 mg/mL level. The effect of phosvitin to HepG2 cells was more pronounced than other cancer cells tested ($p < 0.05$). The IC_{50} value of phosvitin to Hep-2 cells was 9.97 mg/mL (Fig. 1G), which is more resistant to phosvitin than HeLa, AGS, A549, and HepG2 cells. No significant decrease in cell viability at 5-500 μg/mL level was observed.

Inhibitory Effects on H_2O_2-Induced DNA Damage: The comet assay has been used as one of the standard methods for assessing direct and indirect damages to DNA. Reactive oxygen species such as hydrogen peroxide, superoxide anion, singlet oxygen, and hydroxyl radical are well known stress agents for DNA damages. When oxidative stress continuously occurs, it causes DNA damage in cells and raises the risk of diseases such as aging or cancer.

The genetoxic effects of H_2O_2 and the protective ability of phosvitin was assessed in normal human leukocytes using the comet assay (Fig. 2). The protective effect of phosvitin against DNA damages increased in a dose-dependent manner over the range of 50-500 μg/mL. When phosvitin was added at the concentrations of 50-500 μg/mL, the tail portion (%) of DNA (damaged portion) was 23.62%-15.48% while that of the positive control was 35.04%, which was significant reduction from the positive control. At a higher level of phosvitin (500 μg/mL), the damaged DNA was reduced by 55.8%. The concentrations of phosvitin that produced 50% reduction in DNA damage was 424.59 μg/mL.

The results of this study indicated that phosvitin has antigenotoxicity and cytotoxic activity against several human cancer cell lines and has some potential to be used as an anticancer agent for humans.

Table 1. The phosvitin concentrations that inhibit 50% of the cell growth (IC_{50}) on various human cancer cell lines

Cancer cells	IC_{50} (mg/mL)[1]						
	HeLa	MCF-7	AGS	A549	SK-MES-1	HepG2	Hep-2
Phosvitin	5.38±2.25	11.57±1.19	4.78±1.71	6.98±1.82	11.82±1.21	3.93±0.96	9.97±0.18

Values are mean ± standard error ($n = 3$).

[1]IC_{50} value means the effective concentration of phosvitin required for 50% cytotoxic activity under the experimental condition.

Abbreviations: HeLa, human cervix adenocarcinoma; MCF-7, human breast adenocarcinoma; AGS, human stomach adenocarcinoma; A549, human lung adenocarcinoma; SK-MES-1, human lung carcinoma; HepG2, human liver hepatoblastoma; Hep-2, human larynx carcinoma.

Figure 1. The cytotoxic effects[1] of phosvitin on various human cancer cell lines by MTT assay. (A) HeLa cell, (B) MCF-7 cell, (C) AGS cell, (D) A549 cell, (E) SK-MES-1 cell, (F) HepG2 cell, and (G) Hep2 cell. Values are means with standard errors. Values not sharing the same letter are significantly different ($p < 0.05$) (n = 3).
[1]Cytotoxic effect (%) = 1-(absorbance in the samples/absorbance in the control) × 100.

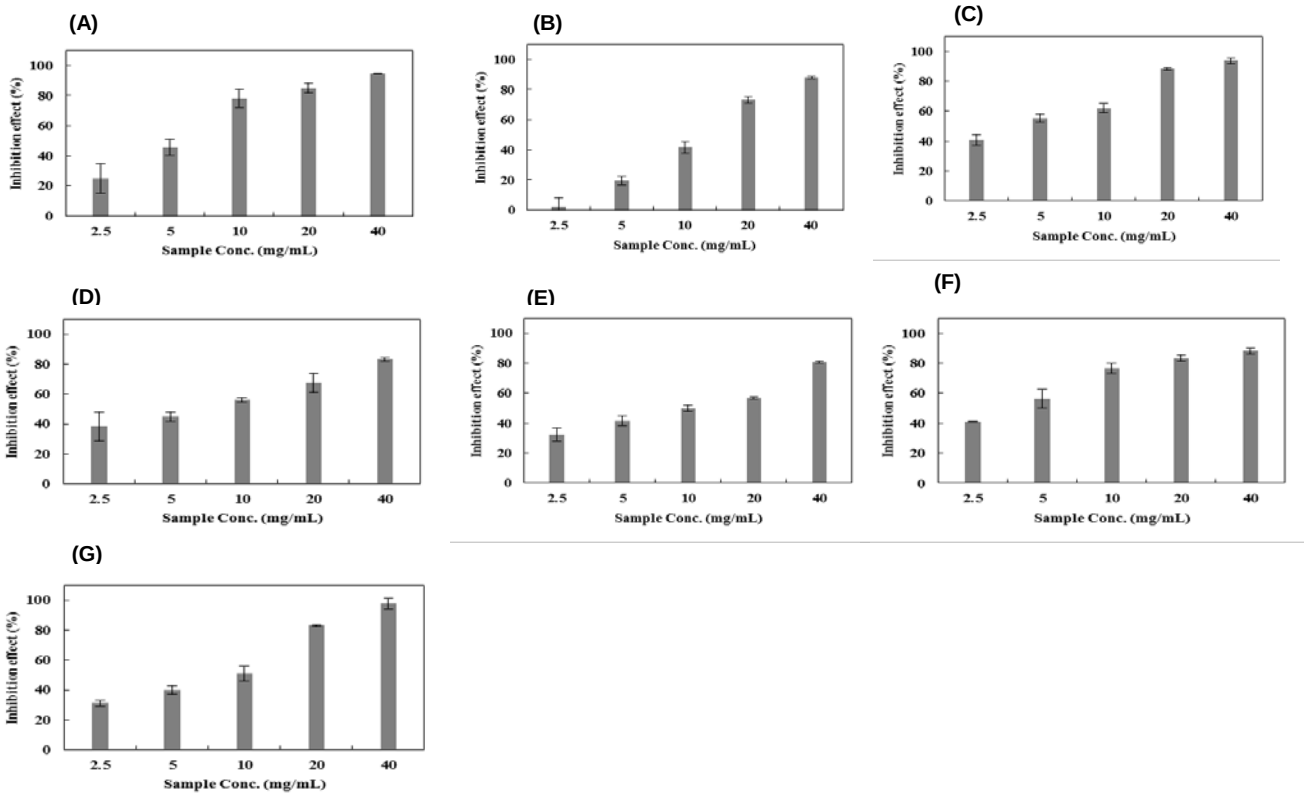

Figure 2. Effect of phosvitin on 200 μM H_2O_2-induced DNA damage by comet assay. Values are means with standard errors. Values not sharing the same letter are significantly different ($P < 0.05$) (n=3).
Abbreviations: NC, negative control (without oxidative stimulus); PC, 200 μM H_2O_2-treated positive control.