Abstract. Phytate, myto-inositol 1,2,3,4,5,6 hexaphosphate (IP6), is recognized as an anti-nutrition phytochemical for decades. Recently, numerous studies have indicated that IP6 and its hydrolysates could suppress colon oncogenesis. However, very little is known concerning the mechanism of IP6 hydrolysates in regulating colon oncogenesis. The aim of the present study was to identify the underlying relationship between IP6 hydrolysates and colon cancer. Three types of human colorectal cancer cells were utilized in the present study. The proliferation inhibition and migration assays were employed to reveal that IP6 hydrolysates inhibited the proliferation of SW620 cells. Real-time PCR, cell-based ELISA and the AKT inhibitor assay were utilized to reveal that 20 and 30% degree of hydrolysis hydrolysates of IP6 inhibited SW620 cell growth by inhibiting the activation of AKT protein. The docking simulation study revealed that IP4 and IP5 could inhibit the activation of AKT by binding to PIP3 receptor. Collectively, our results indicated that the IP6 hydrolysates inhibit SW620 cell proliferation; IP4 and IP5, the probable primary constituents of the 20-30% degree of hydrolysis hydrolysates of IP6, inhibited the proliferation of SW620 cells by competitively inhibiting the AKT protein.

Introduction

According to 2012 Global Cancer Statistics, colorectal cancer has become the second leading cause of cancer-related deaths in developed countries (1). Colorectal cancer also poses a significant threat in developing countries. In China, colorectal cancer was the fifth leading cause of cancer-related deaths in 2015 (2). Compared with other forms of cancer, colorectal oncogenesis is highly correlated with diet (3). Various experts believe that phytate could be effective in preventing colon oncogenesis (4-7).

Phytate, myto-inositol 1,2,3,4,5,6 hexaphosphate (IP6), is ubiquitously distributed worldwide and exists in many types of plant-derived foods (8-10). When IP6 is ingested, it partially degraded by phytase into hydrolysates. Therefore, it is likely that the epithelial cells of the colon are exposed to the mixture of IP6 hydrolysates, but not IP6. Ishizuka et al revealed that the partially degraded IP6 products were responsible for the suppression of colon oncogenesis. It had been demonstrated that IP6 and IP6 hydrolysates were able to suppress HCT116 colon carcinoma cells (11). Based on these findings, we attempted to characterize the underlying antitumour mechanisms of IP6 hydrolysates.

According to our previous study, IP6 exerted inhibitory effects on HT-29 cells via the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signalling axis (10). The PI3K/AKT signalling axis is a major survival pathway. Abnormal activation of the PI3K/AKT pathway is frequently involved in the development and progression of various tumours, including colon cancer (12). In this pathway, AKT is a serine/threonine kinase and plays an essential role. It is activated by the 3'-phosphorylated phosphoinositides 3,4,5-trisphosphate (PIP3) protein and affects the activity of downstream factors, including mTOR, BAD and GSK3β (13-15). AKT contains the pleckstrin homology (PH) domain that has a high affinity for PIP3. In addition, IP6 hydrolysates also possess a similar PH domain as the PIP3 protein. Thus, based on the presumption of a similar structure and evidence from our previous study, we hypothesized that IP6 hydrolysates suppressed the proliferation of colon carcinoma cells through the PI3K/AKT pathway.

Materials and methods

Reagents. Inositol hexaphosphate (IP6) was purchased from Muster Biological Science Technology Company (Sichuan, China). Ascorbic acid, ammonium molybdate, antimony potassium tartrate, monopotassium phosphate, potassium peroxydisulfate and sulfuric acid were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo.
Molecular Technologies, Inc. (Kumamoto, Japan). RT-PCR was performed by a Two-Step RT-PCR QuantScript RT kit (KR103) and a Real-Master/SYBR-Green kit (FR202) which were both purchased from the Tiangen Biotech (Beijing) Co., Ltd. (Beijing, China). The primers were designed using Primer Premier 5.0 (PREMIER Biosoft, Inc., Palo Alto, CA, USA) and Oligo 6.0 (Molecular Biology Insights, Inc., Colorado Springs, CO, USA) software and were synthesized by Shanghai Biological Engineering Company (Shanghai, China). The cell-based ELISA kits were supplied by ImmunoWay Biotechnology Company (Plano, TX, USA).

Hydrolysis curve. The hydrolysis curve assays were performed using the total phosphorus ammonium molybdate spectrophotometric method. Different concentrations of IP6 (0.25, 1, 4, 16, 64, 250, 1,000, 4,000, 16,000 and 64,000 µg/ml) were suspended in water, and potassium peroxydisulfate (50 g/l) was added for digestion. After digestion, molybdate and ascorbic acid were added to the solution and allowed to develop for 15 min. The absorbance of the solution at 690 nm was determined using a microplate reader. Each experiment was repeated three times. The optical density (OD) was recorded to draw the hydrolysis curve.

Determination of the IP6 hydrolysis rate. Forty milligrams IP6 (final concentration, 200 µg/ml) and 6 µg of phytase were suspended in 200 ml of 50 mM sodium acetate (pH 5.5) and incubated at 37°C for 1-8 h. Two types of phytase (EcAppA from Escherichia coli and phytase from wheat) were assessed in the present study. The pretreated hydrolysis solutions were then evaluated using the total phosphorus ammonium molybdate spectrophotometric method aforementioned without digestion. The degree of hydrolysis (DH) of IP6 was calculated as follows:

\[
DH = \frac{C_1}{C_2}
\]

\[C_1\text{---Hydrolysis phosphorus, m/l}
\]

\[C_2\text{---Total phosphorus, m/l}
\]

IP6 and its hydrolysates ranging from 10 to 90% DH were concentrated via vacuum freeze dehydration and stored in a cold dark place.

Cell culture. SW620, HCT116 and HT29 cells obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were cultured in a humidified incubator. Decreased serum media [25 ml/l phosphate-buffered saline (PBS)] was used for all experiments.

Cell proliferation assay. The cell proliferation assay was performed in order to investigate the time- and concentration-dependent effects of IP6 on the growth of SW620, HCT116 and HT29 cells. Logarithmic phase cells (1x10⁴ cells/well) were seeded onto 96-well plates at 5x10⁴ cells/cm². When appropriate, the cells were serum-starved for 4 h and stimulated with IP6 hydrolysates for 10 min. After stimulation, the cells were fixed with 4% formaldehyde in PBS for 20 min to room temperature and washed three times with washing buffer. The cells were then incubated in quench buffer for 20 min, washed three times with washing buffer, blocked with 10% fetal calf serum buffer for 1 h and incubated overnight with various dilutions of a primary antibody at 4°C. The following day, the cells were washed three times with washing buffer for 5 min and incubated with a secondary antibody (peroxidase-conjugated goat anti-rabbit antibody; dilution 1:100) in PBS and Triton with 5% BSA for 1 h at room temperature and then washed three times with washing buffer for 5 min and twice with PBS. Subsequently, the cells were incubated with 50 µl of substrate development solution for 15 min at room temperature in the dark. The reaction was stopped with 50 µl of stop solution. The absorbance was assessed, and the SD values were determined using a microplate reader.

ACT-inhibited assay. Similar to the cell proliferation assay, logarithmic phase cells (1x10⁴ cells/well) were seeded onto
96-well tissue culture plates in 5% CO₂ at 37°C. After 12 h, the medium was replaced with fresh medium containing 0.1 µmol/l of MK2206 (IC₅₀ concentration in SW620 cells) and 10 µg/ml of IP6, and the cells were incubated in CO₂ at 37°C for 48 h. The CCK-8 reagent was added to the cells, and the plates were incubated at 37°C for 2 h. After incubation, the absorbance at 490 nm was determined using a microplate reader. A decrease in the absorbance was considered to reflect a loss of cell viability. Each experiment was repeated three times.

Molecular docking simulation study. A docking study was performed to examine the qualified binding positions of IP6 hydrolysates against AKT. The crystallographic structure of AKT with its ligand was obtained from the RCSB Protein Data Bank (PDB ID, 4EKL). The main protein structure was obtained by removing the 4EKL ligand (2S)-2-(4-chlorophenyl)-1-{4-[(5R,7R)-7-hydroxy-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl]piperazin-1-yl}-3-(propan-2-ylamino)propan-1-one and the water molecules using AutoDock 4.2. After removing the heteroatoms and adding the hydrogen atoms, the protein was suitable for docking simulation with respect to obtaining the best ligand binding results. Thirteen two-dimensional structures of the IP6 hydrolysate constituents were drawn using ChemDraw 8.0. All two-dimensional IP6 hydrolysates were transformed into three-dimensional structures using Avogadro 1.0.3 and converted into PDB files with Open Babel 2.3.2. AutoDock 4.2, an open-source program, was used for the docking simulation. Grid boxes 126 x 126 x 126 points in size with spacing of 0.375 Å between the points were generated to cover almost the entire favourable protein binding site. The X, Y and Z centres were 22.15, 2.53 and 15.74, respectively. The binding aspects of the AKT residues and their corresponding binding affinity scores were regarded as the best molecular interactions. The results were analysed using UCSF Chimera and LigPlot (v.1.4.5). The two-dimensional images of the IP6 hydrolysate-AKT interactions were calculated using LigPlot v.1.4.5 (European Bioinformatics Institute, London, England). All docking simulations were performed using an Intel Core TM i5-2520 M CPU @ 2.50 GHz with Windows 8.1 and a 64-bit operating system.

Statistical analysis. All reactions were performed for three times and each independent experiment was carried out in duplicate or triplicate according to the manufacturer’s instructions. A completely randomized design (CRD) was used for the statistical analysis of the physical and chemical data. All data were subjected to analysis of variance (ANOVA), and

Table I. Real-time-PCR primer sequences and product sizes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>F 5'-CCTGGCACACCAGCACAAAT-3’</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>R 5’-GGGCAGGACTGTCATAC-3’</td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td>F 5’-CTTTTCCCCACAAATCCTCA-3’</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>R 5’-CAGTGTGCCCCATCCCCTCAT-3’</td>
<td></td>
</tr>
<tr>
<td>AKT</td>
<td>F 5’-AGCGGAAGGAGGATGAAGAT-3’</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>R 5’-GGAAAAACGGAGACTTAGG-3’</td>
<td></td>
</tr>
<tr>
<td>BAD</td>
<td>F 5’-GGTTTCTGAGGGAGACTGA-3’</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>R 5’-CTCTGGGCTGAGGACAAAG-3’</td>
<td></td>
</tr>
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</table>

F, forward; R, reverse; PI3K, phosphatidylinositol 3-kinase.

Figure 1. Hydrolysis curve and hydrolysis efficiency of phytases from wheat or Escherichia coli. (A and B) The hydrolysis curve was drawn by the content of total phosphorus. Different concentrations of IP6 were completely digested by potassium peroxydisulfate. (C) The hydrolysis efficiency curve of phytases from wheat or Escherichia coli. The free phosphorusime point was assessed in the solution by the phosphorus ammonium molybdate spectrophotometric method each hour for 8 h. The results are expressed as the mean ± SD of at least three independent experiments; *P<0.05, **P<0.01. IP6, inositol hexaphosphate; SD, standard deviation.
Results

Comparison of the hydrolysis efficiencies of different phytases. To determine the proper IP6 hydrolysis condition,
a series of IP6 concentrations ranging from 0.25 µg/ml to 64 mg/ml were tested in the present study. All the free phosphoric acid was released after IP6 was fully hydrolyzed by potassium peroxydisulfate. The free phosphoric acid was detected with a microplate reader. According to Fig. 1A, when the IP6 concentration was increased to 16 mg/ml, the total reaction system reached its limit and the OD 690 value markedly decreased. According to Fig. 1B, the free phosphoric acid concentration released from IP6 at concentrations ranging from 0.25 to 250 µg/ml exhibited suitable reaction efficiencies (y=0.0078x + 0.0892, R²=0.9980). To analyze the effects of different phytases on IP6 hydrolysis, we assessed the two phytases, one extracted from wheat and one from Escherichia coli. Free phosphorus of IP6 was determined per hour for 8 h. Compared with 37°C control group, the two phytases showed significantly enhanced hydrolysis effects. However, the hydrolysis efficiency of the wheat phytase was low and unstable (y=0.0805x + 0.0026, R²=0.9293), shown in Fig. 1C. In contrast, the hydrolysis efficiency of EcAppA was high and stable (y=0.1507x-0.0027, R²=0.9921). These results indicated that the EcAppA phytase was more suitable for preparing IP6 hydrolysates. Using the hydrolysis curve and the proper phytase, we were able to assess the dynamic hydrolysis condition and calculate the IP6 hydrolysis rate. We concentrated 10-90% DH IP6 hydrolysates via vacuum freeze dehydration.

**IP6 hydrolysates inhibits of SW620, HCT116 and HT29 cell growth.** After preparing the different DH hydrolysates, we used the cell proliferation assay to identify the inhibitory effects of three main colorectal cancer cell lines with various concentrations. We were surprised to find that the SW620 cells were very sensitive to 20, 30 and 90% DH hydrolysates. DH hydrolysates (20, 30 and 90%) in 100 and 10 µg/ml concentration groups exhibited better tumour-suppressor activity compared with the 1 µg/ml concentration group (Fig. 2A). HT29 cells were not sensitive to the majority of the hydrolysates of each concentration tested. Differences were observed in two groups which were 90% DH hydrolysates in the 1 and 100 µg/ml concentration groups (Fig. 2B). HCT116 cells were not sensitive to the majority of the hydrolysates of each concentration tested. IP6 hydrolysates do not alter the mRNA expression of PI3K, Akt, and BAD in the SW620 cells. To explore the effects of IP6 hydrolysates on SW620 cells, we measured the expression of PI3K, AKT and BAD mRNA using real-time PCR. Compared with the control group, the expression of PI3K, AKT and BAD mRNA in the SW620 cells did not exhibit any differences. Results (Fig. 4) indicated that IP6 hydrolysates did not affect the expression of PI3K, AKT and BAD mRNA.
**Table II. Molecular interactions between the IP4 isomers, IP5 and the AKT protein activator PIP3.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding energy</th>
<th>No. of H-bonds</th>
<th>H-bond interacting residues</th>
<th>Van der Waals interacting residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3,4 IP4</td>
<td>6.85</td>
<td>4</td>
<td>Lys284:HZ1 Lys284:HZ2</td>
<td>Try175 Try176</td>
</tr>
<tr>
<td>1,2,3,5 IP5</td>
<td>6.56</td>
<td>5</td>
<td>Lys284:HZ1 Lys284:HZ2</td>
<td>Try175 Try176</td>
</tr>
<tr>
<td>1,2,4,5 IP6</td>
<td>6.02</td>
<td>3</td>
<td>Tyr175:HZ1 Lys214:HZ2</td>
<td>Ala212 Try176 Glu228</td>
</tr>
<tr>
<td>IP5</td>
<td>5.88</td>
<td>4</td>
<td>Lys214:HZ2 Lys214:HZ3</td>
<td>Ala212 Ser478 Ala476</td>
</tr>
<tr>
<td>PIP3</td>
<td>10</td>
<td>1</td>
<td>Lys214:HZ2</td>
<td>Arg174 Ser478 Ala476 Gln203</td>
</tr>
</tbody>
</table>

IP6, inositol hexaphosphate.

Figure 6. IP6 hydrolysates plus with MK2206 inhibit the cell growth of SW620 for 48 h. Cells were treated with IP6 hydrolysates (10 µg/ml) and MK2206 (0.1 µM) concurrently. The cell viability was assessed using a cell proliferation assay. The results are expressed as the means ± SD from three independent experiments; *P<0.05, **P<0.01. IP6, inositol hexaphosphate.

**Discussion**

In the present study, IP6 hydrolysates exerted inhibitory effects on SW620 cells, and certain hydrolysates inhibited AKT activation as AKT protein inhibitors. We first examined the effects of 10-90% DH hydrolysates on three colorectal tumour cells (HT29, HCT116 and SW620) by cell proliferation assays. DH hydrolysates (20, 30 and 90%) significantly inhibited the proliferation of SW620 cells. The data from the wound healing assay confirmed the results of the cell proliferation assay in SW620 cells. Thus, we cautiously speculated that metastatic SW620 tumour cells may be susceptible to IP6 hydrolysates.

Next, we employed molecular docking studies to obtain the predictions of the protein-ligand interaction geometries of the IP6 hydrolysates and the AKT protein. Thirteen IP6 hydrolysates were used as control ligands for validation in AutoDock 4.2. The docking scores of the IP6 hydrolysates with the interacting residues, the number of hydrogen bonds formed between the interacting residues and the residues exhibiting van der Waals interacting force are shown in Table II. The activity of the thirteen IP6 hydrolysates against AKT was correlated with the binding energy, and the number of hydrogen bonds formed at the active site. The thirteen hydrolysates were arranged to dock with the AKT protein; however, only IP5 and 3 of the IP4 isomers could bind to the AKT protein (Fig. 7). Inositol and the IP1, IP2 and IP3 isomers could not bind to the AKT protein.

**IP6 hydrolysates affect the expression of Akt and pAkt in SW620 cells.** Next, using cell-based ELISA, we investigated whether the IP6 hydrolysate treatments affected the expression of proteins related to the PI3K/AKT pathway. The results (Fig. 5) indicated that after hydrolysate treatment (20, 30 and 90%), the protein expression of total Akt did not change. In contrast, the protein expression of pAkt changed significantly in the 20 and 30% DH groups. Based on these results, IP6 hydrolysate treatment inhibited the expression of pAkt in the SW620 cells.

**IP6 hydrolysates inhibit SW620 cell proliferation when the AKT protein is inhibited.** According to our results, certain IP6 hydrolysates inhibited the proliferation of SW620 cells with MK2206. As shown in Fig. 6, after treating SW620 cells with MK2206, 10, 20, 30, 40, 50, 60 and 70% DH hydrolysates did not inhibit cell proliferation. SW620 cells were treated with 80 and 90% DH hydrolysates and IP6 plus MK2206 exhibited a slight decrease in the growth rate of SW620 cells.

**IP4 and IP5 bind to the AKT protein.** Next, we employed molecular docking studies to obtain the predictions of the protein-ligand interaction geometries of the IP6 hydrolysates and the AKT protein. Thirteen IP6 hydrolysates were used as control ligands for validation in AutoDock 4.2. The docking scores of the IP6 hydrolysates with the interacting residues, the number of hydrogen bonds formed between the interacting residues and the residues exhibiting van der Waals interacting force are shown in Table II. The activity of the thirteen IP6 hydrolysates against AKT was correlated with the binding energy, and the number of hydrogen bonds formed at the active site. The thirteen hydrolysates were arranged to dock with the AKT protein; however, only IP5 and 3 of the IP4 isomers could bind to the AKT protein (Fig. 7). Inositol and the IP1, IP2 and IP3 isomers could not bind to the AKT protein.

**Discussion**

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Next, as aforementioned, IP6 hydrolysates possess an analogous domain as PIP3. Thus, we wondered whether IP6 hydrolysates could affect the activation of the AKT protein. A real-time PCR assay was utilized to clarify whether IP6 hydrolysates affected the mRNA expression of AKT. After treatment with IP6 hydrolysates, AKT and its upstream factor, PI3K, and downstream factors, BAD, did not exhibit any differences with the control group in SW620 cells. Since IP6 hydrolysates could not affect the expression of AKT mRNA, we investigated the AKT protein expression using cell-based ELISA. Cell-based ELISA is a new protein detection technique which permits
Figure 7. IP4s, IP5 and PIP3 bind to the active site of AKT. The structural ligands were analysed by AutoDock 4.2 to depict the docked conformation of these compounds with AKT (PDB ID, 4EKL). Hydrogen bonding and hydrophobic interactions for the (A) 1'2'3'4IP4, (B) 1'2'3'5IP4 and (C) 1'2'4'5IP4.
direct detection of cell proteins without extraction. This technique has significant advantages in phosphoprotein analysis. Compared with traditional western blot assay, cell-based ELISA detects the expression of phosphoproteins quickly and decreases the unnecessary phosphoproteins lost from extraction and phosphatise degradation. Thus, we chose cell-based ELISA for pAKT and total protein detection. In Fig. 5, the total AKT protein did not exhibit any difference in each group, but the 20 and 30% DH hydrolysates inhibited the activation of the AKT protein by inhibiting the expression of the pAKT protein. The results indicated that certain IP6 hydrolysates at 20 and 30% DH may have a close relationship with the activation of AKT protein.

The result of cell-based ELISA was insufficient to conclude that hydrolysates may induce changes in the AKT protein. Hence, the classical AKT inhibitor MK2206 was utilized in an AKT inhibition assay to study the relationship between hydrolysates and the AKT protein. In the present study, 20 and 30% DH hydrolysates did not suppress the proliferation of SW620 cells when the AKT protein was inhibited. It was proposed that 20-30% DH hydrolysates inhibited tumour proliferation which was closely related to the AKT protein. In contrast, 90% DH hydrolysates continually inhibited cell growth. This implied that 90% DH hydrolysates inhibited SW620 cell growth through a different mechanism.

Lastly, the molecular docking simulation study was performed to predict the binding energies and the specific binding site of hydrolysates on AKT and to identify the interacting residues using AutoDock software. According to our data, IP5 and the isomers of IP4 exhibited a similar binding pattern as PIP3. IP1, IP2, IP3 and IP6 could not bind to the AKT protein. According to a study from Yuying, when

Figure 7. Continued. IP4s, IP5 and PIP3 bind to the active site of AKT. (A-E) The structural ligands were analysed by AutoDock 4.2 to depict the docked conformation of these compounds with AKT (PDB ID, 4EKL). Hydrogen bonding and hydrophobic interactions for the (D) IP5 and (E) PIP3.
the DH reached 16.67 and 33.36%, the main contents of the hydrolysates of IP6 were IP5 and IP4 (16). Hence we carefully deduced that the main contents of the 20-30% DH IP6 hydrolysates were IP4 isomers and IP5. The results of the docking simulation study confirmed that IP4 isomers and IP5 could attach to the binding area of AKT and inhibit the activation of the AKT protein. This finding explained why 20 and 30% DH hydrolysates inhibited the activation of the AKT protein and slowed the tumour growth rate. Collectively, with the data of the PCR analysis, the AKT inhibited assay and the molecular docking simulation study we confirmed our hypothesis that IP6 hydrolysates, IP5 and IP4, contain a similar structural domain with PIP3, and may bind to the PIP3 receptor of AKT. These hydrolysates occupied the receptor, but could not exhibit any biological functions. Therefore, we concluded that 20 and 30% DH of IP6 hydrolysates inhibited the activation of the AKT protein to suppress the proliferation of SW620 cells, and these hydrolysates inhibited AKT protein activation mainly through competitive inhibition to the PIP3 receptor.

Although our results revealed that IP6 hydrolysates act as an AKT protein inhibitor, there were a few limitations. Separation of the unique hydrolysate was the first issue. With the help of our laboratory colleagues, over a period of two years, we attempted to separate the unique hydrolysate and its isomers by several different methods; we utilized classic chromatography, colorimetric, HPLC, paper chromatography, thin layer chromatography, NMR, dialysis membrane and other methods (17-24). Despite our best efforts, we were unsuccessful. However, we are not discouraged. We are going to deal with this problem in the future and we appreciate the researchers who motivated us. Although we were unable to separate the unique hydrolysate, the mixture of IP6 hydrolysates was still worth being investigated. When IP6 was obtained from the ingestion of food, it was hydrolysed into a group of hydrolysates by intestinal bacteria phytase. These hydrolysate mixtures may just be the exposure substances for colon epithelial cells. Therefore, studying the IP6 hydrolysate mixtures is biologically relevant. DH hydrolysates (90%) was the second issue. This is a very special mixture of the present study. These hydrolysates in 90% DH inhibited the proliferation of SW620 cells, but did not inhibit AKT activation (shown in Fig. 2). According to the results reported by Dinicola et al, inositol efficiently slowed the rate of differentiation and the dissemination of breast cancer cells (25-30). According to IP6 hydrolysis progression, the more free phosphates of IP6 hydrolysed, the less binding phosphates remained on the inositol molecular skeleton. According to the study of Fu et al, when the DH reached to 83 and 100%, the main contents of the hydrolysates of IP6 were IP1 and inositol. We speculate that the main content of the 90% DH hydrolysates was inositol. DH hydrolysed (90%) worked as an antitumour agent in a different manner compared with the 20 and 30% DH hydrolysates which may be investigated in our future study.

The present study, was greatly motivated by previous studies which investigated the relationship between IP6 and its hydrolysates in colon cancer. However, there were some differences that should be mentioned. According to Ishizuka et al, IP6 and its hydrolysates were able to suppress HCT116 colon cells. They also indicated that partially degraded IP6 products inhibited cell proliferation via different mechanisms from those of intact IP6 (11). Suzuki et al believed that IP6 hydrolysates induced F-actin ring formation, the key factor in the phytate-mediated anticancer function in HT29 cells (23). Their results were similar to our findings; we all contended that partly hydrolysed IP6 inhibited the colon cell growth. However, there were still some differences that remained. Their studies suggested that IP6 and IP6 hydrolysates inhibited HT29 and HCT116 cells, but we did not find any differences in these two cell lines. The differences may be derived from the different intervention concentrations. The concentrations in the study of Ishizuka et al and Suzuki and Hara, were 2.1-4.1 g/l and 66-3,000 mg/l, separately (11,23). According to the study of Ishizuka et al, the hydrolysates could not affect the protein phosphorylation when the concentration did not reach 1 mM (300-500 mg/l) in HT29 cells. This concentration was higher than the one we used (1-100 mg/l) in the present study. In vitro, results may markedly change when the concentrations are different. The concentrations were 3-40 times different when comparing the present study to theirs, which explained why the differences in the HCT116 and HT29 cells could not be observed in the present study.

In addition to the lower hydrolysates and IP6 concentrations used in the present study, we also investigated the whole DH hydrolysates of IP6 against colon cancer for the first time. The mixture of IP6 hydrolysates is the absorbed form of IP6 in the colon. The intestine epithelial cells may not be exposed to IP6 actually, however, they are commonly exposed to the IP6 hydrolysed mixture. We confirmed that the anticancer properties of IP6 are primarily due to its hydrolysates which competitively inhibited the activation of the AKT protein. The present study first used the molecular docking simulation method to indicate that IP4 and IP5 may be effective components of IP6 hydrolysates. In the past few years, IP3 has been recognized as the main component of all IP6 hydrolysates. However, according to our molecular docking simulation data, IP4 and IP5 also play an important role in preventing oncogenesis.

Moreover, our results indicated that IP6 hydrolysates could be a protective factor against colon carcinoma. The protective functions may be significantly dependent on the condition of colon bacteria. Intestinal flora disturbances may decrease colon phytase secretion, which may hinder the IP6 hydrolysates to prevent oncogenesis. Hence, we hypothesize that the enteric flora disturbances may be a new mechanism employed in colon oncogenesis.

In conclusion, IP6 hydrolysates were able to suppress SW620 colon carcinoma cells and inhibited the activation of the AKT protein. The 20 and 30% DH IP6 hydrolysates suppressed the proliferation and inhibited the AKT protein. IP6 has been known to be an anti-nutrition phytochemical for decades. Our findings in the present study suggest that IP6 and its hydrolysates may be a valuable agent for cancer prevention and treatment, however further investigation is required.

Acknowledgements

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References


