

# Effect of PP2A concentration on the carcinogenicity of MCs

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## Abstract

Microcystins (MCs) are normal FHAB toxins that are harmful to humans. The toxicological mechanism of MCs is to form covalent bonds with serine protein phosphatases and disrupt the phosphorylation process of cells. Which leads to hyperphosphorylation, which leads to tumor formation. This study tries to study whether keep the process of phosphorylation of cells by increasing the PP2A concentration. Method The experiment will use the animal model, Western blot test for Akt activation, Thr308 and Ser473 within Akt, and PP2A activated acid test.

**Keywords:** PP2A, MCs, protein activation, specific protein phosphatase, tumor

## 1. Introduction

Nowadays, numerous waterways around the world support frequent and intense freshwater harmful algal blooms (FHABs). Large accumulations of cyanobacteria contain toxins harmful to humans who live near the water no matter long-term or short-term. MCs are one of the major FHABs toxins and the most commonly found hepatotoxins in fresh and brackish waters[1]. Organization (WHO) established a tolerable daily intake (TDI) of 0.04 µg MC-LR(kind of very common and well-known MCs) per kg b.w. per day[1](WHO 1998). Therefore, a more suitable treatment for Microcystins is still needed to be developed.

The protein phosphorylation is important for regulating cell apoptosis and protein activity. And it's also responsible for signal transduction. However, the MCs can combine with the Serine and Threonine specific protein phosphatase in chronic exposure, which is proven to cause cancer. According to the research on the toxicology mechanism for MCs, we have a target to predict and treat cancer caused by MCs. Because the MCs caused cancer by replacing the substrate and combining it with the Threonine specific protein phosphatase. So we can use some medicine to treat or predict cancer by overexpressing the Threonine specific protein phosphatase.

PP2A is a highly conserved eukaryotic serine/threonine protein phosphatase of the PPP family of phosphatases with basic cellular functions. PP2A plays a key role in the positive and negative regulation of a large number of cellular processes because it targets a large number of the cellular substrates phosphorylated on Ser/Thr residues[2] Genetic engineering is a good way to figure out the problem caused by MCs in chronic exposure. Genetic engineering is the use of molecular biology techniques to modify DNA sequence(s) in genomes, using a variety of approaches.[3]. We can use genetic engineering

technology to make the increase and Threonine specific protein phosphatase in the human body.

MCs result in morphological and cytoskeletal transformation of human liver cells(HL7702) by inhibiting the activity of PP2A, which damages liver tissue structure and leads to cancer. Meanwhile, PP2A has direct or indirect effects on the insulin signaling pathway. The Akt pathway in human hepatocyte HL7702 is most closely related to it. Inhibition of PP2A activity changes the level of phosphorylation of Akt, a key protein in the insulin signaling pathway[6]. Phosphorylation of Thr308 and Ser473 within Akt is important. In this study, the phosphorylation levels of Thr308 and Ser473 will be tested by Western blot, to determine the activity status of PP2A with different concentrations under MCs exposure.

The overexpression of PP2A, however, may cause the side effect. The most well-known one is protein hyperphosphorylation. The protein hyperphosphorylation will cause the occurrence of the diastolic stage of cardiomyocytes which leads to Ca<sup>2+</sup> leakage, and arrhythmia. The occurrence may be solved by Calyculin A.[4]

As a result, to support the hypothesis that increasing the molarity of the threonine-protein phosphatase in the human liver can decline the cancerogenic effect of the MCs, an experiment should be designed. This experiment will measure phosphorylation of known PP2A substrates by phosphorylation site-specific western blot and measure tumor appearance by cell counts or weight/size of tumors in animals injected with the PP2A + or - cells and animals exposed to microcystin.

## 2. Method

### 2.1 Toxins treatment [5]

MC-LR was dissolved in sterile deionized water containing 5% diunit sulfoxide (DMSO) . The solution of

0.5mM storage is been created.

Liquid divided and stored at -20°C.

Human hepatocytes (HL7702) will be used, and a total of 30 cells are prepared, 10 from high PP2A concentrations, 10 from normal mice, and 10 from cells without PP2A.

The high concentration of PP2A was used for  $8 \times 10^{-2}$ .

## 2.2 Cell culture method

The culture was prepared by primary digestion method:

1. Cut the liver tissue into small pieces of 2 to 4 cubic millimeters and wash it twice with a BSS solution without calcium and magnesium.

2. Move into 1:10.1% trypsin and 0.1% collagenase (both in calcium-free magnesium-BSS configuration), 4°C cold digestion for 10 to 12 hours, filtered through 250 microns and 64-micron nylon mesh or stainless steel mesh.

3. Collect cells and wash BSS solution 1 or 2, count them, and inoculate the culture.

## 2.3 Experiment grouping and cell treatment

Human hepatocytes (HL7702) will be used, and a total of 30 cells are prepared, 10 from high PP2A concentrations, 10 from normal mice, and 10 from cells without PP2A.

The high concentration of PP2A was used for  $8 \times 10^{-2}$ .

Three groups of PP2A were infected by MCs and tested for PP2A activity and Western blot protein expression

## 2.4 Protein phosphorylation test

Test 3group of cells(HL7702) and treat them by SDS-Gel Electrophoresis, Sodium dodecyl sulfate denatures proteins and coats them with negative charges.  $\beta$ -Mercaptoethanol reduces S-S bonds. Migration in the gel becomes a function of the molecular weight of the protein monomers. After electrophoresis, visualize proteins with a stain that binds to them. the concentration was measured with the BCA kit.

## 2.5 Protein activation test

Using the Rat phospholipase (PP2A) Activity Detection Kit, ELISA

The cells group is already been prepared

PP2A activate acid

The HL7702 phospholipase (PP2A) PP2A activate acid (ELISA is purchased from JENKESHENGWU testing will be performed according to the direction from JENKESHENGWU. The controlling group for the experiment is PP2A with nothing.

## 2.6 Animal Model

The mice will be divided into three groups of 8: positive control, negative control, and PP2A increasing express group with the much PPP express caused by genetic engineering, make sure the PP2A expression of these mice

are on the same quantity. The positive control will use cytostatic treatment. The negative control will use PBS. All 3 groups of tumors are caused by MCs long-term exposure. All the mice are the same age. The cell counts, weight, and size of tumors in animals injected will be measured

## 2.7 Statistical Analysis

The statistical significance of all numerical data gathered through Western Blot, Protein activation test, and animal model will be analyzed using the student's GraphPad Prism®T-Test at ( $p < 0.05$ ).

## 3. Results

Possible Results of total reaction (The overview of nine possible results is also shown in Table 1 )

### 3.1 Possible result 1

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are smaller than the tumor treated by cytostatic treatment. The results of the activation test show a positive correlation between the concentration of PP2A in MCs situation and PP2A activated.

Besides, the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473.

The result shows that the extra PP2A in the mouse body can treat cancer caused by MCs better than the treatment of cytostatic. The increasing concentration of PP2A can keep their activation and ability of phosphorylation control from the MCs effect.

### 3.2 Possible result 2

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are smaller than the tumor treated by cytostatic treatment. The result of the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473. The result of the activation test shows no correlation between the concentration of PP2A and its' activation in MCs situation.

The result shows that the extra PP2A in the mouse body can treat cancer caused by MCs better than the treatment of cytostatic. The increasing concentration of PP2A can keep their ability of phosphorylation controlling in MCs situation. The increasing concentration of PP2A cannot affect the activation of PP2A in MCs situations.

### **3.3 Possible result 3**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are smaller than the tumor treated by cytostatic treatment. Increasing PP2A concentration only keeps the phosphorylation levels of Thr308 and Ser473 in Akt. Another experiment shows no correlation between the PP2A concentration and its activation in the MCs concentration.

The result shows that the extra PP2A in the mouse body can treat cancer caused by MCs better than the treatment of cytostatic. The increasing concentration of PP2A only has little influence on keeping their activation controlling ability in MCs situation.

### **3.4 Possible result 4**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are bigger than the tumor treated by cytostatic treatment and smaller than the tumor treated by PBS treatment. Both results of the activation test and western blot test of phosphorylation show a positive correlation with the concentration of PP2A in MCs situation and PP2A activated. Besides, the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473.

The result shows that the treatment of extra PP2A does affect, but does not affect as well as the cytostatic treatment. The increasing concentration of PP2A can keep their ability of phosphorylation controlling in MCs situation. The increasing concentration of PP2A cannot affect the activation of PP2A in MCs situations.

### **3.5 Possible result 5**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are bigger than the tumor treated by cytostatic treatment and smaller than the tumor treated by PBS treatment. The result of the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473. The result of the activation test shows no correlation between the concentration of PP2A and its activation in MCs situation.

The result shows that the treatment of extra PP2A does affect, but does not affect as well as the cytostatic treatment. The increasing concentration of PP2A can keep their ability of phosphorylation controlling in MCs situation. The increasing concentration of PP2A cannot

affect the activation of PP2A in MCs situations.

### **3.6 Possible result 6**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected are bigger than the tumor treated by cytostatic treatment and smaller than the tumor treated by PBS treatment. Increasing PP2A concentration only keeps the phosphorylation levels of Thr308 and Ser473 in Akt. Another experiment shows no correlation between the PP2A concentration and its activation in the MCs concentration.

The result shows that the treatment of extra PP2A does affect, but does not affect as well as the cytostatic treatment. The increasing concentration of PP2A only has little influence on keeping their activation controlling ability in MCs situation.

### **3.7 Possible result 7**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected is no smaller than the tumor treated by PBS treatment. Both results of the activation test and western blot test of phosphorylation show a positive correlation with the concentration of PP2A in MCs situation and PP2A activated. Besides, the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473.

The result shows that the treatment of extra PP2A does not affect. The increasing concentration of PP2A can keep their activation and ability of phosphorylation control from the MCs effect.

### **3.8 Possible result 8**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected is no smaller than the tumor treated by PBS treatment. The result of the western blot test of AKT phosphorylation shows that increasing the PP2A concentration can keep the Total protein (TP)level and the phosphorylation levels of Thr308 and Ser473. The result of the activation test shows no correlation between the concentration of PP2A and its activation in MCs situation.

The result shows that the treatment of extra PP2A does not affect. The increasing concentration of PP2A can keep their ability of phosphorylation controlling in MCs situation. The increasing concentration of PP2A cannot affect the activation of PP2A in MCs situations.

### **3.9 Possible effect 9**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected is no smaller than the tumor treated by PBS treatment. Increasing PP2A concentration only keeps the phosphorylation levels of Thr308 and Ser473 in Akt. Another experiment shows no correlation between the PP2A concentration and its activation in the MCs concentration.

The result shows that the treatment of extra PP2A does not affect. The increasing concentration of PP2A can keep their activation in MCs situation. The increasing concentration of PP2A only has little influence on keeping their activation controlling ability in MCs situation.

### **3.10 Possible result 10**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected is no smaller than the tumor treated by PBS treatment. Increasing PP2A concentration only keeps the phosphorylation levels of Thr308 and Ser473 in Akt. Neither the result of the activation test nor the western blot test of Akt phosphorylation shows a positive correlation with the concentration of PP2A in MCs situation.

The increasing PP2A concentration works, but effect too little.

### **3.11 Possible result 11**

The result of the animal model shows that the measurement of tumor appearance by cell counts, weight, and size of tumors in animals injected is no smaller than the tumor treated by PBS treatment. The results of the activation test show no correlation between the concentration of PP2A in MCs situation and PP2A activated. Besides, the western blot test of AKT phosphorylation shows that increasing the PP2A concentration cannot keep the Total protein (TP) level and the phosphorylation levels of Thr308 and Ser473.

The increasing PP2A concentration does not work.

## **4. Discussion**

A previous study on the influence of different concentrations of PP2A on the treatment of cancer caused by MCs. The study uses the experience of animal models, protein activation tests, and western blot. These experiments show the feasibility by measurement of tumor appearance by cell counts, weight, size of tumors in animals injected, the activation of the PP2A, and the case of the phosphorylation. The goal of these experiments is to prove the hypothesis that increasing the expression of the PP2A that targets a large number of the cellular substrates

phosphorylated on Ser/Thr residues in the human liver can decline the cancerogenic effect of the Microcystins.

For possible results 1 to 3, the result of the animal model experiment supports the hypothesis. All the experiments in possible result 1 support the hypothesis. Possible result 2 shows the increase of PP2A is not powerful enough to keep the PP2A activated. Besides, the possible result 3 shows the PP2A increasing treatment may not keep the Total protein (TP) level. This shows that keeping the PP2A phosphorylation by increasing PP2A does not work. So, by the result of Possible result 2 and 3, there must be another way that PP2A influence the cancerogenic effect of the Microcystins. This has a big potential value to be researched.

For possible results 4 to 6, the experiment of the animal model shows that the increasing PP2A concentration can decrease the influence caused by MCs, but with less effect than positive control does. This result supports the hypothesis. For results 5 and 6, the PP2A concentration cannot keep the activation of PP2A in HL7702. And result 6 shows it cannot affect the TP level. So, by the result of Possible result 5 and 6, there must be another way that PP2A influence the cancerogenic effect of the Microcystins. This is worthy to be research.

For possible results 7 to 10, the experiment of the animal model contradicts the hypothesis in this paper. These possible results of the experiment show that increasing PP2A cannot decrease the cancerogenic of MCs. However, the mechanism and function of the extra concentration of PP2A that keep the activation and the ability of phosphorylation are worthy to explore. However, the potential value of PP2A activated and TP level keeping in possible result 8 and possible result 9 still have the benefit to research.

## **5. Conclusion**

Generally, this study explores the concentration of PP2A effect of the treatment caused by MCs through a variety of experiments. The result of our study tries to support the hypothesis that PP2A concentration increasing can decrease the cancerogenic effect caused by Microcystins. In addition, this experiment determines whether the concentration increasing of PP2A can keep its activation and ability of Akt phosphorylation in HL7702. The possible controversial results of the animal model, test of PP2A activation, and Western blot test of Akt phosphorylation will also indicate the potential relationship between MCs and other Thr residues in the human liver, which should be investigated in future studies on treatment caused by Thr residue problems. The big problem of this study is that we have no efficient way

to increase the concentration of PP2A in the human liver except through genetic engineering technology. Besides, the overestimation of PP2A has terrible side effects for

the human body, which cannot be ignored. The study may have not had enough influence before these problems have been solved.

### Supplemental Tables and Figures

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Result of western blot For Akt With High PP2A concentration	+	+	-	+	+	-	+	+	-	-	-
Result of western blot For Akt With normal PP2A concentration	-	-	-	-	-	-	-	-	-	-	-
Result of western blot For Akt With NO PP2A	-	-	-	-	-	-	-	-	-	-	-
Result of western blot For Thr308 and Ser473 within Akt With High PP2A concentration	+	+	+	+	+	+	+	+	+	+	-
Result of western blot For Thr308 and Ser473 within Akt With normal PP2A concentration	-	-	-	-	-	-	-	-	-	-	-
Result of western blot For Thr308 and Ser473 within Akt With NO PP2A	-	-	-	-	-	-	-	-	-	-	-
PP2A Activated acid Test with high PP2A concentration	+	-	-	+	-	-	+	-	-	-	-
PP2A Activated acid Test with normal PP2A concentration	-	-	-	-	-	-	-	-	-	-	-
PP2A Activated acid Test with no PP2A	-	-	-	-	-	-	-	-	-	-	-
The result of animal model	+	+	+	+/-	+/-	+/-	-	-	-	-	-

*Note.*

For the animal model, “+” represents a tumor smaller than the positive control. “+/-” represent a tumor bigger than the positive control, but smaller than the negative control. “-” represents tumors that are not significantly different from the negative control.

For PP2A activated test and the Western blot test, “+” represents the positive correlation between the things we taste and the concentration in HL7702 of PP2A in MCs situation, and “-” means no correlation

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