

Suppressive effect of AHCC on acute inflammation

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AHCC has a biological defense mechanism and it is quite effective on enhancing it to give a resistance against the cancer, as it is a defense mechanism against the infection. If there is an excessive inflammation, it is to be reduced to maintain the biological level. We have conducted the animal experimentation for example to restrict liver diseases on the mice and to inhibit the generation of diabetes, and these have been actually recognized. This time we have conducted the study and we would like to give you a presentation concerning the two areas of restriction.

(Slide 1)

This is the study about the effect of AHCC on the accumulation of inflammatory cells in the peritoneal cavity of mice by inducing the peritoneal inflammation by administrating *Enterococcus faecalis*, intestinal bacteria. By doing this, there is an inflammation in the peritoneal cavity so we investigated how AHCC will effect on the inflammatory cells. This is a very basic and simple model how AHCC will active on the inflammation cells. This is the method. Tap water or 5% AHCC solution were administered to mice by free intake for 2 weeks, then afterwards other mentioned *Enterococcus faecalis* dead cell 1mg/mouse was administered. And after certain period of time, then peritoneal cells were collected. The number of peritoneal cells was counted. If there is an exceeding inflammation, there will be a lot of peritoneal cell accumulation.

(Slide 2)

How does the cells change is the purpose of this study. We talk about peritoneal cells and we use the method by injecting saline water. Peritoneal cells were more diluted in the mice which were administered AHCC compared with the mice which were taking a tap water, it is more clear. We counted the number of cells. You can find as like difference of accumulation of cells. Left side is tap water and right side is AHCC mice. This is a kind

of cell populations. Twenty-four hours later, mostly a neutrophil, both of the tap water and AHCC, a kind of the accumulation of peritoneal cells is not different.

(Slide 3)

In order to prove the reproducibility of administered dead cells of *Enterococcus faecalis*, the number of peritoneal cells was counted. This is the number of peritoneal cells upon mouse 6 hours later, 24 hours later and 72 hours later. You can see that 6 hours later and 24 hours later AHCC group was less than tap water group. Seventy-two hours later, it was almost the same. We now know that AHCC can suppress this effect.

(Slide 4)

When we dissected at the mouse abdominal and we see the fat in the peritoneal cavity. AHCC was administered for 2 weeks and then the fat in the peritoneal cavity was much less. This is the fat that is attached to the testicular. Seven hours later, AHCC group had less amount of fat in the peritoneal cavity compared with tap water group however their body weight was almost the same.

(Slide 5)

And we have studied about the effect of AHCC on triglyceride in the blood. You can find that AHCC group is less than tap water group. When AHCC was administered, it reduced the amount of fat. In this case, the injection of *E. faecalis* is given and it is necessary to do a comparative study without injection of *E. faecalis*.

(Slide 6)

Another topic that I would like to mention is effect of AHCC on apoptosis induced by calprotectin. Calprotectin is a protein in neutrophil and this is quite abundant. It induces the apoptosis to many cells such as cancer cells or normal fibroblast; that is to say calprotectin would kill cancer cells. It is known that calprotectin markedly increase in the blood of the patients who have an inflammation or in the body for it in the area where there is inflammation. We wonder that exceeding amount of calprotectin in the inflammatory tissue. There is a possibility that it becomes apoptotic. We

studied the impedance effect of AHCC against apoptosis by calprotectin and the method is here. Anti-inflammatory effect and AHCC effect on fibroblast had to be studied. For the experiment we just simplified and used cancer cells. Calprotectin and AHCC were added to the cancer cells and incubated. And then death of cells was assayed.

(Slide 7)

This upper photograph is no calprotectin and lower one has added calprotectin. You can find some of anti-apoptotic effects when AHCC is added. This is the rate of impedance on the vertical axis. Rate of impedance was increased in AHCC, so there is higher ratio of impedance.

(Side 8)

This left graph shows what components of AHCC are working. We have done some of dialysis process. In the case of only AHCC, impedance effect was seen. And then in the case of dialyzed AHCC, impedance effect or activity was reduced. So, low molecular compound seems to be working on the impedance. The other possibility is that the effect of calprotectin of neutrophil with divalent cation is reduced. It seems that divalent cation is working for the deprivation of impedance. We try to deprive divalent cation to see the effect on impedance by AHCC and there was a little difference. High molecular nothing to do with divalent cation but a factor inhibits the activity. This is the calprotectin apoptotic effect seems to be contributing to the production of active oxygen. AHCC may have some components for anti-oxygen, so I mentioned about impedance of excessive reaction.

《Questions and answers》

Hosokawa : We will talk about biological body that there is various systems to inhibit. AHCC seems to be active and helping this.

Iwamoto : Anti-inflammatory effect of AHCC and when looked from qualitative point of view compared with steroid hormone, what is the difference against the steroid hormone? AHCC modify T-cells like suppressor and helper. It is known that it should not be used for some quaint and for the colon cancer. It was recognized that it should not be used for patients suffering from thyroid however a patient suffering from a cancer of the liver but he also had thyroid problems and he was complaining. Hashimoto thyroid problem is often connected with rheumatoid arthritis. And for these patients, it has been mentioned that AHCC should not be used. But later this person had liver cancer, and it was a quite extensive part of colon cancer and there was a metastasis and the doctor mentioned that there was a possibility of metastasis as well as relapse. And the doctor suggested the chemotherapy however the patient studied about chemotherapy and he did not want to receive the chemotherapy and he wanted AHCC. Three months later, it was advised by the doctor, his finger joints of rheumatoid arthritis these became quite better, so clinical doctor and I were very impressed. What is the qualitative difference of AHCC and cortical steroid? And do you think that AHCC can be used for rheumatoid arthritis patients?

Yui : About calprotectin that I talked about, this is found in a large amount in the rheumatoid patients. It seems that their might be working against patients when calprotectin is to be inhibited by some compound. It seems that it is becoming effective for example by some compound and I hope that AHCC would be beneficial for that and it would been done animal experimentation. Compared with steroid hormone, I have not

done any extensive study. How different they are but rather AHCC may work upon steroid hormone and it may work as the anti-inflammatory and these something that I have to study in the future.

Hosokawa : I think that something has to be made clear, this is the dead bacteria. That's why AHCC was effective on the biological body when the body has to defend it from bacteria. We have to consider that AHCC is regulating some excessive amount, is that so?

Yui : Yes, something very important is that how it is working in effective. According to our experiments, something missing is the quality of cells. We were only studying on the amount not at quality for example cytokines from the peritoneal cavity exuded cells. They may disappear but those cells might be attached to some part of peritoneal cavity. When it is activated by cytokines, there is transparent, disappearance or reduction. There is a reduction but each cell is activated, it is quite possible. We must base a source on that until see generation of cytokines.

Slide 1

I AHCC の急性炎症モデルに対する効果

[目的]

Enterococcus faecalis 死菌投与により腹腔炎症を惹起し、腹腔局所への炎症細胞の集積に対するAHCCの影響を調べる。

[方法]

C3H/He ♂マウス

↓ 水道水 もしくは

↓ 5% AHCC 水溶液 自由摂取

14日後

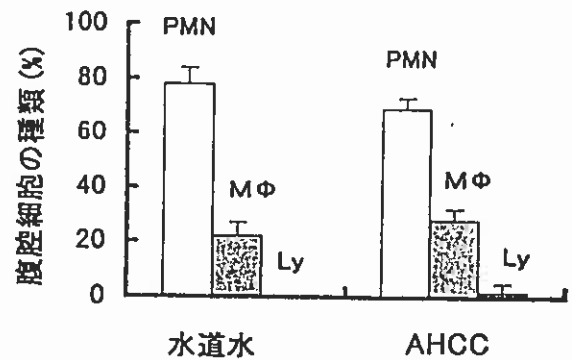
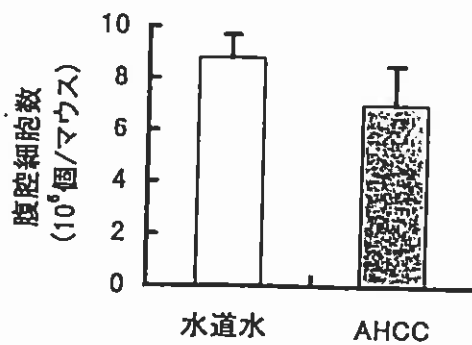
← *E. faecalis* 死菌 1 mg/マウス

↓ i.p. 投与

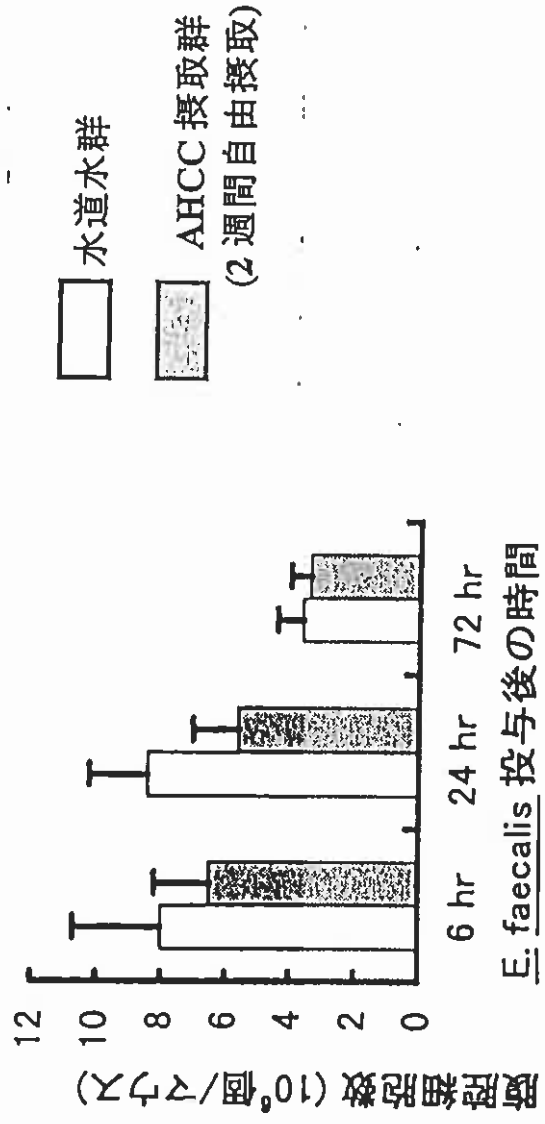
7、24、48時間後の
腹腔細胞採取

Slide 2

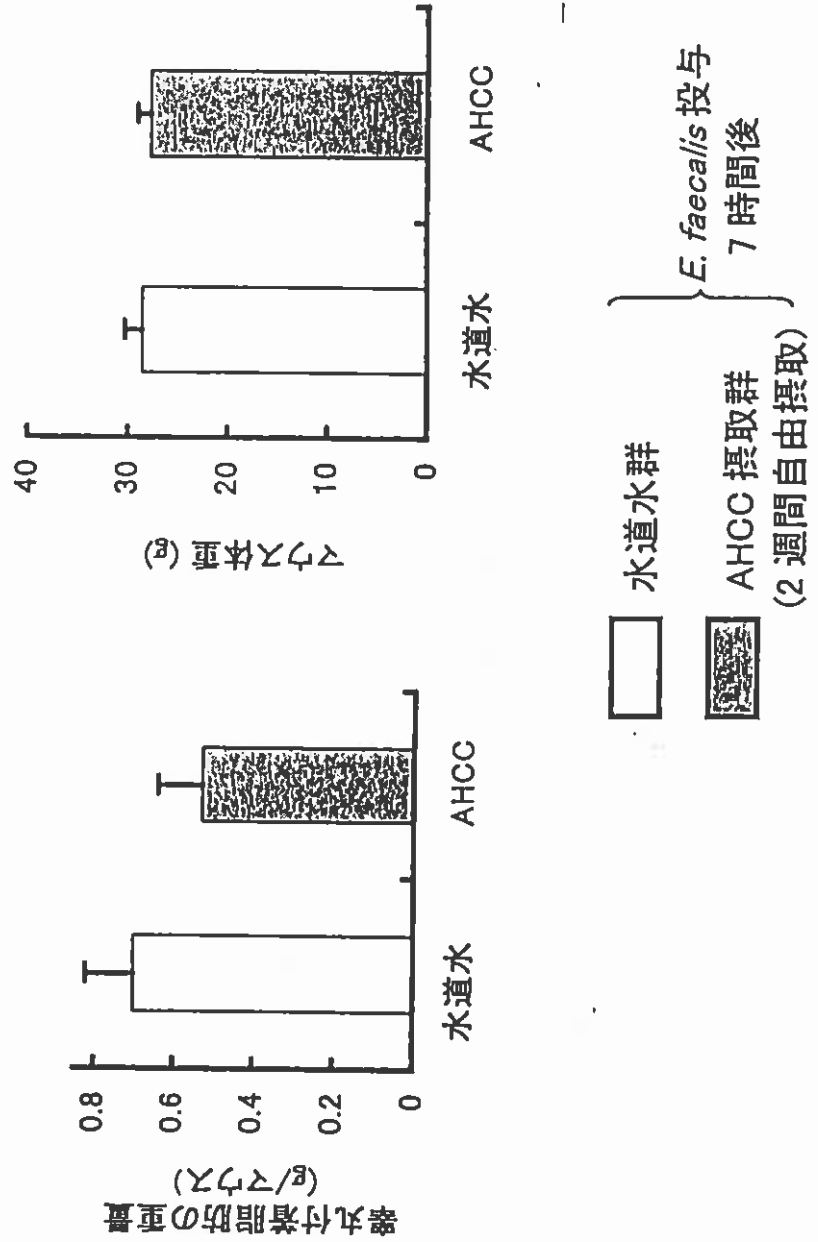
Enterococcus faecalis 死菌投与 24 時間後の腹腔細胞数に及ぼす AHCC の影響



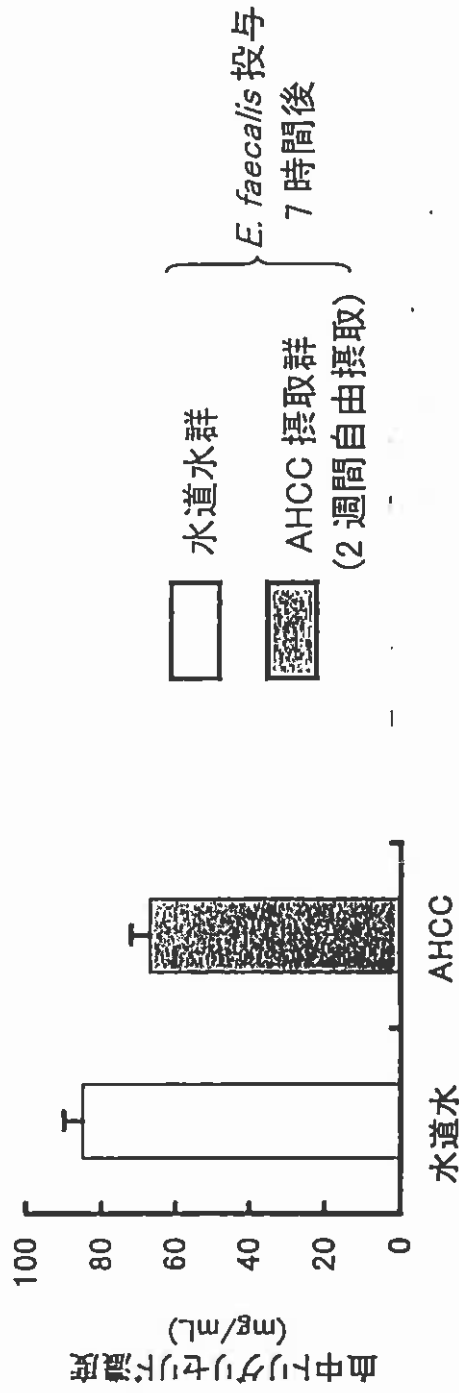
Enterococcus faecalis 死菌投与後の腹腔細胞数に及ぼす
AHCC の影響 (タイムコース)



腹腔内の脂肪重量に及ぼす AHCC の影響



血中の中性脂肪濃度に対する AHCC の影響



II カルプロテクチンの細胞傷害性に対する AHCC の効果 (*In vitro*)

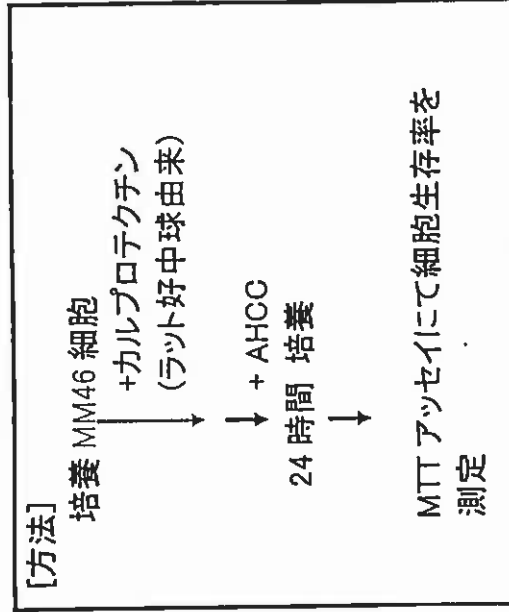
[目的]

カルプロテクチンは、好中球由来のタンパク質で、癌細胞や正常線維芽細胞など多くの細胞に対してアポトーシスを誘導する。

また、カルプロテクチンは、炎症疾患をもつ患者の血液中、または炎症局所の体液中において、顕著に増加することが知られている。



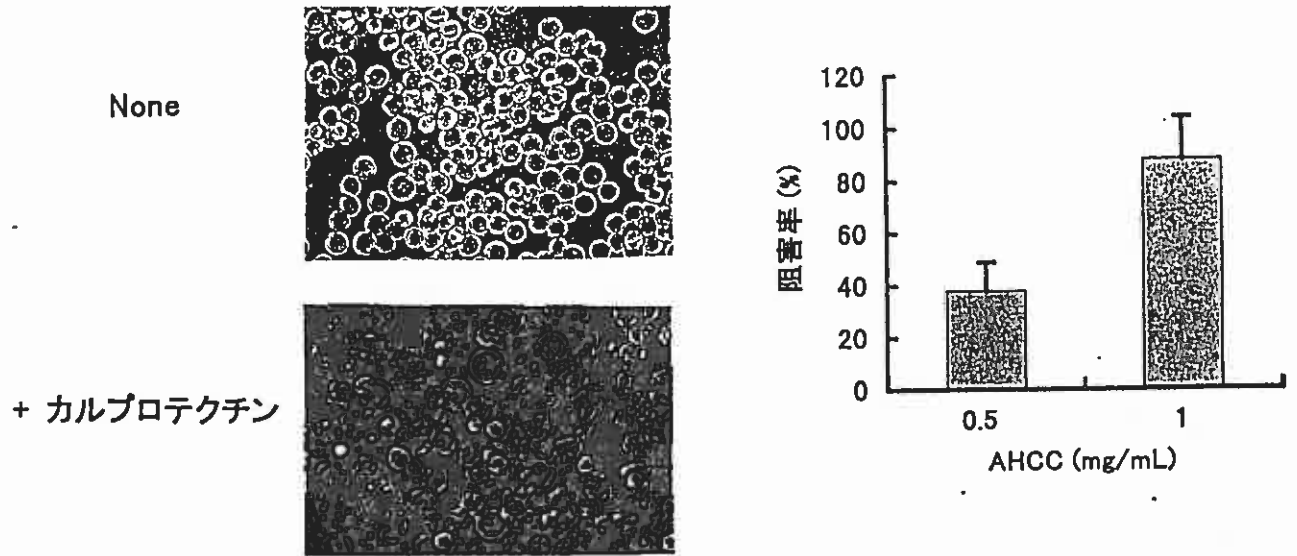
カルプロテクチンは、過剰に炎症組織に存在すると組織傷害因子としてはたらく可能性がある。



カルプロテクチンの細胞傷害作用(アポトーシス誘導)にたいする AHCC の阻害効果を調べる。

Slide 7

カルプロテクチンの細胞傷害作用(アポトーシス誘導)
に対する AHCC の影響



Slide 8

AHCC によるカルプロテクチンの細胞傷害作用の阻害
= 透析処理と脱 2 価金属イオン処理の影響 =

