Iraqi Journal of Cancer and Medical Genetics

Study the Effect of Vitamin C in Increasing the Oxidation Damage on Two Types of Cell Lines

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Abstract :

A method of testing the cytotoxicity by in vitro exposing different cells to vitamin C, intracellular metabolizing and then detecting cell damage in these cells as an indication of the cytotoxicity. From this data there was a great variation between the two cell lines, in which vitamin C effect on vero cell line was much better than Hep-2 cell line, this was due to the type of the cell, in which Hep-2 cell are encompassed of squamous cells and the vero cell line are encompassed of the Monkey kidney cells, which means different respond to cancer and transformed cell to vitamin C.

Keyword: *Vitamin C, Cytoxcity, Oxidation damage.*

Introduction:

Tumors are made up of cells that are reproducing at abnormally high rates. Normal cells known to stop reproducing (or dividing) when they come into contact with other cells. In the case of a tumor, this mechanism is missing, causing cells to continue to divide over and over. (1)

The vitamin C is a water-soluble and is known as ascorbic acid (meaning "without scurvy," the disease caused by a vitamin C deficiency). We depend on ascorbic acid for many aspects of our biochemical functioning; yet human beings are among only a handful of animal species that cannot produce their own supply of vitamin C. Like these other animals, including primates and guinea pigs, we have no choice but to obtain this nutrient in our diet. Considering the many benefits vitamin C may provide, that mandate is deceptively simple. (2)

In fact, this nutrient plays a major role in the manufacturing and defense of our connective tissue, the elaborate matrix that holds the body together. It serves as a primary ingredient of collagen, a glue-like substance that binds cells together to form tissues. Vitamin C helps some of our most important body systems. First it helps the immune system to fight off foreign invaders and tumor cells. Vitamin C also supports the cardiovascular system by facilitating fat metabolism and protecting tissues from free radical damage, and it assists the nervous system by converting certain amino acids into neurotransmitters. (3).

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The warns against big doses of Vitamin C during cancer treatment, that are cancer patients who take large doses of vitamin C in the hope of a cure might actually make their disease worse by inadvertently protecting their tumors from radiation and chemotherapy, new research suggests, they cannot prove the vitamin is harmful during cancer treatment. But they say there are strong biological reasons to think mega doses could be bad. The concern is based on the discovery that some cells actually contain large amounts of vitamin C, which appears to protect them from oxygen damage. Many cancer treatments, especially radiation therapy, work by triggering oxygen damage to the genes of cancer cells. Vitamin C has many adherents, in part because it is an antioxidant, a substance that protects the body from potentially harmful oxygen particles known as free radicals. Oxidation - the same process that rusts iron - is suspected of triggering cancer and other disease.In 1993, Golde's team discovered how vitamin C gets into human cells. They found that an oxidized form of vitamin C called dehydroascorbic acid enters cells through the same opening used by sugar. Once inside, it is immediately converted back to vitamin C.They said a key feature of many cancers is they have many more of these sugar openings than do ordinary cells. This allows them to take in the energy they need to grow.

But they said cancer cells often also have very high concentrations of vitamin C. The exact function of the vitamin inside cancer is unknown. The cancer cell wants vitamin C because it wants antioxidant protection.(4)

A recent in vitro study found that low levels of vitamin C inhibited tumor growth, but high levels increased tumor growth (5).

In 2005 in vitro (test tube) research funded by the National Institutes of Health indicated that vitamin C administered in pharmacological concentrations (i.e. intravenous) was

preferentially toxic to several strains of cancer cells. Vitamin C supplementation may interfere with effective cancer chemotherapy. A study found that vitamin C was taken up by cancer cells and protected the cells from chemotherapy drugs; raising the possibility that vitamin C might impair the effectiveness of chemotherapy (6 , 7).

Material and methods:

VitaminC

Vitamin C was obtained from (Samara Drug Industry company) at concentration of 500mg/tablet, 200 mg of vitamin c was dissolved in 10 ml of sterile phosphate buffer saline to make a stock solution (20mg/ml), and from this solution we made the concentrations of (0.625, 1.25, 2.5, 5, 10, 20) mg/ml.

Cell lines

African green monkey kidney (Vero) cells. The Vero cell was initiated from the kidney of a normal adult Africa green monkey in 1962 by Y. Yasumura and Y. Kawakita at the Chiba University in chiba (8). In this study , a passage 230 was used and the cells were maintained in MEM medium, and Hep-2 (human larynx epidermoid cancinoma cell) passage 295 was used to study the cytotoxic effects of vitamin C. Hep-2 cell were grown in MEM at 37Cº and 5% CO2 (9).

Maintenance of the cell line

Cell line preparation for cytotoxicity

According to Freshney 2005(10), the growth media was discarded .two to three ml of trypsin – versene was added to the cell sheet and the flask recked gently .after approximately 30 sec at 37 C, the trypsine versene detached from the flask. Cells were farther dispensed by pipeting in growth medium. After warred, 200µl of cell suspension was added to each well of sterile 96 well micro titration plate , sealed with self adherence film and incubated at 37 C.

When the cells are at exponential growth i.e. after lag phase, the medium was removed and a serial dilution of vitamin C in free serum media (20000 ,10000, 5000, 2500, 1250, 625µg / ml) and added to the well.

Three replicate were used for each concentration of vtamin C .the plates were incubated at 37C for exposure times (24, 48, and 72).

Cytotoxicity assay

After the end of each exposure period, micro titer plate's supernatant was removed from the wells at serial conditions. After the end of exposure time, the micro titer plate was stained by neutral red and left for 2 h in incubator, 50 µl PBS and ethanol in 1:1. The result was study by Eliza at 492 nm.

Detect the viable cell by :

$$\text{Proliferation cell count} = \frac{\text{Test}}{\text{Control}} \text{X } 100$$

$$\text{Inhibition cell count} = \frac{\text{Control - Test}}{\text{Control}} \text{X } 100$$

Statistical analysis

The analysis of data was done on the SPSS program to the value of LSD.

Results:

This experiment were designated to view the effect of different concentrations of vitamin C on two selected cell line Hep-2 and vero and in three time of exposures (24,48 and 72 hr.)

Figure (1) and (2) show the significant variations between the concentrations and the exposure times.

A method of testing the cytotoxicity of vitamin C by exposing different cell lines to it in vitro, intracellular metabolizing and then detecting cell damage in the test cells as an indication of the cytotoxicity .

In Hep-2 cell line exposed to vitamin C, a maximum inhibition concentration was in 48 h. of exposure in a concentrations (20000 µg/ml,10000 µg/ml and 5000 µg/ml) with std error(2.7)($p>0.05$). in the same time the other concentration showed an induction of proliferation.

The most induction of proliferation of cancer cell was at 24h. (385%) in a concentrations (2500 µg/ml,1250 µg/ml, 625 µg/ml)

For the vero cell line exposing to vitamin C showed a maximum inhibition of cancer cell was at 24h. of exposing in (20000 µg/ml) is (83%)std (8) ($p>0.05$).

While at 24h. of exposure showed an inhibition of (13% and11%) at (20000 µg/ml and 10000 µg/ml) .there was an increase in the proliferation of cancer cell and reach it maximum effect in 625 µg/ml at 72h.of exposure.

From these results we conclude that the most effective concentration of vitamin C is (20000 µg/ml) at 48h. for the two cell line.percentage.

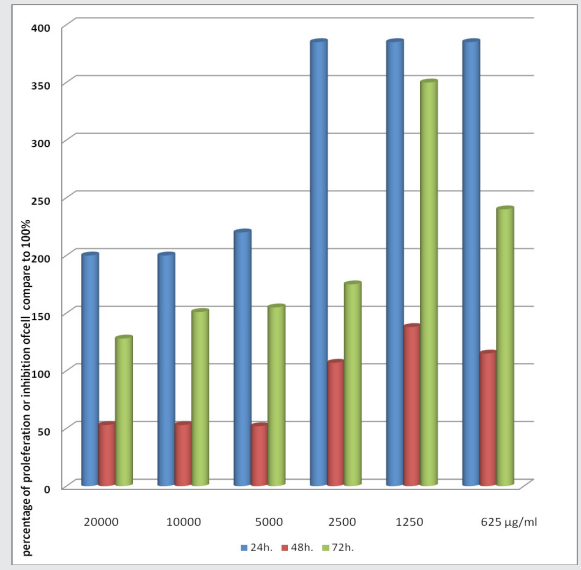

Figure (1): Show the effect of vitamin C on the cell line Hep-2 on different concentrations and different times.

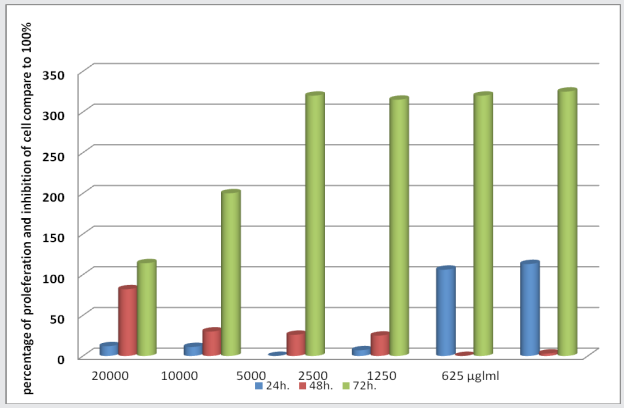

Figure (2): Show the effect of vitamin C on the cell line Vero on different concentrations and different times.

Discussions:

From these data there was a great variation between the two cell lines, in which vitamin C effects vero cell line much better than hep-2 cell line this was due to the type of cell, in which Hep-2 cell are encompassed of squamous cells and the vero cell line are encompassed of the Monkey kidney cells, these two cells show a different effect to vitaminC.

In a recent presentation at the American Cancer Society meeting, Dr. David Golde of Memorial Sloan-Kettering Cancer Center speculated that supplemental vitamin C may be harmful to cancer patients. Dr. Golde had shown that vitamin C gets into and accumulates in cancer cells. Golde and others are concerned that the extra vitamin C in cancer cells may enhance their growth or protect them from the cell-killing free radicals produced by radiation and some chemotherapeutic drugs.

While different cancer cells may respond differently to vitamin C, it is important to view these concerns in the context of the experimental in small animal, and human clinical studies (4).

Vitamin C has enhanced cancer cell growth. Dr. Chan Park has found that the growth of leukemic cells from some leukemia patients put into culture was enhanced by vitamin C. The growth of cells taken from other leukemia patients was either inhibited or unaffected by vitamin C. It is unknown whether similar effects would have been observed in the same patients taking supplemental vitamin C (11).

Dr. Joel Schwartz of the National Institutes of Health has published studies in which supplemental vitamin C enhanced the growth of tumors induced in hamsters by a chemical carcinogen. Interestingly, the growth of tumors was significantly inhibited by supplemental vitamin E and by a mixture of antioxidants, including beta-carotene, vitamin E, and vitamin C (12).

Dr. Constance Tsao and her colleagues at LPI showed that supplemental vitamin C (sometimes combined with oxidation products of vitamin C) inhibited the growth of human colon, lung, and mammary tumors implanted into mice. LPI investigations also demonstrated that vitamin C and its derivatives have anticancer effects against a number of cancer cell lines in culture (13).

In 1979, Drs. Cameron and Pauling noted that little information was available on the interaction between vitamin C and chemotherapeutic drugs. They cautioned that patients undergoing aggressive chemotherapy expected to be curative should refrain from taking large doses of vitamin C at the same time in case the vitamin interfered with the drug action. There is some evidence that vitamin C increases the activity of liver enzymes that detoxify xenobiotics, including drugs. When the chemotherapy was merely palliative, they did not believe that the use of concurrent vitamin C was contraindicated. They believed that vitamin C potentiates radiation, and even many clinicians who disagree on this point nevertheless agree that supplemental vitamin C given after radiation ameliorates radiation sickness (14).

Interestingly, Dr. Hoffer's regimen is remarkably similar to that recommended by Dr. Kedar Prasad of the University of Colorado and his colleagues, who advocate the use of a combination of B vitamins, large doses of calcium ascorbate (vitamin C), vitamin E, and beta-carotene for cancer patients undergoing either chemotherapy or radiation (15).

Dr. Prasad acknowledges the accumulation of antioxidant vitamins in cancer cells, but argues that this has favorable biochemical effects, including the inhibition of oncogenes and the induction of factors that inhibit cell growth, favor differentiation, or induce apoptosis (programmed cell death). In an extensive and well-referenced recent review published in the Journal of the American College of Nutrition, Dr. Prasad presented results from cell culture experiments demonstrating that the killing effect of many cancer drugs or radiation on mouse and human cancer cells is enhanced in the presence of vitamins C or E. Of course, cell culture studies (or animal studies) cannot always predict what will happen in humans (16). In another extensive review published in Alternative Medicine Review in 1999, Drs. Lamson and Brignall reached conclusions similar to those of Dr. Prasad. These authors noted that "considerable data exists showing increased effectiveness of many cancer therapeutic agents, as well as a decrease in adverse effects, when given.

Conclusion:

- Vitamin C harms effect on cancer cells in low concentration.
- Vitamin C had different effect on different cell types with different concentrations.

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دراسة تأثير فيتامين C في زيادة الاكسدة المحطمة على نوعين من الخلايا

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الخلاصة:

إن طريقة اختبار القابلية السمية لفيتامين ج عن طريق تعريض انواع من الخلايا خارج جسم الكائن الحي للمادة, ويحدد بعد ان تتعرض له الخلايا ويدخل ضمن الايض الغذائي لها وتحديد الخلايا المتحطمة .

هذه الدراسة اعتمدت نوعين من خطوط الخلايا Vero و Hep-2 وعرضت الى تراكيز مختلفة من فيتامين ج في الاوقات 24 و 48 و 72 ساعة.

اظهرت النتائج ان هناك تباين واضح لتاثير الفايتمين ج على نوعي الخلايا حيث اثر على خلايا اكثر من تبعا لنوع الخلايا حيث إن هوالخلايا البشرية و الفيرو هو خلايا الكلية للقرود . مما يعني ان انواع السرطان يوثر بصورة مختلفة على الخلايا المختلفة.