STUDIES ON THE EFFECT OF CISPLATIN ON MALIGNANT AND NORMAL CELLS: PRELIMINARY INVESTIGATIONS ON CISPLATIN COMBINATION CHEMOTHERAPY

ABSTRACT

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M.Sc

THESIS

Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in Zoology

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July, 1995

A B S T R A C T

STUDIES ON THE EFFECT OF CISPLATIN ON MALIGNANT AND NORMAL CELLS: PRELIMINARY INVESTIGATIONS ON CISPLATIN COMBINATION CHEMOTHERAPY

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Studies on the tumor growth pattern and antitumor activity of cisplatin against ascites Dalton's lymphoma adapted to Swiss albino mice show that a regular increase in ascites tumor volume could be noted with time following tumor transplation. During the initial phase (2-12 days) of tumor growth ascites fluid increases more rapidly in the ascites tumor so that the ratio of ascites fluid to tumor cell pellet reached about two during the middle period of tumor growth.

Cisplatin treatment of the tumor-bearing hosts on the 10th day post-tumor transplantation resulted in a significant reduction in tumor volume indicating effective tumor regression.

Measurement of carbohydrate and protein contents in tumor supernantant, and glucose contents in serum as

well as tumor supernatant of the tumor-bearing hosts treated with or without cisplatin revealed that following 3-4 days of cisplatin treatment, the carbohydrate contents markedly increased in the ascites fluid of the tumor-bearing hosts. However, the protein contents in the ascites fluid decreased by about three fold following 2-4 days of cisplatin treatment. The serum glucose level of tumor-bearing hosts was found to be comparatively lower than the normal animals and 1-3 days of cisplatin treatment of the tumor-bearing hosts resulted in a significant increase in serum glucose level. In the tumor supernatant however, it is found that in the tumor-bearing hosts very low concentration of glucose was present which increased progressively following 1-4 days of cisplatin treatment.

It is suggested that since ascites fluid is the direct nutritional source to the tumor cells, the rapid increase in ascites fluid during tumor growth could possibly be a means to meet more nutritional requirements of tumor cells. This is evident from the rich carbohydrate and protein contents in the ascites fluid and the presence of numerous surface membrane ruffles and cytoplasmic processes all over the tumor cells which could play a role in nutritional exchange with ascites fluid. The observed lower serum glucose level in tumur-bearing hosts than the normal animals may indicate higher rate of glucose uptake by tumor cells. And

an increase in a carbohydrate contents in ascites fluid following 3-4 days of cisplatin treatment could be due to utilize the carbohydrates present in the ascites fluid as well as release of surface mucopolysaccharides and sialic acid moieties from tumor cells. Studies on the rate of glucose consumption by tumor cells and tumor cell sialic acid content also revealed a decrease in the rate of glucose consumption and also decrease in sialic acid content in the tumor cells.

Metaphase chromosome analysis of Daltion's lymphoma cells show that cisplatin (8 mg/kg b.w.) for 1-4 days resulted in a very high frequency (80-90%) of aberrant metaphases. Pulverized (severely damaged) cells were also frequently observed.

Light microscopical studies show that the percentage ratio of leukocytes (neutrophils, monocytes, lymphocytes; based on nuclear shape and size) to tumor cells increased about three times by 1-4 days of cisplatin treatment than the control animals. In control ascites tumor very few leukocytes were seen among tumor cells which were round in shape. After 8-96 hr of cisplatin treatment many leukocytes are found coming closer to the tumor cells and surround them finally resulting in the formation and shedding of membrane vesicles, and disintegration of plasmamembrane

leading to the lysis of tumor cells.

Measurement of Ca^{2+} concentrations in various tissues i.e., liver, kidney, brain, spleen and tumor cells show that following cisplatin treatment resulted in significant increase could be noted in tumor cells followed by kidney and brain tissues. The potassium concentration decreased significantly in the kidney and tumor cells following cisplatin treatment, whereas liver and spleen tissues showed no significant change. Significant variations in the rate of oxygen consumption (QO_2) were observed in the tumor cells following cisplatin treatment. Tumor cells showed about 40% increase in QO_2 following one day of cisplatin treatment. However, following 2-4 days of the treatment, QO_2 decreased steadily.

Scanning electron microscopic observations show that during tumor regression following cisplatin treatment definitie changes in the pattern of surface membrane ruffles/ blebs also occur along with the infiltration of leukocytes towards tumor cells and sharp decrease in ascites fluid. Control tumor cells showed the presence of fine ruffles/ blebs distributed evenly over the cell membrane. After 8 hr of cisplatin resulted in the infiltration of leukocytes surrounding tumor cells and forming connections with the latter. Also it leads to a definite movement of ruffles/

blebs from the top surface of the tumor cells towards the marginal areas. One day of cisplatin treatment showed formation of broader tumor cell-leukocyte connections and the appearance of fine microvilli like processes extending from tumor cells. At 2-3 days of the treatment, thick blebs are formed over the surface membrane of tumor cells and lysis starts. There is disappearance of thin cellular processes, formation of membrane vacuoles and breaking of plasmamembrane of tumor cells after 4 days of cisplatin treatment which leads to the lysis of tumor cells.

It is suggested that cisplatin has a definite effect on the cell surface membrane. And the disintegration of plasmamembrane of tumor cells surrounded/connected by leukocytes could be due to the release of some toxic factors from leukocytes.

Studies on the glucose-6-phosphatase enzyme activity in the liver tissues show that the normal animals exhibit higher activity than the tumor-bearing animals. Cisplatin treatment of the tumor-bearing hosts resulted in a steady increase in enzyme activity. In the kidney tissues the enzyme activity decreased till the 2nd day of cisplatin treatment and showed sign of recovery thereafter.

Studies on lactate dehydrogenase (LDH) enzyme acti-

vity show that except for liver all other tissues studies definite patterns of changes in enzyme activity could be noted. It is observed that the LDH activity in serum and liver of the tumor-bearing animals were comparatively higher than the normal animals. Following cisplatin treatment except for tumor cells, in other tissues an overall increase in enzyme activity could be observed. In the tumor cells however, a progressive decrease in LDH activity was noted following cisplatin treatment.

LDH isozyme analysis in serum, kidney, liver, tumor supernatant and tumor cells following cisplatin treatment of the tumor-bearing host *in vivo* show that in the serum and kidney tissues only all the 5 isozymes are present. In the other three tissues distinct variations are notable as regards to the number and nature of the various isozyme bands. In the liver tissue only 3 isozyme bands (LDH-3, LDH-4, and LDH-5) are seen. Following 1-4 days of cisplatin treatment of the tumor-bearing hosts. LDH-3 and LDH-4 showed marked variations in band intensities (decreasing) indicating changes in activity and at the 4th day of the treatment LDH-5 was found to be the only isozyme form. In the tumor supernatant besides LDH-5, LDH-4 isozyme could also be seen. But, in the tumor cells LDH-5 is the only isozyme form present.

It has also been noted that one extra band near the cathodic end could be found in the serum of tumor-bearing as well as cisplatin treated group which may also be seen in tumor supernatant and tumor cells; but absent from liver, kidney and serum normal animals.

Assay of Na^++K^+-ATPase activity in the tumor cells as well as in tumor supernatant show that a gradual decrease in enzyme activity could be noted in the tumor cells following 2-4 days of cisplatin the enzyme activity increased upto 2nd day of the treatment, but; following 3-4 days of the treatment decreased to that of the control level.

5'-Nucleotidase (5'-ND) activity in liver of tumor-bearing hosts was found to be about 2.5 times higher than the normal animals which progressively decreased following cisplatin treatment. However, in the kidney tissues the enzyme activity increased following cisplatin treatment.

Studies on the activity of arginase in liver, kidney as well as in tumor supernatant revealed that tumor-bearing animals exhibit significantly lower enzyme activity than the normal animals which decreased further following 3-4 days of cisplatin treatment of the tumor-bearing hosts. An almost similar pattern of arginase activity was also observed in the kidney tissues. In the tumor supernatant

however, the arginase activity increased steadily following 8 hr to 96 hr (4 days) of cisplatin treatment of the tumor-bearing hosts.

Measurement of cathepsin activity in serum as well as in tumor supernatant revealed that following 2-4 days of cisplatin treatment of the tumor-bearing hosts, the activity of both cathepsin B and cathepsin H increased progressively and by 3-4 days of the treatment very high activity could be recorded for both the enzymes. Similar trends were also observed for cathepsin B and cathepsin H activity in tumor supernatant.

It is suggested that although DNA is considered as the primary target for cisplatin for its anticancer activity, however, it may have multilevel targets and act through changes in the activity of various enzymes and isozymes of metabolic importance.

Combination chemotherapeutic studies with subtherapeutical dose of cisplatin (4.0 mg/kg b.w.) and vitamin C (0.5% in drinking water) revealed that tumor-bearing hosts receiving either cisplatin or vitamin C alone show almost similar survival patterns and 50% animals survived upto 35 days.

The combined administration of 0.5% vitamin C in

drinking water from the first day and cisplatin (i.p., 4.0 mg/kg b.w.) on the 10th day resulted in 70% survivals upto 55 days and 40% of the treated mice were found to be tumor free. The increase in body weight in this group of mice was very slow indicating effective retardation of tumor growth. This combined treatment was found to be sequence dependent since cisplatin treatment first and vitamin C treatment started second did not result in any synergistic effect. Changing the host strain from C3H/He mice to Swiss albinomice did not alter the result thus indicating that the observed synergistic antitumor activity between vitamin C and cisplatin may not be host strain specific.

Studies on the effect on thymus and spleen weight, and tumor pH revealed that combined treatment of 0.5% vitamin C and cisplatin (4.0 mg/kg b.w.) significantly increased the weight of spleen and thymus, and the average tumor pH decreased to 6.27 as compared to 6.93 found in the controls.

It is also noted that tumor-bearing animals have a lower serum ascorbic acid level (7.11 µg/ml) which increased significantly (13.44 µg/ml) in the vitamin C treated group. The combined treatment of vitamin C and cisplatin also resulted in a more sustained increased in total leukocyte count in the blood as compared to the treatment of cisplatin as a single agent.

Agglutination studies on ascites Dalton's lymphoma (DL) using conconavalin A (Con A) show that control DL cells show high degree of Con A agglutination. However, combined treatment with vitamin C plus cisplatin markedly decreased the degree of agglutination as compared to that when these agents were treated separately.

Fluorescence labelling of DL cells with Con A fluorescentisothyocyanate (Con A - FITC) show uniformly distribed bright even fluorescence all over the surface. However, treatment with cisplatin or vitamin plus cisplatin for 15-60 min resulted in rearrangemtn/loss/removal of labelled Con A from the cell surface thus reducing fluorescence intensity. This effect was more rapid in case of the combined treated groups than the groups treated separately.

Studies on the effect of vitamin C on cisplatin induced mutagenicity revealed that treatment of the tumor-bearing hosts with 0.5% vitamin C prior to cisplatin treatment significantly reduced the incidence of chromosomal aberrations, micronuclei in bonemarrow cells and sperm head abnormalities in mice thus indicating that vitamin C may have a chemoprotective effect against cisplatin induced mutagenicity in the hosts.

Studies on the cisplatin induced nephrotoxicity

as observed from serum uric acid as well as serum urea level show that cisplatin causes nephrotoxicity which is dose dependent in mice. However, pretreatment with vitamin C significantly reduces the serum urea level thus suggesting that vitamin C may have some protective effect against cisplatin induced nephrotoxicity in Swiss albino mice.

It is concluded from the present study that:

i) Cisplatin treatment brings about definite changes in the ascites fluid as well as in tumor cells in terms of nutritional requirements, degree of infiltration of leukocytes towards tumor cells finally leading to the death of the tumor cells.

ii) Cisplatin has some definite effect on the arrangement and movement of ruffles/blebs over the surface of tumor cells and also leads to the formation of membrane vesicles/cellular vacuoles/thick blebs all of which ultimately favour the tumor cell death.

iii) The enzyme lactate dehydrogenase showed comparatively increased/decreased activity in tumor cells (↓), ascites fluid (↑), serum (↑) and kidney (↘) following cisplatin treatment. In addition to this the appearance of a new isozyme which is here named as LDH-T was noted in the serum and tumor cells of the tumor-bearing hosts.

iv) Vitamin C showed synergistic effect with cispla-

tin and it may be used with subtherapeutical dose of cisplatin in protecting the host against cisplatin induced nephrotoxicity without losing the therapeutic efficacy.

v) Along with the enhanced therapeutic efficacy of cisplatin by vitamin C, it may also protect the host against cisplatin induced mutagenicity.

vi) These studies further indicate the involvement of multistep and multilevel effects of cisplatin resulting the tumor regression in the host.