CHAPTER VIII

GENERAL DISCUSSION

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Malignant lymphoma is one of the most chemo sensitive neoplasm. A number of metal based anti cancer agents have demonstrated their anti-tumor effect. Cu (II) complex coded as BRH_2 (5 – phenylazo – 3 – methoxy salicylidene thiosemicarbazone) has been synthesized and deployed against the growth inhibition of P 388 lymphocytic leukemia cell sensitive and resistant to adriamycin (Patil et al., 1989). The present investigation shows that the intraperitoneal administration of BRH_2 in DAL implanted mice can increase the life span by 70% over the respective control.

Lactic dehydrogenase is an older enzyme (Schwartz, 1991) and has been established as tumor marker in nasopharyngeal carcinoma (NPC) patients with liver metastases and multiple organs site metastase (Liaw et al., 1997). LDH is remarkable as marker in having five electrophoretically separable isoenzymes. Further, this group of authors concluded that serum LDH levels are high in metastatic NPC and this is a good marker for monitoring the response to chemotherapy.

The present investigation revealed the significant reduction of LDH activity in DAL implanted BRH_2 treated mice in all the four types of tissues. The reduction of LDH values clearly indicates anticancer activity of BRH_2, which extends a possible support that there is inhibition in the reversible catabolic activity of the LDH.

The differences in the LDH isoenzyme composition in malignant tissues reflect the difference in metabolic requirement in them. Earlier, Good friend et al.(1965) observed inhibition of LDH_5 activity in presence of low pyruvate level of malignant tissues. On the other hand, LDH_3, LDH_4 and LDH_5 have been seen to contain predominantly in malignancy (Richards et al., 1972). The response of this glycolytic enzyme to the copper based BRH_2 exposed malignant mice was significant in all the organs (ANOVA, $P < 0.01$). The appearance of LDH_1, LDH_2 & LDH_3 as prominent bands in DAL implanted mice ***(Table. 5.2, 5.3, 5.4 and 5.5)*** have been considered as marker in malignant lymphoma of this investigation.

The reduction of LDH_3 (Pi 90), LDH_4 (Pi 0) and LDH_5 (Pi 55) in liver, LDH_2 (Pi 80), LDH_5 (Pi 80) in spleen, LDH_2 (Pi 70), LDH_3 (Pi 85) and LDH_5 (Pi 60) in kidney and LDH_1 (Pi 90), LDH_2 (Pi 0) and LDH_4 (Pi 50) in bone marrow after the administration of BRH_2 suggested that either total LDH or LDH isoenzyme could specify the recovery during and after chemotherapy which were in accordance with the finding of Von Eyben et al. (1992).

The element profile has been considered as diagnostic markers (Lal et al., 1989) in a variety of malignant tissues (Muller et al., 1988, Ranade & Pandey, 1984).

A negative correlation is evident between the Cu & Se concentration and LDH activity in all the cases of this investigation ***(Fig: 8.1, 8.2, 8.3, & 8.4)***.

Reduction of Cu & Se with the elevation of LDH activity suggest the inverse relationship during the process of carcinogenesis, while the Zn presented a positive approach towards the LDH activity of the Liver, spleen, kidney & bone marrow in the lymphoma bearing host ***(Fig: 8.1, 8.2, 8.3, & 8.4)***. Introduction of BRH_2 in the group D and E mice caused positive relationship between the LDH activity and Zn and negative relationship between the LDH activity and Cu & Se of this investigation. Both the LDH activity and the Zn concentration had decreased in group D & E while the Cu & Se increased in both these groups in case of all the tissues.

Considerably higher amount of Zinc, Iron, Cobalt, and Antimony was earlier observed in the DNA isolated from sarcoma M – 1 and Walker 256 – carcinosarcoma (Andronikashvili et al., 1970). The experiments with animals proved that some trace elements are required for neoplastic growth, the first of which is zinc (Petering et al., 1967; De Wys et al., 1970; Mc Quitty et al., 1970). It has been established that zinc alone is responsible for the nucleic acid biosynthesis in regenerating rat liver (Fujioka and Liberman 1964; Weser et al., 1969).

Correlation of zinc content with Rous sarcoma development was also confirmed by Rubin (1972). Variation of other trace elements in the dynamics of tumor development has been shown by Zimmer et al., (1970) and Neporandy (1963). The presence of Zn in DNA and RNA polymerase explains the observations of Fujoka and Liberman (1964) and those of

Weser et al. (1969) on the participation of Zn in nucleic acid synthesis of the regenerating rat liver.

Although there are no direct indications that the uncontrolled reduplication in the neoplastic growth is determined by DNA polymerase, it is natural to assume that the enzyme with its active Zn center plays an important role in the synthesis of enhanced amount of DNA in the malignant tissues. The increased Zn level in different malignant tissues ***(Fig: 8.1, 8.2, 8.3, & 8.4)*** of the present investigation shows that it is an important element for the fast – dividing cells that are characterized by the intensive DNA and RNA synthesis.

The enhanced concentration of Cu in the BRH_2 treated tissues of kidney, liver, spleen and bone marrow was higher than the malignant group (B & C) involved in maintaining the concentration of this element. The concentration of Cu observed in the lymphoma implanted tissue of liver, kidney and spleen are lower than those observed in the normal control ***(Table 6.1).***

Recently Reddy et al. (2003) recorded the reduced level of Cu and Zn in the cancerous tissue of stomach suggested that the deficiency or excess of certain elements is correlated to carcinogenesis. However, Horst et al. (2000) stressed that the serum Cu level remained more or less unchanged during and after hyperfractionated radio-chemotherapy (HFRCT) of patients with locally advanced pancreatic adenocarcinoma. Songchitsomboom et al.

(1999) suggested that the use of serum Cu / Zn ratio as marker for the diagnosis of cancer or for the staging tumors must be interpreted cautiously.

A negative correlation is evident between the Se concentration and the LDH activity in all the tissues of this investigation ***(Fig: 8.1, 8.2, 8.3, & 8.4)***. Reduction of Se with the elevation of LDH activity suggest the inverse relationship during the process of carcinogenesis. Haung et al. (1999) reported that the mean serum Se level was significantly lower than the control. Dobrowolski et al. (2002) showed that Zn and Se are strongly decreased in the neoplastic mass of Renal cell carcinoma, while depleted Zn and enhanced Se was noticed in the BRH_2 exposed animals in the present experiment.

In the BRH_2 treated i.e. D & E groups the concentration of Se is higher than normal control and lymphoma implanted tissues. Se and Zn with preventing function against cancer have been observed also by Fassina et al. (1990). Recently, Reddy et al. (2003) observed that the concentration of Se are lower in the cancerous tissue of kidney than those observed in the normal tissue. In this study lower levels of Se are observed in the tissue of kidney affected with cancer when compared with those in the tissue of normal kidney, which supports the hypothesis that Se acts as a possible protective agent against cancer. In various epidemiologic studies, association between low Se levels with an increased risk of cancer incidence has been highlighted (Willet et al., 1983; Salonen et al., 1984; Salonen et al., 1985;

Clark, 1985; Menkes 1986; Newberne et al., 1986; Coates et al., 1988; Iyengar and Woittiez, 1988; Knekt et al., 1988; Ringstad et al., 1988; Knekt et al., 1990; Pawlowicz et al., 1991; Turan and Delibasi, 1992; Pawlowicz et al., 1993; Torun et al., 1995). The potential of selenium as natural anticancer agent is well documented by epidemiologic evidence and clinical interventional trials (Willet et al., 1983; Salonen et al., 1984; Salonen et al., 1985; Clark, 1985; Menkes 1986; Newberne et al., 1986; Coates et al., 1988; Iyengar and Woittiez, 1988; Knekt et al., 1988; Ringstad et al., 1988; Knekt et al., 1990; Shu - You et al., 1991; Turan and Delibasi, 1992; Torun et al., 1995).

A striking parallel has been observed between the BRH_2 and the SEM images of the Dalton's lymphoma cells. The loss of microvilli formation of ruffles and cellular processes ***(Plate 7.4)*** suggested high surface activity in malignancy and confirmed the earlier observations in intestinal as well as gastric epithelia of human (Schuman et al., 1978; Isomaki, 1979). Appearance of large number of ruffles,, ballooning of cells, cell-cell association ***(Fig. 7.5 d,e)***, movement of ruffles from surface top to periphery, invagination and formation of large sized hole on plasma membrane ***(Fig. 7.5 f, g)*** and the disintegration and lysis of cell membrane ***(Fig. 7.5 h)*** of DAL cells in the BRH_2 treatment tissue was evident.

The development of cytoplasmic projection after cisplatin treatment in VX_2 carcinoma of rabbit (Hsieh, 1990) and ruffle formation in Dalton's lymphoma

(Prasad & Arjun, 1991) and in rat malignant esophageal region (Sharma et al., 1991) strongly advocated the hypothesis of the high cell surface activity during chemotherapy. The potentiation of the antitumor activity of the cisplatin was induced by disintegration of the cell membrane of the malignant tissue and the tumor cell lysis was evident in the Dalton's lymphoma (Prasad & Arjun, 1991) and in the Methyl Cholanthrene induced oral carcinogenesis of rat (Sharma et al., 1991).

Thus this present findings extend the Cu (II) based drug BRH_2 which is potential to prevent further growth of the Dalton's lymphoma and enhance the life span of the tumor bearing C_3H / He mice ***(Table – 4.1)***. It also supports the hypothesis that one of the main biological action mechanisms of this BRH_2 is to facilitate Cu (II) ion uptake into cells (Conato et al., 2001). However, further studies must be developed to determine the relationship between the cytotoxicity and the reaction with cell thiols in the compound.

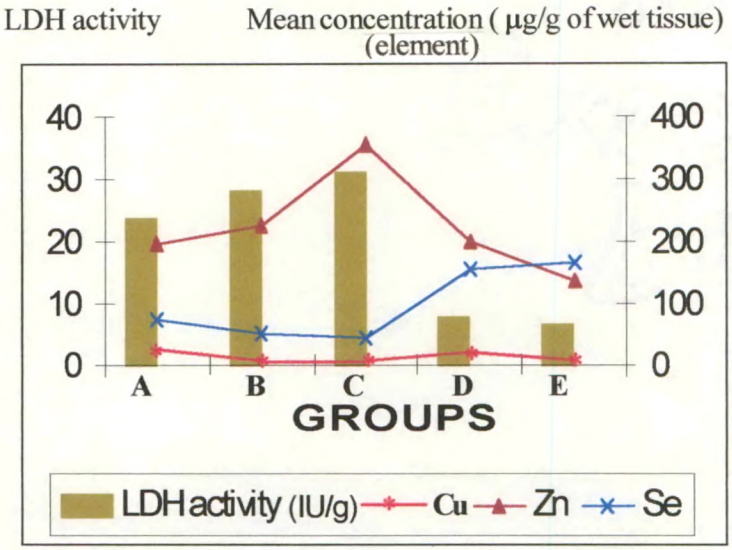

FIG. 8.1 **MEAN CONCENTRATION OF LDH ACTIVITIES AND ELEMENT CONCENTRATION IN THE LIVER OF DIFFERENT TREATMENT GROUPS OF C_3H/He MICE.**

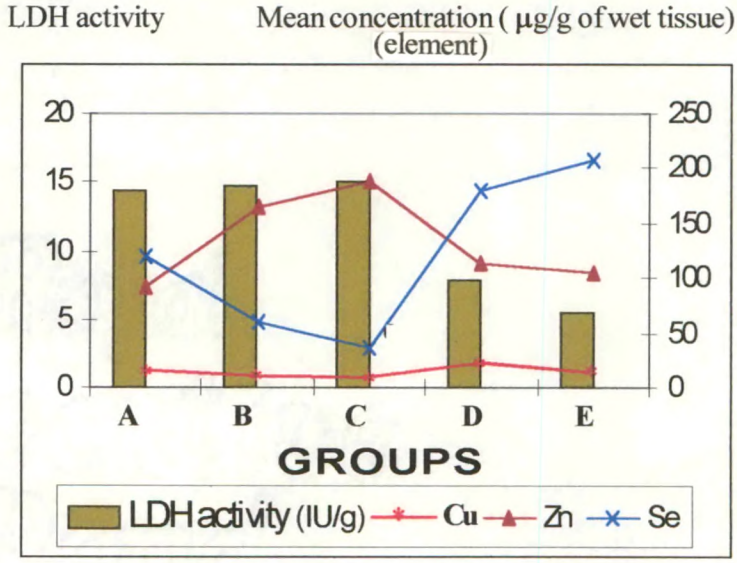

FIG. 8.2 **MEAN CONCENTRATION OF LDH ACTIVITIES AND ELEMENT CONCENTRATION IN THE SPLEEN OF DIFFERENT TREATMENT GROUPS OF C_3H/He MICE.**

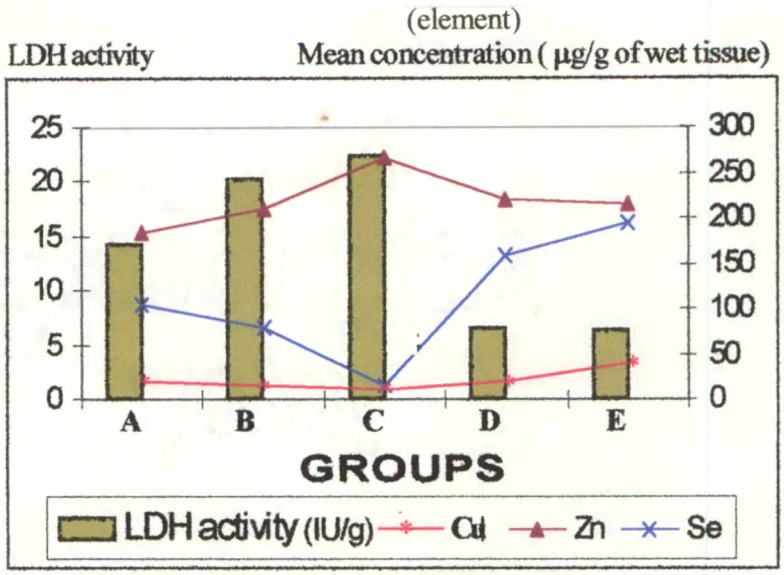

FIG. 8.3 **MEAN CONCENTRATION OF LDH ACTIVITIES AND ELEMENT CONCENTRATION IN THE KIDNEY OF DIFFERENT TREATMENT GROUPS OF C_3H/He MICE.**

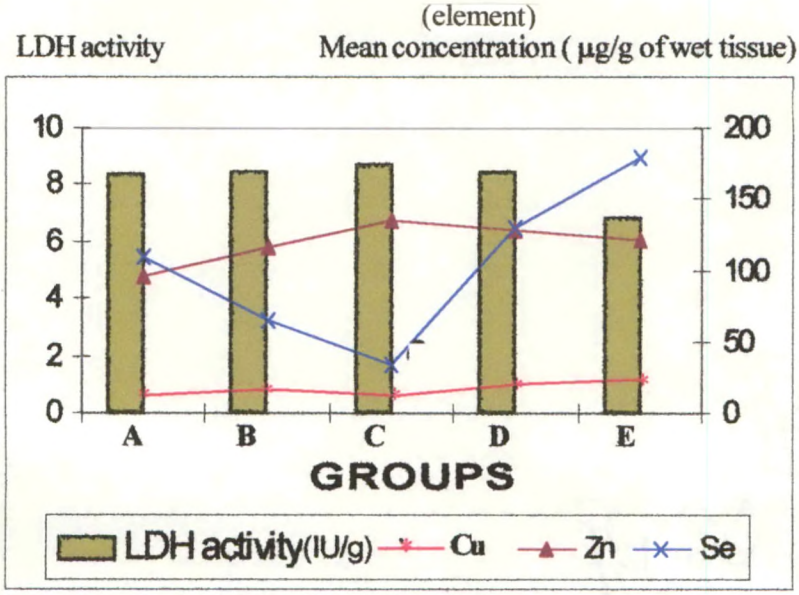

FIG. 8.4 **MEAN CONCENTRATION OF LDH ACTIVITIES AND ELEMENT CONCENTRATION IN THE BONE MARROW OF DIFFERENT TREATMENT GROUPS OF C_3H/He MICE.**

Summary:

1. The BRH_2 can increases the life span of DAL implanted mice for a period of above 35 day (above 70%) over the respective lone DAL implanted mice.

2. The LDH has been used as prognostic marker to assess the anticancer activity of BRH_2.

3. The correlation between the elements and the BRH_2 suggest certain positive role.

4. The effect of BRH_2 on the Dalton's lymphoma cell surface topography is evident, hence it may be suggested as the anti-malignant agent.