CHAPTER VII

ULTRASTUCTURE OF DALTON'S LYMPHOMA AND BRH_2 TREATED LIVER, SPLEEN, KIDNEY AND SURFACE TOPOGRAPHY OF DALTON'S LYMPHOMA CELL

ULTRASTRUCTURE OF DAL AND BRH_2 TREATED LIVER, SPLEEN, KIDNEY AND SURFACE TOPOGRAPHY OF DAL CELL

A change in the surface topography is a common feature associated with the process of malignant transformation and such change may contribute to the pathological outcome of cancer with regard to the process of neoplastic invasion. Many structural and functional properties of malignant cells are related to changes in the cell surface cell membrane (Hynes, 1979) including cell-to-cell contact (Hoshino, 1963), cell association (Hayashi et al., 1981) and cell communication (Lowenstein, 1975; Good enough, 1976). Several workers (Carter et al., 1978; Hodges, 1978; Nelson, 1979) described significantly different surface structures in neoplastic cells from their normal cellular architecture. Direct observations under microscope are devoid of a subjective error, hence the recorded observations under light microscope (LM) is pertinent. The potential use of Scanning Electron Microscope (SEM) in the detection of cell surface changes and their correlation with malignancies has been getting wide attention (Spriggs and Boddington, 1968; Vesely and Boyde, 1973; Legrand and Pariente, 1975).

Malignant epithelial cells display abundantly long pleomorphic microvilli, especially those present in efflusions (Berliner et al., 1978; Kenemans et al., 1981). The appearance of long pleomorphic microvilli provides an early

marker of irreversible neoplastic transformation (Gaeta et al., 1977; Hodges, 1978; Croft et al., 1979). Gradual decrease in the number and length of cell surface microvilli (Mickey et al., 1977; Trump et al., 1980; Hsieh, 1990), appearance of exotropic blebs, ruffles etc (Carter et al., 1978; Hodges, 1978; Nelson et al., 1979) was observed in neoplastic cells. Meanwhile, pleomorphic microvilli has already gained importance and drawn wide attention as a tumor marker (Hodges, 1978; Croft et al., 1979 & Price et al., 1980). Cell surface microridges indicate normal cellular morphology, while cell surface microvilli indicate abnormality.

Microvilli are cylindrical membrane bound strands of cytoplasm that extend from the cell surface of 0.1 μm in diameter and normally not more than 2 – 5 μm in length. Microvilli contain a parallel arrangement of filamentous components (microfilaments), which extends outward from the dense lattice of randomly oriented (6 nm) filaments in the cell cortex just under the membrane. Extremely variable numbers, distributions shapes and sizes microvilli are seen on transformed cells. The microvilli occurring in several different form or changing form of microvilli in several different times during its life cycle is called pleomorphic microvilli.

Contact between cells results in a modification of the cell membrane. In epithelial cells four types of junctional differentiation of cell membranes are present which might be associated with neoplastic transformation (Weinstein et al., 1980).

Since several major hepatic functions involve reactions that occur at the surfaces of the various types of liver cells, examination of the surface features of normal and abnormal livers may provide new information that assists to understand hepatic pathology. Sequence of changes for carcinogenesis in mouse liver had already been described (Squire and Levitt, 1975; Lipsky et al., 1979). Wanson et al., et al. (1980) described the alteration in the cell surfaces of the diethylnitrosamine induced preneoplastic and hyperplastic hepatocytes in situ. Neoplastic cells in the hepatocellular carcinoma were poorly differentiated, forming imperfect plates and lacking bile canaliculi (Nopanitaya et al., 1977). Instead of surfaces of the neoplastic hepatocytes contained microridges and a few irregularly shaped microvilli, which alternated with smooth areas. A progressive loss of microvilli from tumor cells in increasingly undifferentiated malignant tumors has been observed (Tannenbaum et al., 1978). SEM images revealed the presence of proliferated aciner cells, duct cells islet cells with intracellular attachments, microvilli and stenosis around the aciner lobules of pancreatic carcinoma (Nonomura et al., 1992). Loss of microvilli was an invariable feature of malignancy while pleomorphic microvilli were found to exclusively cover the entire surface of the free floating metastatic

carcinoma cells (Gaeta et al., 1977; Trump et al., 1980; Koss and and Domagala; 1980) and the premalignant cells of human ectocervical epithelium (Kenemans et al., 1980). But contours and density of microvilli are important in the diagnosis of neoplasia (Berliner et al., 1978).

The presence of cell-cell contact has been described in solid tumors and in few ascitic tumors (Hayashi & Ishimoru, 1981). The pattern of cell distribution cell-cell association and the effect of metal based drug may provide an insight in the mechanism of growth inhibition of lymphoma cells. SEM studies give major clue on the pattern of cell surface topography and cell-cell association with reference to the effect of cisplatin on ascitic Dalton's lymphoma cells (Prasad & Arjun, 1991).

The Scanning Electron Microscopic (SEM) studies were undertaken to find the pattern of cell surface topography and cell-cell association with reference to the effect of BRH_2 on ascites Dalton's lymphoma cells in vivo and also light microscopic studies of liver, spleen and kidney of control, malignant and BRH_2 treated mice to observe their structural differences.

Materials and methodology:

Details of the materials and methodology have been presented in the chapter II.

Results:

The histological observation under light microscope of liver, spleen and kidney in control (A); malignant (C) and chemotherapy (D & E) given mice and the SEM images of the surface topography of normal and BRH_2 exposed Dalton's lymphoma cells exhibit structural changes.

LIVER

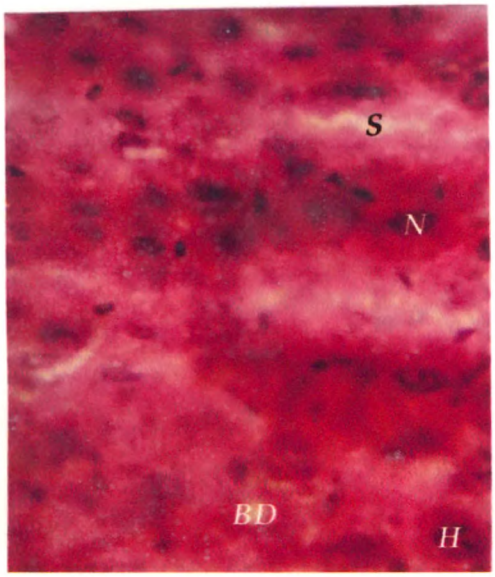
The control liver exhibited the normal nuclear structure round with a smooth surface, with a few scattered chromatic clumps and one or more prominent nucleoli. Bile canaliculi are also seen between the cells. ***(Plate – 7.1a).***

Lymphoma implanted liver cell after 20^{th} day showed more vacuolated nucleus, rupturing of nuclear membrane, diffused nuclei, reduced bile canaliculi ***(Plate – 7.1b).***

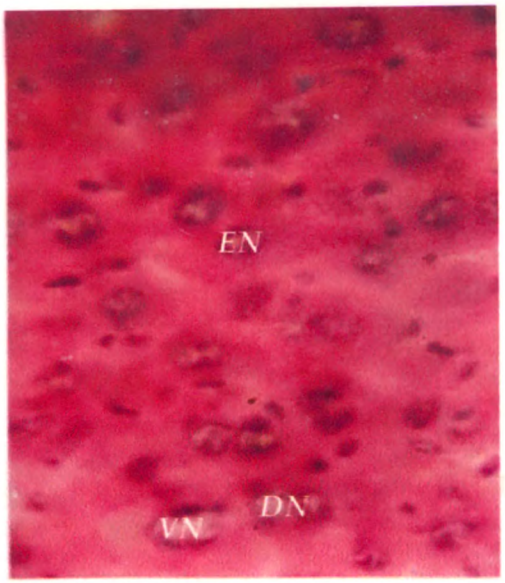
Lymphoma implanted BRH_2 treated liver cell after 20^{th} day exhibit some similarities with that of normal cellular structure, bile canaliculi and hepatic sinusoids. ***(Plate – 7.1c).***

Lymphoma implanted BRH_2 treated liver after 35^{th} day displayed large size vacuolated nucleus, rupture of nuclear membrane, loss of cellular integrity, with loose parenchymal cells. ***(Plaete – 7.1d)***

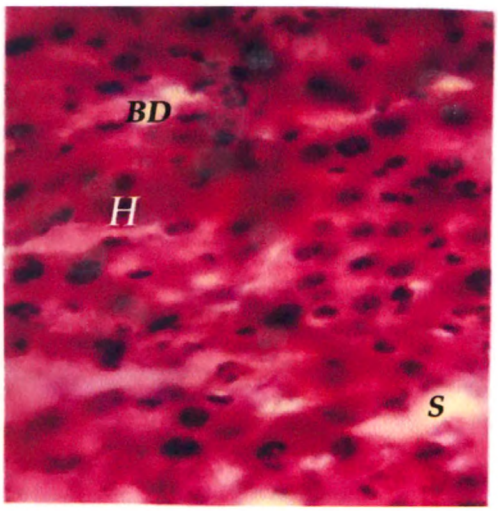
LIVER



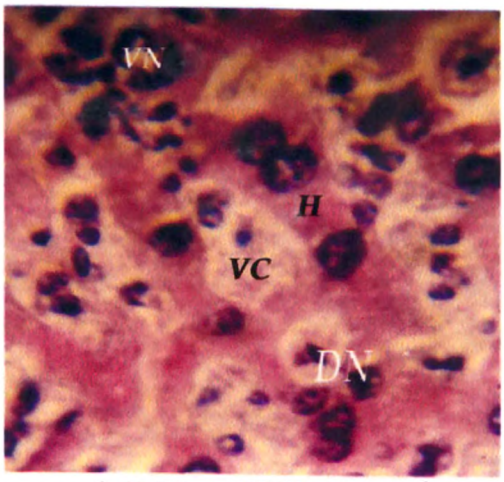
a. Control X 1000



b. DAL implanted (after 20 days) X 1000



c. DAL + BRH_2 treated (after 20 days)X 1000



d. DAL + BRH_2 treated (after 35 days) X1000

Plate 7.1 a. Showing normal cellular structure and regularly spaced nuclei.

Plate 7.1 b. Showing loss of normal cellular pattern and breaking down of nuclear membrane.

Plate 7.1 c. Showing nearest to the normal structural pattern of cell and nuclei.

Plate 7.1 d. Showing large sized vacuolated nuclei with breaking down of nuclear membrane.

N= Nuclei , BD = Bile duct, VN= Vacuolated nuclei, DN = Diffused nuclei, EN= Enlarged nuclei, S= Sinusoid, VC= Vacuolated cell , H=Hepatocyte.

SPLEEN

The control splenic pulp or parenchyma is of two distinct types – the white pulp and the red pulp. The white pulp consists of ordinary lymphoid tissue and is distributed as roughly spherical or fusiform masses. The red pulp consists of a reticular meshwork traversed by numerous venous sinuses. It is infiltered with all elements of the circulating blood such as lymphocytes; plasma cells, macrophages, other leucocytes, giant cells and erythrocytes ***(Plate 7.2a)***

The section of the 20^{th} day DAL implanted mice spleen displayed cell structure abnormalities. The membrane of some R.B.C in the red pulp were found to be wrinkled and some vacuolation also observed in the peripheral blood of this group of mice ***(Plate 7.2b)***

The spleen of DAL implanted BRH_2 treated on or after 20^{th} day demonstrated less structural abnormalities of cell. The majority of the RBC present in the splenic pulp displayed normal smooth membrane structure ***(Plate 7.2c)***

DAL implanted treated with BRH_2 spleen on 35 day displayed more irregular structure of RBC, macrophages, platelets, a few plasma cells and lymphocytes. ***(Plate 7.2d).***

SPLEEN

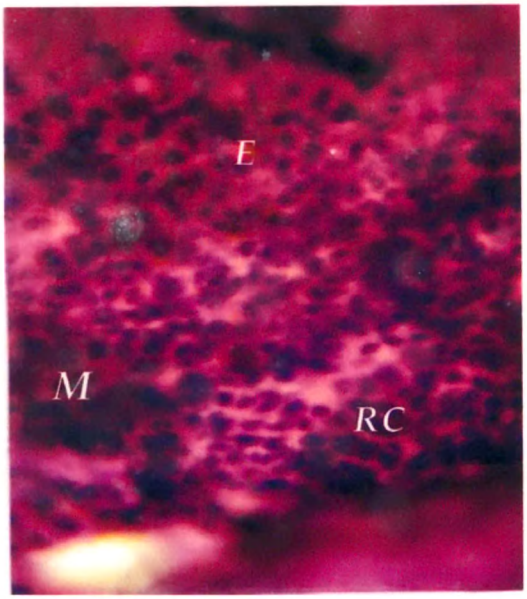


Plate 7.2 a. Control X1000

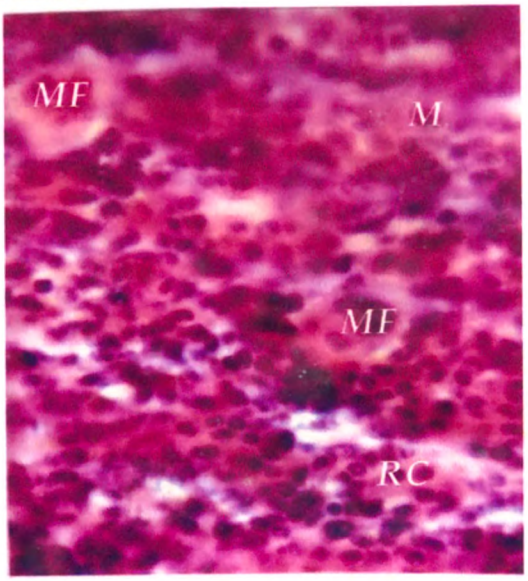


Plate 7.2 b. DAL implanted (after 20 days) X 1000

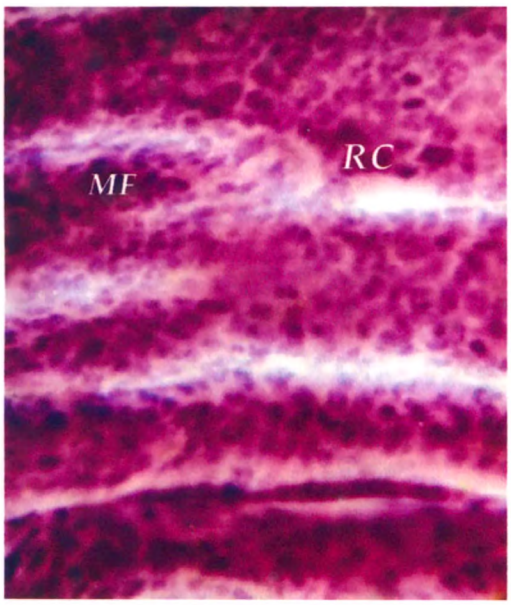


Plate 7.2. c. DAL + BRH_2 treated (after 20 days) X1000

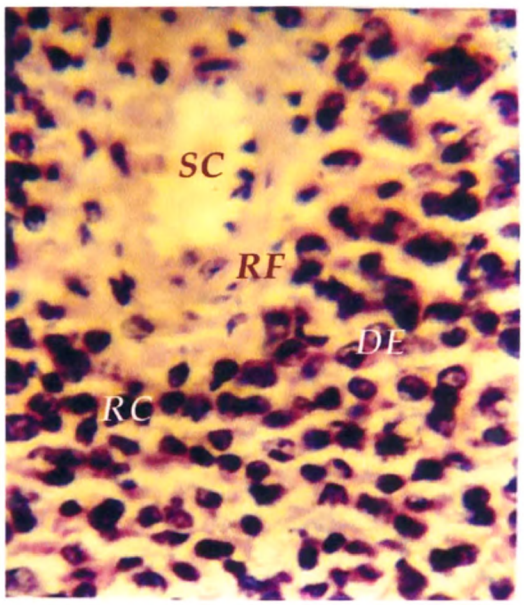


Plate 7.2 d. DAL + BRH_2 treated (after 35 days) X1000

Plate 7.2 a. Control spleen with normal cellular structure.

Plate 7.2 b. Showing macrophages with some changes of cellular pattern.

Plate 7.2 c. Showing some changes of cellular pattern, some similarities with normal control.

Plate 7.2 d. Showing some deformed cellular structure.

(MF = Macrophages, M = Monocyte, E = Erythrocyte,
RF = Reticular fibers, RC = Reticular cells,
SC = Splenic cord, DE = Deformed erythrocyte).

KIDNEY

The control section of kidney showed normal structure of proximal and distal convoluted tubules. The free surface of the proximal convoluted tubules has an epithelial cell lining which bears a deeper brush border and the distal convoluted tubules has a thinner brush border and prominent dense bodies in the cytoplasm ***(Plate 7.3a).***

DAL implanted section of the kidney after 20^{th} day exhibit abnormal structure of nuclear membrane and also fusion of some nuclei ***(Plate 7.3b).***

Lymphoma implanted BRH_2 treated kidney after 20^{th} day displayed similarities with that of normal cellular structure, capillaries, proximal & distal convoluted tubules ***(Plate 7.3c).***

DAL implated BRH_2 treated kidney cell on 35 day displayed large sized vacuolated nucleus and also showed more vacuolated space between the proximal and distal convoluted tubules ***(Plate 7.3d).***

Control Daltons lymphoma cell collected on the 20^{th} day of tumor transplantation were noticed to be in various advanced stages of the formation of cellular connections with neighboring cells ***(Plate 7.4 a - f).*** More than one cellular process – connection could arise on a cell, each appearing on the side of the cell exactly opposite to the process connection arising on the adjacent cell ***(Plate 7.4 c - f).*** Tumor cells showed the

KIDNEY

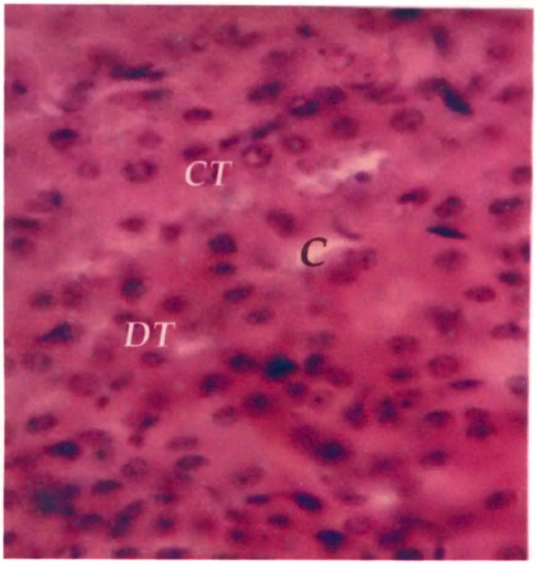


Plate 7.3 a. Control X1000

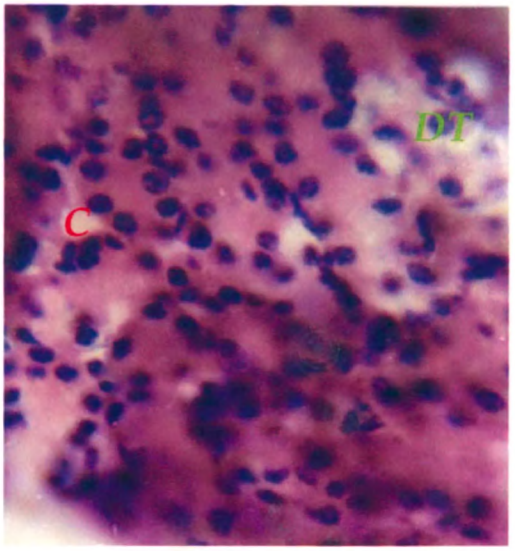


Plate 7.3 b. DAL implanted (after 20 days) X 1000

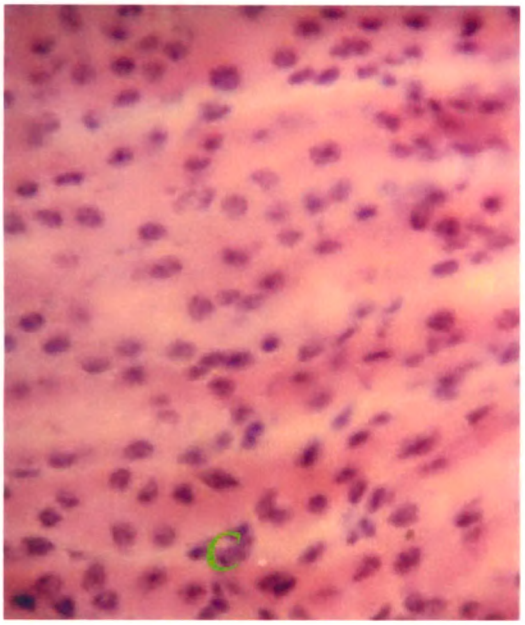
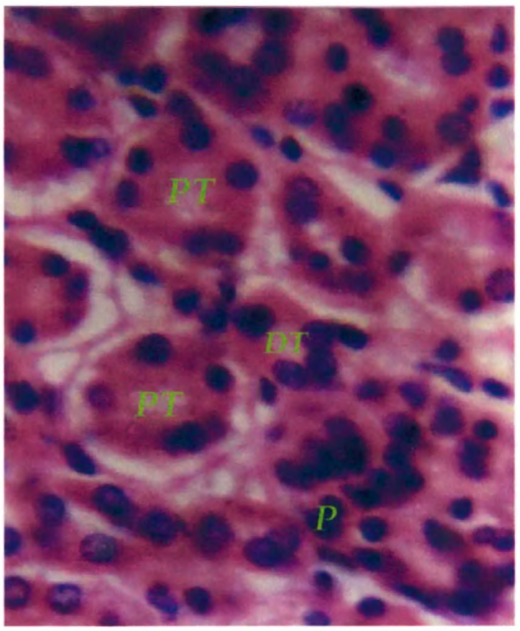


Plate 7.3 c. DAL + BRH_2 treated (after 20 days) X1000



Palte 7.3 d. DAL + BRH_2 treated (after 35 days) X1000

Plate 7.3 a. Control kidney cell normal cellular structure.

Plate 7.3 b. Showing loss of normal cellular pattern.

Plate 7.3 c. Showing some changes in structural pattern of cells.

Plate 7.3 d. Showing some structural changes in the proximal and distal tubule.

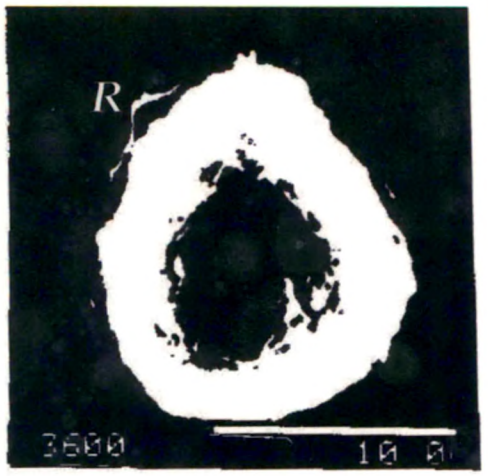
(C = Capillaries, CT = Collecting tubule

P = Podocytes, DT = Distal tubule

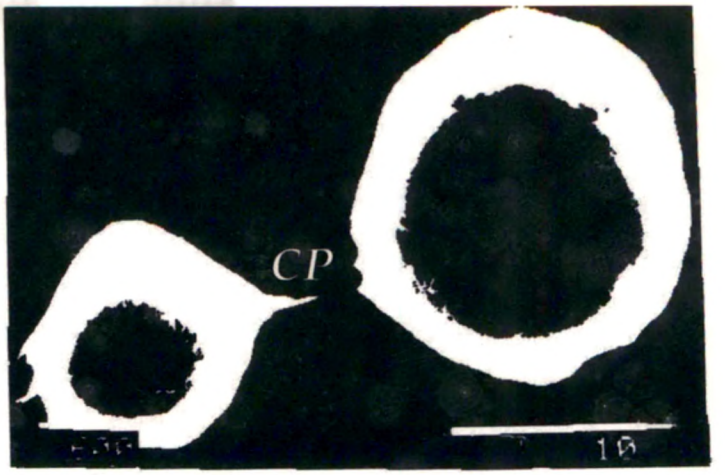
PT = Proximal tubule).

presence of blebs and ruffles all over the cell ***(Plate 7.4a).*** Fusion of 2 – 4 cells, resulting in the formation of multinucleated tumor cell ***(Plate 7.4 d, e, f)*** were noticed.

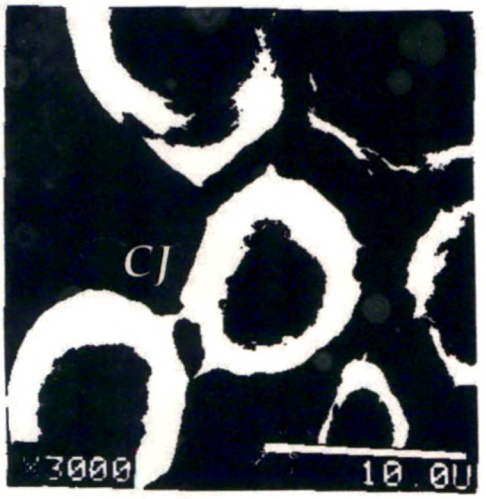
BRH_2 exposed Dalton's lymphoma cells in vivo does not show significant changes in the pattern of cell-cell association; but it was able to create certain changes or reorientation in the arrangement of ruffles and blebs over the tumor cell. After 20^{th} days of BRH_2 treatment the appearance of thick, pronounced, flat ruffles, blebs and also some small size hole on the surface of plasma membrane of lymphoma cell were recorded ***(Plate 7.5 a, b, c).*** Movement of blebs – ruffles from the top surface of the cells towards the marginal areas was also seen ***(Plate 7.5 b).*** After 35 days of BRH_2 treatment many small dense ruffles are seen towards the cell margin ***(Plate 7.5 d, e, f)***. More disintegration of large size hole and breaking of the plasma membrane were noticed with the removal of some cellular material, ***(Plate 7.5 g, h).***



a



b



c

d

e

f

Plate 7.4 SEM images of control ascite Dalton's lymphoma cells after 20 days of implantation (a,b,c,d,e,f).
R = Ruffles ,
CP = Cellular process, CC = Cell-cell connection,
CJ = Cellular joining, FC = Fusion of cell.

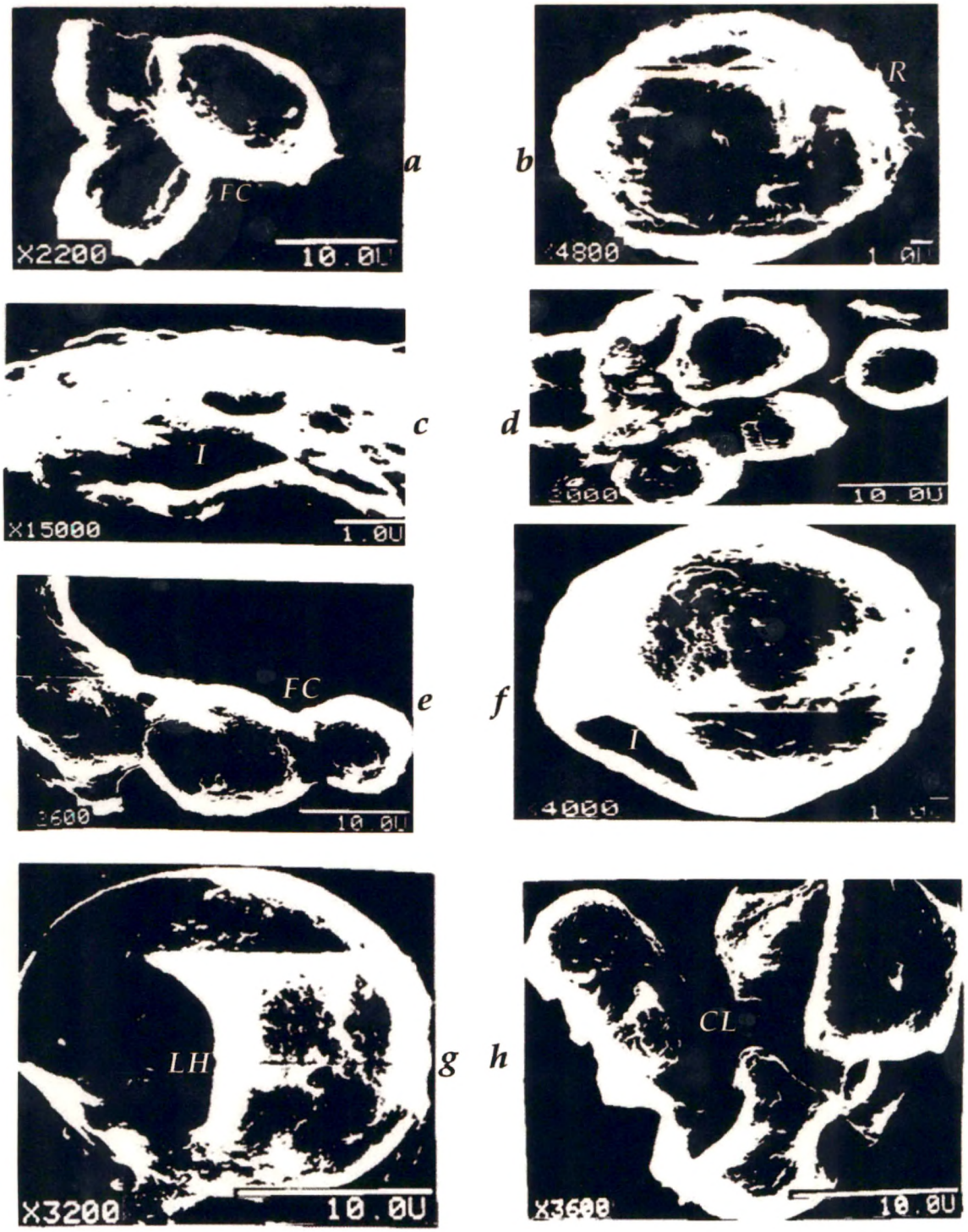


Plate 7.5 SEM images of Dalton's lymphoma cells treated with BRH_2 a, b & c after 20 days; d, e, f, g & h after 35 days.

FC = Fusion of cells, R = Ruffles,
I = Invegination, LH = Large size hole,
CL = Cytolysis

Discussion:

Light Microscopic images of normal vs. DAL implanted and DAL implanted vs. chemotherapy exposed host tissue extend excellent opportunities for characterization of the epithelial cell changes. Morphological changes of the cell are among the first alteration when the normal cells begin to transform due to the action of specific gene (Ambros et al., 1975).

Sequence of changes for carcinogenesis in mouse liver had already been described (Squire and Levitt, 1975; Lipsky et al., 1979). The DAL implanted tissue demonstrated cytoplasmic vacuolation with multinucleated cells ***(Plate 7.1b, 7.2b, and 7.3b)***. Hsich (1990) observed nucleated giant cells on rabbit VX_2 carcinoma after 10 days (i/p) injection of cisplatin. The DAL implanted BRH_2 treated liver tissue after 20 days have clearly shown somewhat near normal structure; but after 35 days it shows cellular lysis under light microscope. The DAL implanted and BRH_2 treated spleen and kidney after 20^{th} days shows approximate normal structure, however, after 35^{th} day vacuolation and cellular lysis occur.

Dalton's lymphoma cells in the ascites fluid were observed to be mainly distributed in islands of 2 – 3 or 4, 5 or more cells connected together or as single cells. It is observed that the cells were noticed to have tendencies to form cellular connections with the neighbouring cells ***(Plate 7.4 b, c, d, e, f)***. Cells showed the appearance of one or more cellular connections –

processes arising exactly opposite to the cellular processes at the adjacent cell ***(Plate 7.4 c, d)***. The presence of groups of 2 – 3 or more cells in ascites fluid may be helpful in the acquisition of the characteristic growth properties of tumor cells in the host. Cell – to – cell adhesion in malignant cells has been suggested to be a multifunctional process and it regulates the pattern of growth and behaviour of malignancy in tumors (Curtis, 1973). Fusion of 2 – 3 cells resulting in the formation of a multinucleated tumor Dalton's lymphoma cell ***(Plate 7.4. c, d, e, f; 7.5. a, d, e)***, which probably acquires a more stable metabolic existence. Cell connections from the channels which probably regulate the hydrophilic pathway between adjacent cells and thus help in transport of ions and small molecules from one cell to another (Loewenstein, 1975 and Good enough, 1976).

Ascites Dalton's lymphoma cells showed the presence of numerous cytoplasmic blebs and ruffles all over the cells with some infolding of plasma membrane ***(Plate 7.4 a; 7.5 b, c, f)***. The membrane infoldings have been well marked in ascitic cells (Gupta et al., 1985). Porter et al. (1973) reported the presence of unusual ruffles on several virally and spontaneously transformed Balb /C_3T_3 Cells and showed that ruffles appeared around the cell margin and occupied a significant part of the top surface of the cells, a feature probably related to the known capacity of tumor cells to phagocytose their environment. Other transformed cells that have been reported to have large numbers of ruffles on the surface of interphase cells include mouse sarcoma 180, mouse thymoma cells, rat sarcoma 4337,

mouse hepatoma 129 cells (Porter et al., 1973), and malignant melanoma A 375 cells (Gonda et al., 1976). Blebs have also been reported to appear in unusual numbers on many transformed cell lines, e.g. adenovirus type – 5 – transformed hamster embryo cells, human carcinoma A 549, rhabdomyosarcoma A1186, and mouse embryo cells transformed chemically (Gonda et al., 1976). It is likely that blebs result from alterations of the cortical microfilament network (Allred et al., 1979).

BRH_2 treatment of Dalton's lymphoma cells in vivo did not show significant changes in the pattern of cell - cell association in groups or as single cells which in turn suggests that it does not change cell-cell connections. However, BRH_2 treatment showed significant changes in the arrangement – movement of ruffles and blebs over the cells and some holes of variable size on the plasma membrane ***(Plate 7.5 a, b, c, f).*** The more larger hole and disintegration and breaking of plasma membrane observed at 35^{th} day of the BRH_2 treatment, which could lead to the lysis of tumor cells into small fragments. Ribereau – Gayon et al. (1986) reported that bacterially fermented mistletoe preparation (BFMP) treatment brings significant modifications of cell surface of rat hepatoma cells and disintegration of the plasma membrane takes place in the anti-tumor effect of BFMP. In the present studies also reorientation in the arrangement of cell surface ruffles-blebs and the disintegration in the plasma membrane resulting from BRH_2 treatment seem to be the direct cause of tumor cell lysis. The direct disintegration and braking in the plasma membrane was more pronounced

after treatment of cisplatin within 6 to 8 days (Prasad & Arjun, 1991). This is supported by the observation at 20th day and 35th day of BRH_2 treatment when most of the plasma membrane is noted to be perforated by hole formation and disintegrated with the removal of some cellular material ***(Plate 7.5 b, c, f, g, h)***.

Present investigation projected significant structural changes in DAL implanted and DAL implanted with BRH_2 treated groups. Therefore, it is believed that the BRH_2 provide some anti-tumor potentiation on the DAL implanted Liver, Spleen and Kidney of white mice.

Summary:

1. Tissue necrosis, loss of cell adherence, cracks, vacuolation, enlargement of nuclei, lysis of the plasma membrane were observed on 20th day DAL implanted tissues under LM.

2. BRH_2 treatment at the initial stage shows some recovery of the cellular structure towards normal but at the later stage showed lysis of the plasma membrane as well as cellular lysis was evident under L.M.

3. Ascite DAL cells are distributed as single cells and in groups of 2 – 3 or more cells connected together that may lead to the fusion of the cells, results in the formation of a multinucleated tumor cell. Presence of numerous cytoplasmic blebs and ruffles all over the cells with some infolding of plasma membrane were evident.

4. BRH_2 treatment of the tumor cells in vivo does not affect the pattern of cell – cell association but it brings about significant changes in the arrangement of ruffles – blebs over the cell and causes disintegration in the plasma membrane to lyze the tumor cells.