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LIVER CANCER: DIFFERENT APPROACHES FOR TARGETING

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ABSTRACT : Cancerous tumors of the liver can be primary cancer – cancer starting in the liver itself or secondary or metastatic cancer – cancer which started in another part of the body and has spread to the liver. Surgery is the most effective treatment for primary liver cancer, but this is not always possible due to the size or position of the tumor and hence chemotherapy is used sometimes to treat these cancerous tumors that cannot be removed. Chemotherapy is also given following surgery to prevent relapse of the cancer. Chemotherapeutic agents used in treatment of liver cancer are not specific for the treatment of liver cancer and suffers from the problem of serious toxicities to the normal cell of the liver as well as to other cells. This can be overcome by targeting drug to the liver by different approaches such as by designing prodrugs which may be distributed to all body tissues but cleaved only in liver or tethering a moiety which binds with the receptor on liver cells. New drugs are being developed that work in a different way from standard chemotherapeutic drugs.
Key words : Liver cancer, Targeting Strategies.

1. INTRODUCTION[1]

Cancerous (malignant) tumors of the liver can be primary cancer – cancer starting in the liver itself or secondary or metastatic cancer – cancer which started in another part of the body and has spread to the liver. There are two different types of primary liver cancer. First type is **Hepatoma** or **hepatocellular carcinoma** (HCC) which arises from the main cells of the liver (the hepatocytes). This type is usually confined to the liver, although occasionally it spreads to other organs. It is more common in men and occurs mostly in people with a liver cirrhosis. The second type of primary liver cancer is **Cholangiocarcinoma or bile duct cancer** is so called because it starts in the cells lining the bile ducts. Cholangiocarcinoma is more common in women.

Some primary tumors in the liver are non-cancerous (benign) and do not spread to other parts of the body. They are usually small and may cause no symptoms. They are often discovered by chance during operations or investigations for other conditions. Unless they are causing symptoms they do not usually need to be removed.

2. TREATMENT OPTIONS

2.1 SURGERY[2]

During the past decade, one of the major changes in the field of oncology has been in the surgical approach to primary and secondary cancer of the liver., but this is not always possible due to the size or position of the tumor. It is also not possible to operate if the cancer has spread beyond the liver. If the liver is severely damaged by cirrhosis it may not be safe to have surgery, but can only be done in a very few cases when the tumor is small (less than 5cm) or there are less than three tumors, all smaller than 3cm in size. Various types of surgeries that can be performed depending on tumor size, tumor location includes liver resection, lobectomy, liver transplant, radio surgery and theraspheres.

2.2 TUMOR ABLATION[3]

This type of treatment is used for tumors less than 5cm (2 inches) in diameter. Liquids such as alcohol (ethanol) or acetic acid are injected through the skin and into the tumor. The liquids destroy the cancer cells. Ultrasound can be used to guide the needle

directly into the tumor. If the tumor grows again, the treatment can be repeated.

2.3 LASER OR RADIOFREQUENCY (THERMAL) ABLATION[4]

This treatment uses a laser or electrical generator to destroy the cancer cells. Under local anesthetic, a fine needle is inserted into the centre of the tumor. Powerful laser light or radio waves are then passed through the needle and into the tumor; these heat the cancer cells and destroy them.

2.4 RADIOTHERAPY[5]

Radiotherapy is the use of high-energy x-rays to destroy cancer cells, while doing as little harm as possible to normal cells. It is not usually used to treat hepatomas, but it may be used to treat Cholangiocarcinoma.

2.5 OTHER TREATMENTS

Cryosurgery or cryotherapy , chemoembolisation, antineoplastons therapy and ethanol injection into tumor are treatments which are still being evaluated as part of research trials.

3. CHEMOTHERAPY FOR LIVER CANCER[6]

Chemotherapy is the use of anti-cancer (cytotoxic) drugs to destroy cancer cells. It is sometimes used to treat primary liver cancers that cannot be removed.

Chemotherapy drugs are usually given by injection into a vein (intravenously) or by injecting the drug directly into the hepatic artery (the blood vessel that takes blood to the liver).

Chemotherapy is often given as a session of treatment, usually lasting a few days. This is followed by a rest period of a few weeks to allow body to recover from any side effects of the treatment. The number of sessions will depend on the type of liver cancer and how well it is responding to the drugs.

Chemotherapy can sometimes cause unpleasant side effects, but it can also make you feel better by relieving the symptoms of the cancer. Any side effects that occur are usually temporary and can often be well controlled with medicine. The main side effects are a reduced resistance to infection, feeling sick, a sore mouth, and hair loss.the drugs most often used for liver cancer Doxorubicin (Adriamycin), Cisplatin, Methotrexate, 5FU (fluorouracil).

Though above mention drugs are used in treatment of Liver cancer, none of them are specific for the treatment of liver cancer and suffer from the problem of serious toxicities to the normal cell of the liver as well as to other cells. This can be overcome by targeting drug to the liver by different approaches such as by designing prodrugs which may be distributed to all body tissues but cleaved only in liver or tethering a moiety which binds with the receptor on liver cells.

4.DIFFERENT APPROACHES FOR SPECIFICALLY TARGETING LIVER CANCER

4.1 TARGETING CORBOXYL ESTERASES (CES) OVER EXPRESSED BY CANCEROUS LIVER CELLS[7]

Since carboxyl estrase-1(CES-1) & carboxyl estrase-2(CES-2) are over expressed in liver cancer cells, the derivative of the drugs can be designed in such way that they will be converted to active form by CES-1 or CES-2 in liver. Thus active form of the drug is available to target cancer cells of liver. Carboxyl esterases are serine esterases that causes hydrolysis of various esters and carbamates. Therefore carbamate prodrugs of some anticancer drugs are used to target liver cancer. The substrate selectivity of carboxyl estrases stems from pairs of recognisation pockets. One pocket is small, rigid cavity that commonly recognizes small alcohol or ester functional group and other is a large flexible pocket capable of accommodating numerous substrates for hydrolysis.

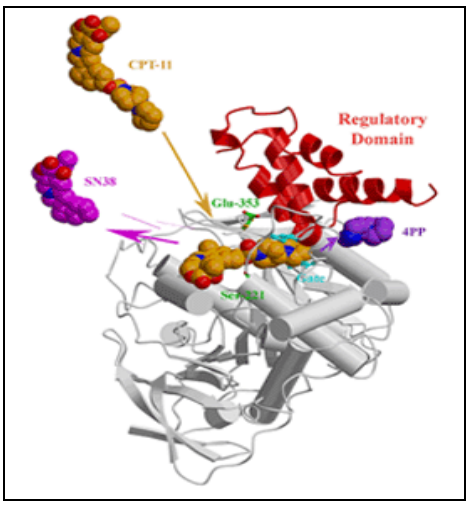

Figure 1 : (carboxylestrase)

4.2 DESIGNING PRODRUGS THAT CLEAVE USING INTRACELLULAR LIVER ENZYMES.

4.2.1 Carbamate prodrugs of doxazolidine[8]

Doxazolidine (Doxaz) is oxazolidine derivative of doxorubicin.

For in vivo treatment of tumor, release of Doxaz from hydrolytically robust prodrugs (carbamate) by a CES enzyme over expressed by liver cells at the site of tumor increases tumor response and minimizes side effects.

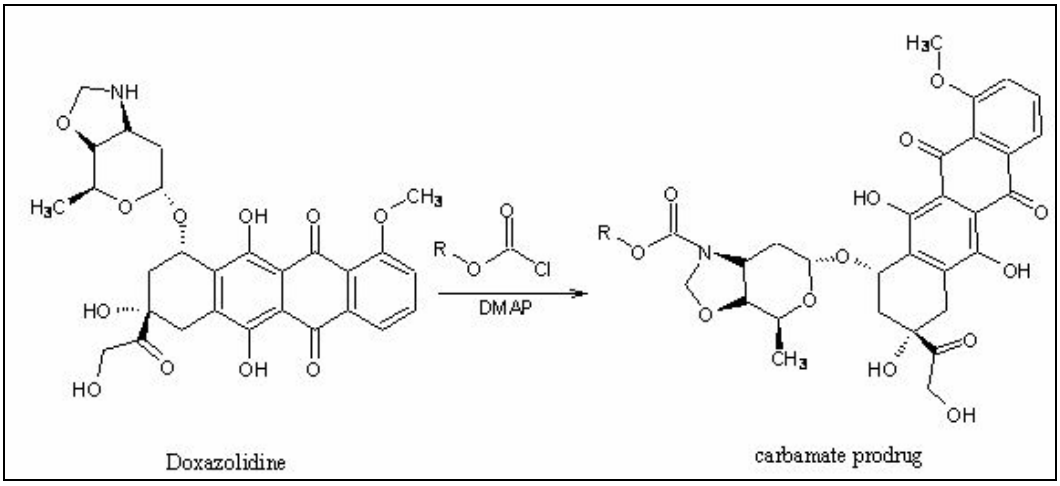

Figure 2 : synthesis of carbamate doxazolidine prodrugs

R=Et	Doxaz ethyl carbamate
R=Bu	Doxaz butyl carbamate
R= pentyl	Pentyl Doxaz ethyl carbamate

The pentyl and butyl carbamate inhibited the growth of cancer cell. Ethyl caramate with least complex structure exhibited poor cancer cell growth inhibition. The relative inactivity of the ethyl carbamate indicate that a substantial lipophillic interaction is required at the active site of the carboxyl esterase to hold the doxaz substrates.

S.E.: cardiac toxicity is relevant because the ultimate product of metabolism and substrate hydrolysis of these carbamates is doxorubicin, which is cardiotoxic.

4.2.2 Prodrugs of doxazolidine carbamates with self eliminating spacer[8]

It was found that the drug efficacy might be improved by adding a self-eliminating spacer between alkyl carbamate functionality and the antracycline. This could the lipophilic carbamate to the enzyme with significantly less steric bulk and increases the rate of enzyme hydrolysis.p- Amino benzyl alcohol(PABA) is used to incorporate spacer (Figure 3).

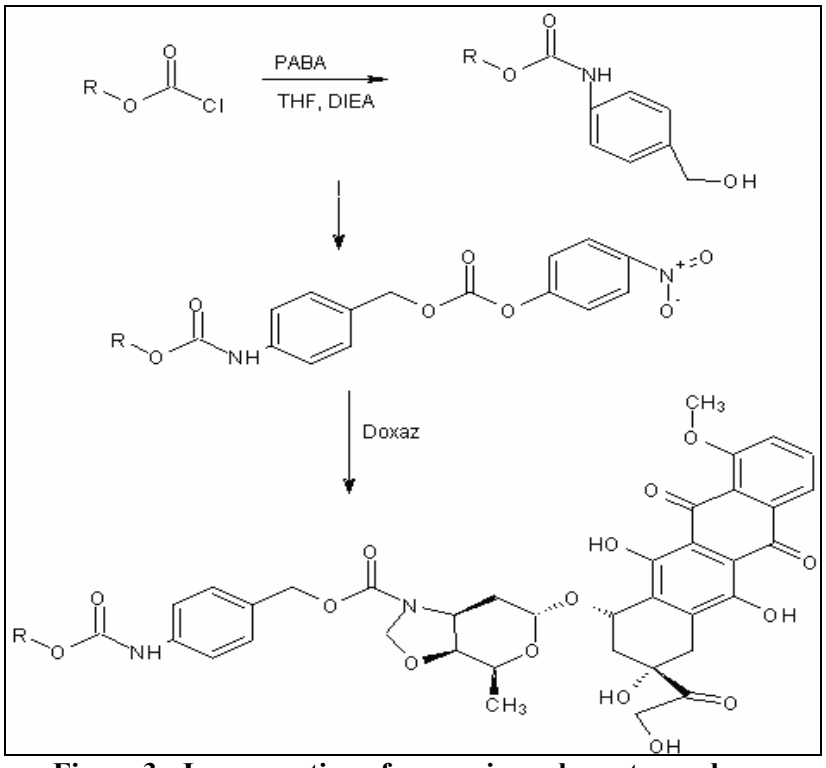

Figure 3 : Incorporation of spacer in carbamate prodrugs

Growth inhibition experiments with butyl and pentyl carbamates bearing PABA self eliminating spacer focused on HEP-G2 liver cancer cells that strongly express CES2 than CES1. These compounds are less cardiotoxic than simple carbamates. Thus these compounds can target liver more efficiently.

4.2.3 Carbamate derivatives of 5-FU[9]

Capcitabine is carbamate prodrugs of 5-FU. Capcitabine is the pentyl carbamate of 5- Flurocytidine derivative that functions as a prodrug of the antitumor compound 5-FU.

In vivo activation requires three enzymatic steps, the first of which is hydrolysis of the pentyl carbamate functional group by carboxylestrases (Figure 4). Capecitabine is a substrate for human CES2 with CES1 being more active enzyme. Thus can function as good cytotoxic agent in HCC as CES is most over expressed it the cancer cells of liver.

4.2.4 Carbamate derivative of camptothecin[10,11]

Irinotecan is the carbamate prodrug of water soluble camptothecin and is hydrolyzed primarily by CES2(Figure 5).

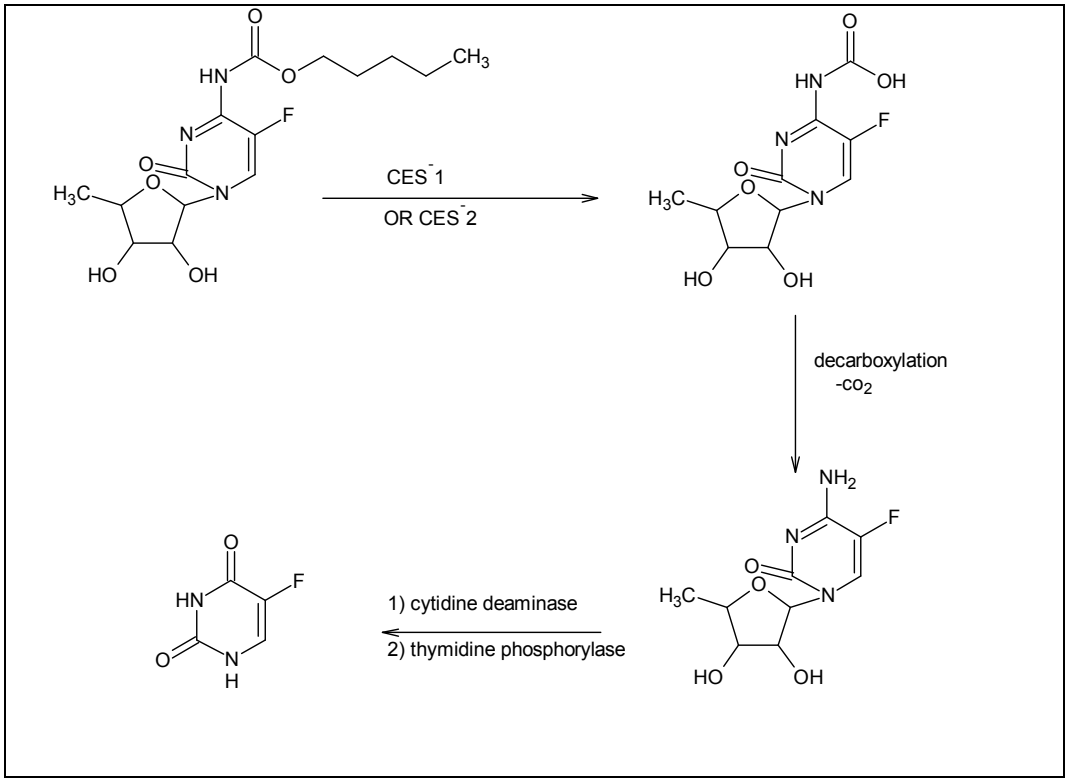

Figure 4 : Proposed enzymatic activation of capecitabine by human CES

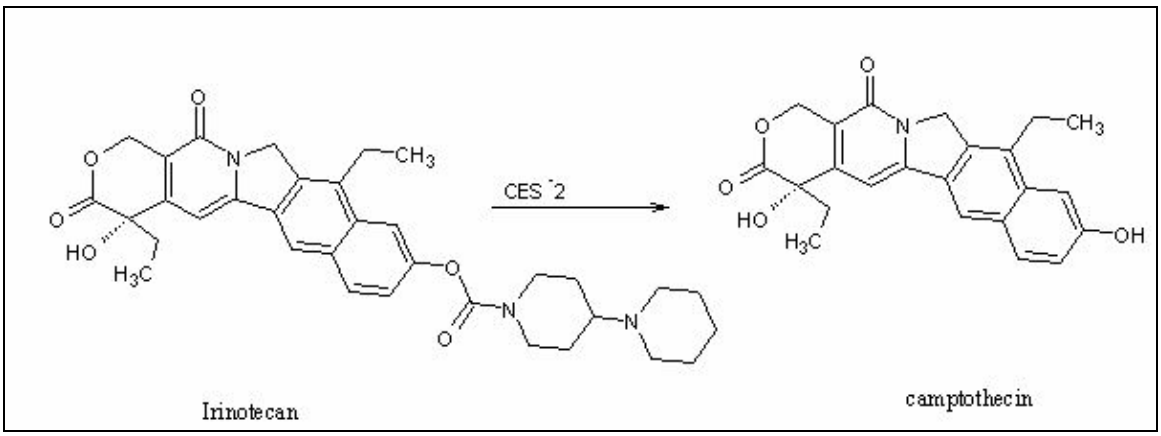

Figure 5 : proposed enzymatic activation of irinitecan by CES2

In spite of the substantial structural differences between capecitabine and irinotecan, they are both hydrolyzed by carboxyl estreses CES1&CES2. But in general CES1 prefers smaller alcohol moiety as in capecitabine and CES2 , a larger alcohol moiety as in irinotecan.

4.3 TETHERING SITE SPECIFIC CARRIERS TO THE DRUG MOLECULES

This approach is specifically used to target platinum anticancer drugs to liver cancer cells.

4.3.1 Tethering carbohydrate moieties[12]

One strategy for selective delivery of platinum anticancer agents is to tether carbohydrate moieties to the platinum center. Such compounds have primarily been designed to target liver cancer because of the galactose receptors are present on the surface of parenchymal cells of liver which will selectively take molecules bound to galactose(Figure 6).

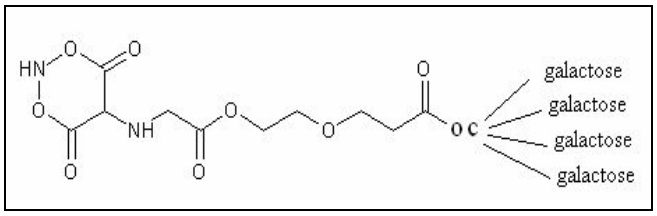

Figure 6 : Molecule tethered to galactose

4.3.2 Tethering bile acids[13]

Another strategy for targeting liver cancer is the use of bile acids, which are effectively taken up by hepatoma cells via sodium independent transport carriers. A series of bile acid-platinum conjugates have been synthesized and their cytotoxicity investigated both in vivo and in vitro.

Conjugate Bamet-UD2 exhibited enhanced uptake in hepatocytes and has got ability similar to that of cisplatin to inhibit tumor growth and tendency to prolong survival time.

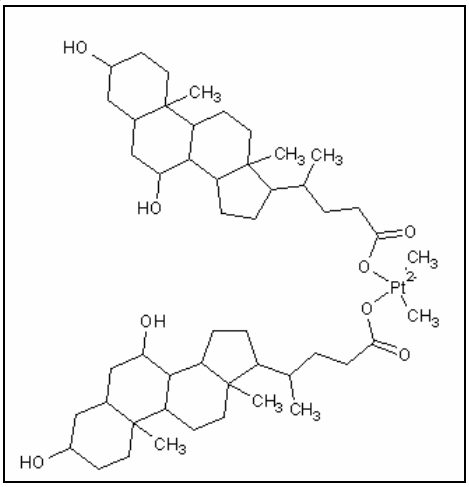

Figure 7 : Bomet UD-2

4.4 DESIGNING PRODRUGS THAT CLEAVES USING INTRACELLULAR LIVER ENZYMES.

4.4.1 Hepdirect prodrugs[14]

A series of phosphate and phosphonate prodrugs, called HepDirect prodrugs, results in liver-targeted drug delivery following a cytochrome P450-catalyzed oxidative cleavage reaction inside hepatocytes.

Liver targeting was demonstrated in rodents for MB07133 [(2*R*,4*S*)-4-amino-1-[5-*O*-[2-oxo-4-(4-pyridyl) -1,3,2-dioxaphosphorinan-2-yl]-β-D-arabino furanosyl]-2(1*H*)-pyrimidinone], a HepDirect prodrug of cytarabine (araC) 5'-monophosphate (Figure 8). Liver targeting led to higher levels of the biologically active form of araC in the liver. Liver targeting also confined production of the prodrug byproduct, an aryl vinyl ketone, to hepatocytes. Glutathione within the hepatocytes rapidly reacted with the byproduct to form a glutathione conjugate. No byproduct-related toxicity was observed in hepatocytes or animals treated with HepDirect prodrugs.

These findings suggest that HepDirect prodrugs represent a potential strategy for targeting drugs to the liver and achieving more effective therapies against chronic liver diseases such as hepatitis B, hepatitis C, and hepatocellular carcinoma.

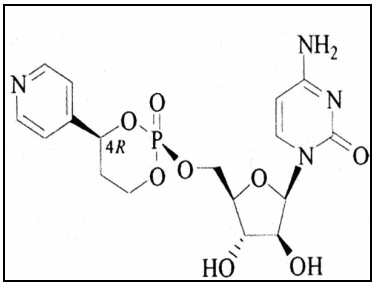

Figure 8 : compound MB07133

Activation process of HepDirect prodrugs

These prodrugs are cyclic 1,3-propanyl esters containing a ring substituent that renders them sensitive to an oxidative cleavage reaction catalyzed by a cytochrome P450 (P450) (Erion et al., 2004✦). Prodrugs with a 4-aryl substituent (Figure 9) are oxidized specifically by the P450 isoenzyme family CYP3A, which is expressed predominantly in the parenchyma cells of the liver and to a lesser extent the enterocytes of the small intestine (de Waziers et al., 1990✦). Oxidation results in ring opening and the generation of a transient negatively charged intermediate (**3**), which is retained inside the cell. A subsequent β-elimination reaction produces the phosphate or phosphonate (**4**) and the prodrug byproduct, i.e., the aryl vinyl ketone (**5**). The latter undergoes rapid conjugation with glutathione (GSH), which exists at mill molar levels in the liver.

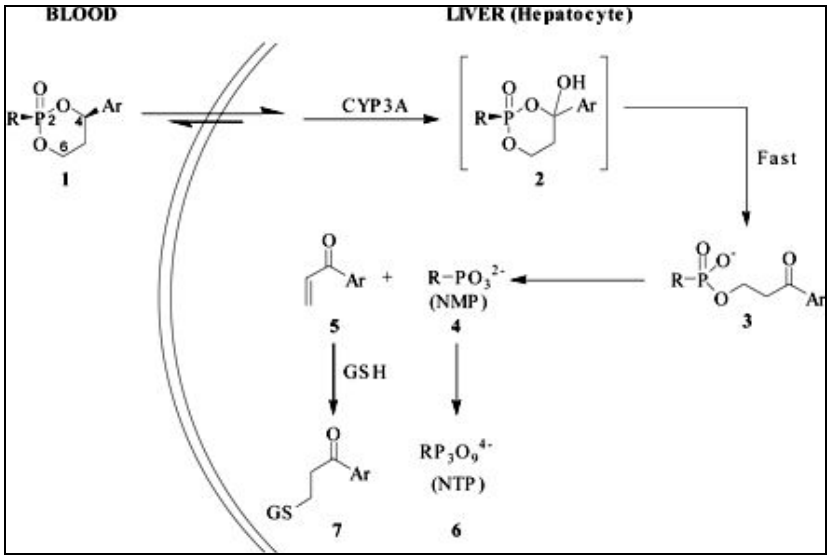

Figure 9 : Prodrug cleavage mechanism and products.

4.4.2 Prodrugs of 5- fluorouracil (5-FU)[15]

Dihydropyrimidine dehydrogenase (DPD) is a NADPH-dependant enzyme and catalyzes first step in the degradation pathway of 5-FU. This process is of major importance since it leads to decreased availability of 5-FU in tumor tissue. DPD has abundant expression mainly in liver and in tumor cells of liver this enzyme is also active.

Inhibiting this enzyme will leads to enhanced availably of 5-FU in liver and increased cytotoxicity to liver tumors.Two inhibitors are available- ethyl uracil & CDHP (5-Chloro-2,4-dihydropyridine)(Figure 10).

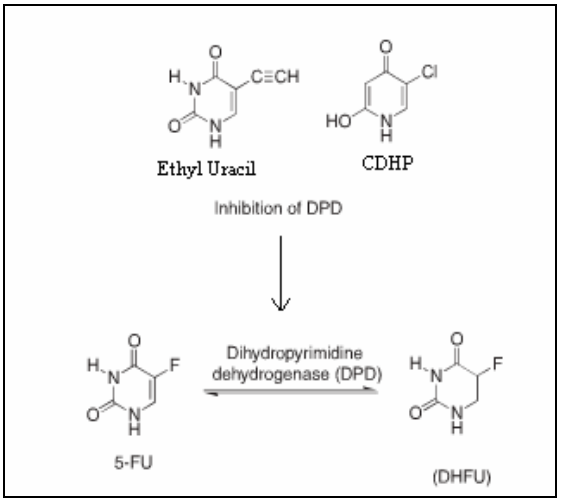

Figure 10 : Prodrugs of 5-FU.

4.5 VEGFR-2 INHIBITORS[16]

The VEGF-receptor family (vascular endothelial growth factor) is involved in the process of the development of new blood vessels, the angiogenesis. The early phase of formation of many tumors is associated with increased angiogenesis. Related to this an increased expression of pro-angiogenic factors like the VEGF-receptors 1-3 is observed. VEGFR-2 is considered as the most important. Vascular endothelial growth factor-2 (VEGFR-2/Flk-1) is a receptor tyrosine kinase (RTK) whose activation regulates angiogenesis in liver cells.

For example, a drug called erlotinib (Tarceva®), which targets VEGFR on cancer cells, has shown some benefit in people with advanced liver cancer in early studies. Several other targeted drugs are now being studied as well.

Li.Sun.et.al have reported Series of 3-substituted indolin-2-ones(Figure 11) with potent and selective inhibitory activity toward VEGF-R2. the general formula for the series is as fallows.

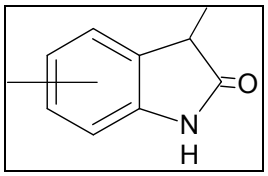

Figure 11 : (3-substituted indolin-2-ones)

4.6 TREATING LIVER CANCER WITH GLASS BEADS[17]

A new method for treating liver cancer with tiny radioactive glass beads has been introduced recently. The treatment consists of injecting millions of tiny, radioactive glass beads into the main artery supplying blood to the liver. The blood carries the beads into the liver, where they deliver localized radiation to malignant cells in liver tumors.

4.7 USE OF CHEMOPREVENTIVE AGENTS:

Chemoprevention is the use of either synthetic drugs or natural products to inhibit, reverse or suppress the development of invasive malignant cancer, either

- by blocking the DNA damage that initiates carcinogenesis
- By arresting or reversing the progression of premalignant cells in which DNA damage has already started.
- Chemoprevention is one of the most direct way to reduce cancer related morbidity and mortality.

4.7.1 Zapotin[18]

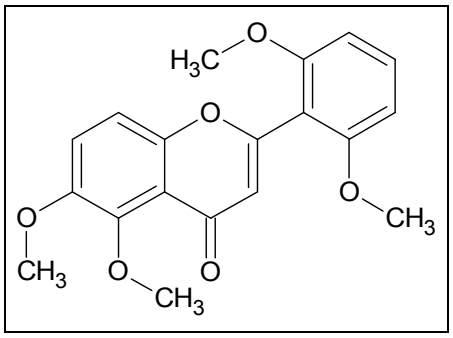

Figure 12 : Zapotin

Zapotin is polyoxymethylated flavonoid from Zapote blanco seeds on Casimiroa edulis, has been found to be non toxic inducer of cellular differentiation.

It causes inhibition of TPA- induced NF-kB activity in HepG2 cells.

Nuclear factor-kB (NF-kB) is an inducible transcription factor for genes involved in cell survival, cell adhesion, differentiation and growth. In normal cell NF-kB is present in cytoplasm binds to the inhibitory IkB proteins which blocks the nuclear localization sequence of NF-kB activation of NF-kB by variety of stimuli such as carcinogens , tumor promoters like cigarette smoke, phorbol ester and okadaic acid promote degradation of IkB and thus unmasks the nuclear localization sequence permitting NF-kB to enter the nucleus and bind to specific sequence in DNA , which in turn results in transcription of targeted gene. Various genes that are involved in tomor cell invasion and angiogenesis have been found to be regulated by NF-kB activation of NF-kB promotes survival and proliferation and down regulation of NF-kB sensitizes the cell to apoptosis. This has encouraged a search for specific inhibitor of NF-kB activation from natural sources which might lead to good chemoprevention. Zapotin displayed promising inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA) induced NF-kB activity with human hepatocellular liver carcinoma cells.

4.7.2 L-carnitine[19]

A research group in King Saud University, Kingdom of Saudi Arabia investigated, for the first time, the role of carnitine, a naturally occurring compound that is synthesized mainly in the liver, during the development of hepatocarcinogenesis. Authors of the study reported that carnitine deficiency is a risk factor and should be viewed as a mechanism in hepatic carcinogenesis, and that long-term L-carnitine supplementation prevents the development of liver cancer. Therefore, carnitine supplementation alone or in combination with other natural chemopreventive compounds could be used to prevent, slow or reverse the occurrence of liver cancer (unpublished data).

5. ADVANCES IN LIVER CANCER RESEARCH

5.1 Genetic link to liver cancer[20]

A gene that causes liver cancer in mice has been identified by researchers at the University of Illinois at Chicago. When the researchers deleted the gene, called Foxm1b, from liver cells in lab mice, the animals didn't develop tumors. The mice with the deleted gene remained cancer-free even when the researchers tried to use artificial means to induce tumors

This is the first time a gene has been directly linked to the growth of cancer cells in live animals. a prototype for a drug to block Foxm1b activity and starve tumor cells of the protein manufactured by Foxm1b is under study.. Depriving tumors of this protein prevents them from multiplying

5.2 Targeting nerve growth factor (NGF) may help cure liver cancer[21]

The study was conducted by researchers from the National Research Council of Italy, Marino Hospital in Rome, Regina Elena Cancer Institute in Rome, and University of Rome led by Dr. Annalucia Serafino.The scientists revealed that in patients' livers troubled with liver cirrhosis and/or hepatocellular carcinoma (HCC), NGF and its receptor TRKA NGF were expressed, whereas these two molecules were not detected in the livers of healthy people. In order to affect a cell by a growth factor, it is necessary that there should be its specific receptor expressed on the surface of the target cell. In the liver of patients, both NGF and its specific receptor are expressed abnormally, NGF is either expressed by liver cells to affect themselves, so called as autocrine, or to affect adjacent cells, called as paracrine, in patients with liver cirrhosis and/or HCC. Based on these discoveries, it can be understood that a critical role is being played by NGF in the development of liver cirrhosis and its progression towards HCC

5.3 Reptin: key role in liver cancer[22]

This protein has been known for about ten years, but this is the first time that a study has clearly shown that it has an important role in the development of HCC.

By conducting an analysis of the proteoma , proteins present in cancer cells were compared with those present in the non-cancerous part of the liver. It was found that reptin, or RUVBL2, were present in higher quantities in the cancerous part of liver.

In order to find out if reptin directly plays a role in tumoral progression, they artificially modified its level of expression in human HCC cells. The scientists then found that a reduction in the quantity of reptin in cancer cells stopped cell growth and subsequently caused cell death, whereas an increase in the quantity of reptin made it possible for cancer cells to form larger tumors

These results therefore throw new light on the mechanisms of hepatic carcinogenesis and suggest that reptin constitutes a new and potentially interesting therapeutic target

5.4 Antibody targeting to liver cancer[23]

A study by Xie et.al. shows that genetic engineering of a monoclonal antibody target human hepatocellular carcinoma (HCC). All results indicates that mAb-95 itself and the genetically engineered scFv95 could potentially be used as targeting agents for HCC immunotherapy or immuno-detection.

5.5 Double targeting in liver cancer[24]

Antibody and Magnetic Nanoparticles Provide Double Targeting to Liver Cancer Cells.Cancer researchers have long sought to harness the tumor-targeting ability of monoclonal antibodies with the cell-killing property of radioisotopes, particularly iodine-^{131} (^{131}I). But clinical results with numerous ^{131}I-antibody formulations have failed to live up to expectations, in large part because the therapy is not specific enough for tumors. In attempt to remedy that problem, a group led by Jin Chen, Ph.D., and Changsheng Xie, Ph.D., both at Huazhong University of Science & Technology in Wuhan, China, has added magnetic nanoparticles to the ^{131}I-antibody preparation, and the preliminary results suggest that this approach could be promising for treating human liver cancer. Writing in the journal *Cancer Letters*, the investigators describe how they coupled dextran-coated magnetic nanoparticles to an ^{131}I-labeled monoclonal antibody that binds to vascular endothelial growth factor (VEGF), a protein found on the surface of the blood vessels that surround most solid tumors. The idea here was that a focused magnetic field could be used as an initial targeting vector that would concentrate radioactively labeled antibody in the vicinity of a tumor. Once there, the antibody would provide a second level of targeting to the blood vessels surrounding the tumor. Radiation would then kill the neighboring malignant cells. Results in mice with implanted human liver tumors found that a focused magnetic field did indeed concentrate the nanoparticle-antibody formulation as desired, with very little of the formulation accumulating in healthy tissue. In a second experiment, animals in which the nanoparticle-antibody formulation was injected into tumors experienced a marked shrinkage of the tumors, with little toxicity as measured by white blood cell production and weight loss.

6. CONCLUSION

Surgery is best possible but not always desired option for treating liver cancer & the present chemotherapy is not very much effective in treating liver cancer. In such scenario targeting drugs to liver tissues is an attractive strategy for enhancing drug efficacy and reducing side effects. Various new approaches such as use of antibodies, gene therapy are in research phase and shows promising evidences for targeting liver cancer.

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