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Glutamic acid analogues used as potent anticancer: A review

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ABSTRACTS

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasin that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. Cancer caused about 13% of all human deaths in 2007 (7.6 million). L-Glutamic acid plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA. The synthesis of L-glutamine is hindered in neoplastic cells due to lower reactivity of L-glutamine synthetase. L-glutamic acid γ-(4-hydroxyanilide) a growth regulatory substance isolated from mushroom Agaricus bisporous was found to inhibit B16 mouse melanoma cells in culture. The L-glutamic acid conjugated with paclitaxel is known to form a new anticancer called poly (LGA)-paclitaxel (PG-TXL) with superior antitumor activity. Glutamic acid and its derivatives will be more promising in its action against cancer with minimal side effects as they are endogenic in nature. Looking at the wide profile of activity, it has been proposed that though L-GA and glutamine were once considered nonessential for health, may now be considered as - 'conditionally essential' amino acids.

Keywords: Glutamic acid, Malignant neoplasm, Paclitaxel, Cathepsin.

INTRODUCTION

Glutamic acid or glutamate is one of the 20 most common natural amino acids. As its name indicates, it is acidic, with a carboxylic acid component to its side chain. The side chain carboxylic acid functional group has pKa of 4.1 and exists in its negatively charged deprotonated

carboxylate form at physiological pH. Glutamic acid is critical for proper cell function, but it is not considered an essential nutrient in humans because the body can manufacture it from simpler compounds[1, 2]. In addition to being one of the building blocks in protein synthesis, it is the most widespread neurotransmitter in brain function, as an excitatory neurotransmitter and as a precursor for the synthesis of GABA in GABAergic neurons. Glutamate activates both ionotropic and metabotropic glutamate receptors[3]. The ionotropic ones being non-NMDA (AMPA and kainate) and NMDA receptors. Free glutamic acid cannot cross the blood-brain barrier in appreciable quantities; instead it is converted into L-glutamine, which the brain uses for fuel and protein synthesis. It is conjectured that glutamate is involved in cognitive functions like learning and memory in the brain, though excessive amounts may cause neuronal damage associated in diseases like amyotrophic lateral sclerosis, lathyrism, and Alzheimer's disease[4, 5]. Also, the drug phencyclidine antagonizes glutamate at the NMDA receptor, causing behavior reminiscent of schizophrenia[6]. The sodium salt of glutamic acid, monosodium glutamate (MSG) is responsible for one of the five basic tastes of the human sense of taste (umami), and MSG is extensively used as a food additive. Glutamic acid is one of the 20 proteinogenic amino acids, and its codons are GAA and GAG[7].

Cancer (malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth, invasin that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumors, which do not invade or metastasize. Each year, the American Cancer Society estimates the number of new cancer cases and deaths expected in the United States in the current year and compiles the most recent data regarding cancer incidence, mortality, and survival based on incidence data from the National Cancer Institute, the Centers for Disease Control and Prevention, and the North American Association of Central Cancer Registries and mortality data from the National Center for Health Statistics[8]. Incidence and death rates are age-standardized to the 2000 US standard million population. A total of 1,529,560 new cancer cases and 569,490 deaths from cancer are projected to occur in the United States in 2010[9]. Overall cancer incidence rates decreased in the most recent time period in both men (1.3% per year from 2000 to 2006) and women (0.5% per year from 1998 to 2006), largely due to decreases in the 3 major cancer sites in men (lung, prostate, and colon and rectum) and 2 major cancer sites in women (breast and colorectum)[10]. This decrease occurred in all racial/ethnic groups in both men and women with the exception of American Indian/Alaska Native women, in whom rates were stable. Cancer caused about 13% of all human deaths in 2007 (7.6 million)[11-13].

Cancer in India

The crude incidence rates of cancer in 1997 as recorded by the urban population based cancer registries under National Cancer Registry Programme (NCRP), varied between 52.9 and 81.5 per 100,000 men; and between 56.8 and 95.6 per 100,000 women[14, 15]. The age standardized incidence rates in these registries ranged from 81.8 to 122.8 per 100,000 men; and from 93.5 to 137.7 per 100,000 women[16]. The rural registry at Barshi (Maharashtra) showed crude incidence rates of 32.6 per 100,000 men and 42.9 per 100,000 women; and age standardized rates of 38.2 per 100,000 men and 49.8 per 100,000 women[17]. Cancer incidence in Indian men is about half to one third of the incidence recorded in USA and Europe. Incidence rates in Indian women are

about half the experience of USA and European women. A global comparison shows that India has high incidence rates of cancers of oral cavity, pharynx, and cervix[18].

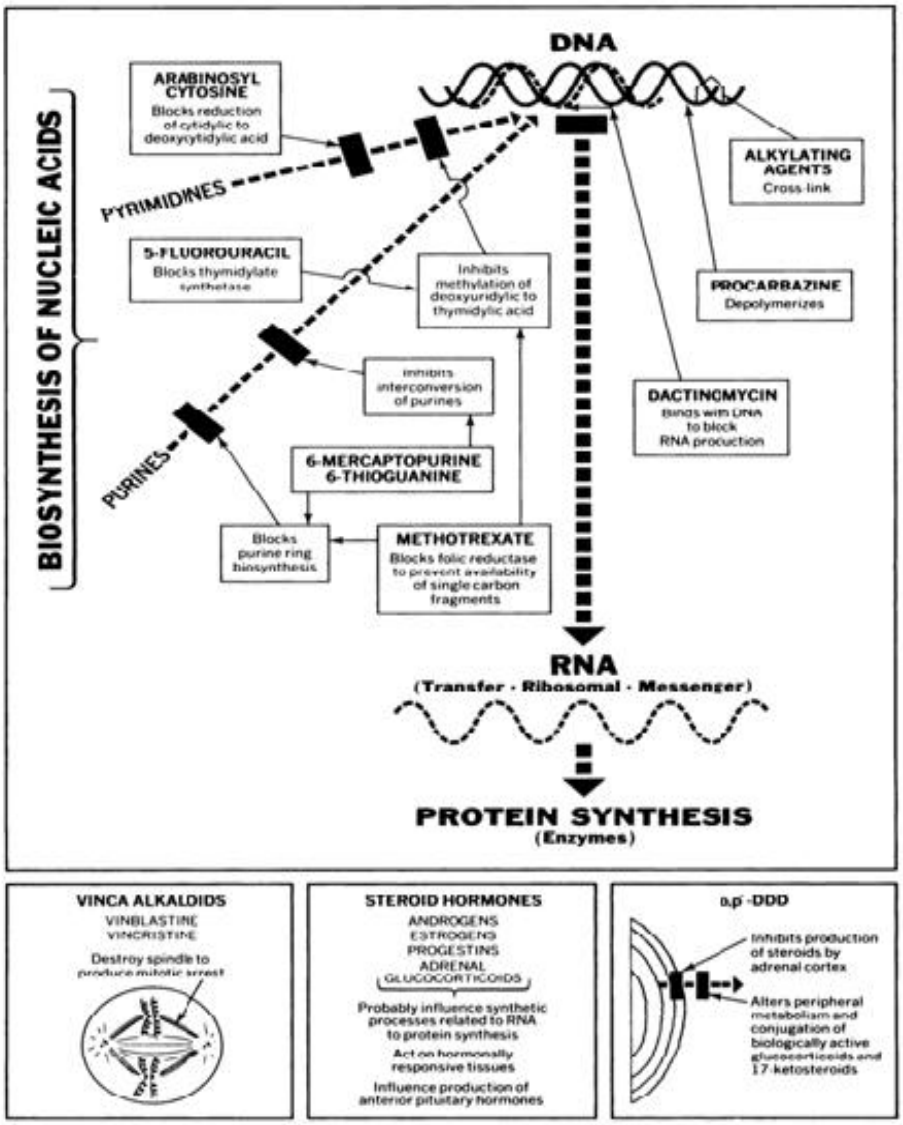

Fig. 1: Mechanism of Action of Anticancer Drugs at a Cellular Level

Cancer Registration in India

Until 1964, information on cancer occurrence in India was available from surveys. Initiation of population based cancer registries at Bombay in 1964, at Pune in 1973, at Aurangabad in 1978, and at Ahmadabad and Nagpur in 1980, started the availability of data on cancer incidence on a continuous basis. However, the boost for cancer registration in India was in 1982, through initiation of NCRP by Indian Council of Medical Research[19, 20]. The NCRP began with three population based (existing Bombay registry and new registries at Bangalore and Madras), and three hospital based registries (at Chandigarh, Dibrugarh and Trivandrum)[21]. Further, expansion

of NCRP saw the initiation of urban population based cancer registries at Bhopal and Delhi in 1987; rural population based cancer registries at Barshi (Maharashtra) in 1987; and hospital based cancer registries at main hospital of PBCRs at Bangalore, Bombay and Madras in 1986. A hospital based cancer registry functioned at Chandigarh from 1982 till 1992. Besides the above mentioned registries population based cancer registries are also functioning at Kolkata, Thiruvananthapuram, Karunagapally (rural Kerala) and Ambillikai (rural Tamil Nadu)[22, 23].

Mechanism of Action of Anticancer Drugs

The available anticancer drugs have distinctly different mechanisms of action which may vary at different drug concentrations and in their effects on different types of normal and neoplastic cells. While not selectively lethal to cancer cells, as such, in many instances these drugs produce more extensive injury and death to certain neoplastic cells than to the normal tissues, presumably because of quantitatively altered metabolic processes in the cancer cell[24]. These selective anticancer effects, thus far, are difficult to anticipate in the individual patient, or to define in terms of demonstrable biochemical differences in the cancer cells. In the great majority of cases, also, initially responsive cancers recur in a form resistant to the previously effective agent[25]. Despite the many unsolved problems, there is a great deal of information on how anticancer drugs act at the cellular level to inhibit the growth of, or to destroy, susceptible cells (Fig. 1).

Cancer Chemotherapy

Chemotherapy drugs, are sometimes feared because of a patient's concern about toxic effects. Their role is to slow and hopefully halt the growth and spread of a cancer. There are three goals associated with the use of the most commonly-used anticancer agents.

1. Damage the DNA of the affected cancer cells.
2. Inhibit the synthesis of new DNA strands to stop the cell from replicating, because the replication of the cell is what allows the tumor to grow.
3. Stop mitosis or the actual splitting of the original cell into two new cells. Stopping mitosis stops cell division (replication) of the cancer and may ultimately halt the progression of the cancer.

L-Glutamic Acid Used As Anticancer

L-Glutamic acid (L-GA), a seaweed ingredient, identified in 1908 by Japanese scientists responsible for enhancing flavor for food is now best known scientifically as monosodium glutamate (MSG)[26]. The other names include – S-(+)-GA, L-GA, 2-aminoglutaric acid, and an anionic form of MSG at physiological pH known as glutamate[27]. The presence of free form of glutamate, not linked to protein is said to enhance flavor in food. It is also called as *palate pleaser*. It is also known as levoglutamide, L-GA 5 amide, L-(+)-2-aminoglutamic acid. It is synthesized in the body from GA and ammonia in an energy requiring reaction. Although nonessential in health, glutamine is conditionally essential in stress and illness. Studies over the last several years have explored the physiological role and therapeutic utility of these molecules in various disease conditions[28-32].

L-Glutamic acid plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA. It is metabolized to L-glutamine by L-glutamine synthetase and this metabolic process is essential for normal maintenance of cells. The synthesis of L-glutamine is hindered in

neoplastic cells due to lower reactivity of L-glutamine synthetase[33]. Thus antagonists of this enzyme can interfere with the metabolic role of L-glutamine and act as anti-cancer agents. Azaserine and 6-diaza-5-oxo-L-norleucine antagonized the metabolic process involving L-glutamine and exhibited antitumor activity in animal models. L-glutamic acid γ-(4-hydroxyanilide) a growth regulatory substance isolated from mushroom *Agaricus bisporous* was found to inhibit B16 mouse melanoma cells in culture[34, 35]. Similarly an arylamide derivative of L-threo-γ-hydroxy glutamic acid isolated from *Justica ghiesbreghtiana* was found to be active against various tumors. The synthetic amides of L-glutamic acid also exhibited activity against Ehrlich ascites carcinoma[36, 37].

Glutamine is one of the most abundant amino acids and participates in a variety of physiological functions, namely - as a major fuel source for enterocytes, as a substrate for neoglucogenesis in kidney, lymphocytes, and monocytes, a nutrient/substrate in muscle protein metabolism in response to infection, inflammation, and muscle trauma[38, 39]. Studies evaluating the role of glutamine have confirmed it's participation in maintaining mucosal integrity of the gastrointestinal tract following it's administration in patients with major bowel surgery. The role of glutamine as a protective agent in hepatobiliary dysfunction and as a supplement in total parenteral nutrition is well established, particularly, in patients under intensive care. L-Glutamic acid (L-GA) physiologically exists as glutamate[40]. Glutamate along with glutamine plays a major role in amino acid metabolism and thus in maintaining nitrogen balance in the body[41]. Glutamate is a well-established excitatory neurotransmitter in the central nervous system. There has been convincing evidence on protective activity of L-GA and α-ketoglutarate in vincristine-induced neurotoxicity. Based on the above information, a large number of studies have been carried out[42-45].

Anticancer activity

The prevalence of cancer-related fatigue in the absence of antineoplastic therapy is said to be 40-75% and is reported to be due to circulating cytokines such as cathepsin-D and Tumor necrosis factor (TNF) (also called asthenins) are proposed to be responsible for metabolic process underlying muscle mass break down observed in cancer patients. It is suggested that nitric oxide could reduce the levels of cathepsin by modulating its synthesis and therefore may benefit cancer patients with fatigue[46]. A double blind placebo-controlled cross over study with oral administration of arginine-glutamate 6 g/day for 6 weeks was reported to increase the endogenous production of NO in patients with endothelial dysfunction. However, the relation between the asthenins and improvement in fatigue is said to require further studies[47, 48].

In 2000, Oldham *et al.* have shown superior anticancer activity of paclitaxel an anticancer agent when combined with L-glutamic acid in human breast cancer. It's anticancer effect has been attributed to it's ability to produce favorable pharmacokinetic profile and distribution of paclitaxel. The L- glutamic acid conjugated with paclitaxel is known to form a new anticancer called poly (LGA)-paclitaxel (PG-TXL) with superior antitumor activity, favorable pharmacokinetic properties and/or mechanism of action different from that of paclitaxel alone. It is suggested that superior activity of PG-TXL may be due to continuous release of paclitaxel. In a double blind placebo-controlled study, administration of glutamic acid prevented the vincristine-induced neurotoxicity, a well known principal limiting side effect[49]. The loss of tendo-Achilles reflex as an objective parameter of vincristine-induced neurotoxicity was reported to be significantly higher in placebo group as compared to glutamic acid group[50]. Intra-thecal

administration of vincristine produced ascending paralysis and death, which was prevented by *i.v.* glutamic acid. Inhibition of disruption of microtubular structures by glutamic acid have been proposed to play a role[51].

The mechanisms through which metastasizing cancer cells cause functional disruption of bone are not fully characterized. Like the brain, bone uses the amino acid glutamate as a specific intercellular signaling molecule, and the tightly regulated glutamatergic processes in bone could become disordered if excess glutamate is present in the environment. Although the detrimental effects of tumor-derived glutamate on host organ functions are well established in malignant glioma, it is unclear whether an analogous process may occur in bone metastasis. Some study establishes that several cancer cell lines relevant to bone metastasis actively secrete glutamate using a transport mechanism that is expressed at the mRNA and protein level, and that this glutamate release can be pharmacologically inhibited. Since it is uncertain whether a peripheral tumor environment would support such conditions, the role of these transporters in tumor cell glutamate release *in-vivo* is undetermined[52, 53]. Vesicular glutamate transporters, involved in glutamate release from presynaptic neurons, have also been identified in a variety of peripheral sites, including pancreas, upper GI, adipose tissue, and testis[54].

Haifeng Ye *et al.* showed that poly(α,L-glutamic acid)-cis-dichlorodiammineplatinum (PGA–CDDP) conjugate was less toxic than the free drug, and could effectively reduce xenografted human breast tumor size in nude mice and prolong the survival of nude mice grafted with Bcap-37 tumor cells[55]. This result suggests that γ-poly(α,L-glutamic acid) (γ-PGA) produced by microbial fermentation may be used as a potential drug delivery system.

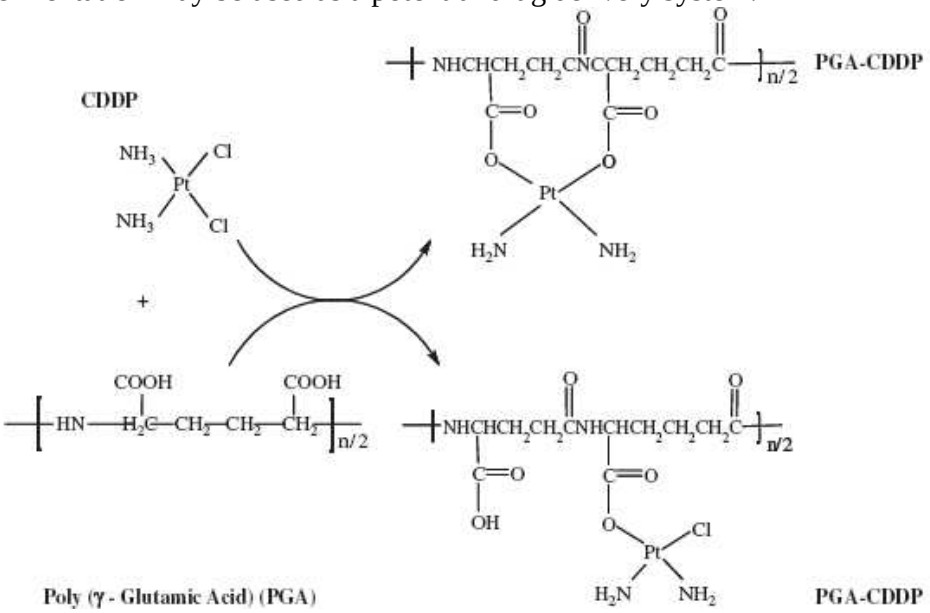

Fig. 2: Chemical structures of CDDP, γ-PGA, and PGA–CDDP conjugates

Several studies have indicated that N-(4-hydroxyphenyl)retinamide (4HPR) treatment is associated with inhibition of angiogenesis and a decreased vascular response *in-vitro* and *in-vivo*. 4HPR was bound to a synthetic polyamino acid poly(L-glutamic acid) (PG)[56, 57]. PG–4HPR was evaluated for its release kinetics and *in-vitro* anti-proliferative and *in-vivo* antitumor activities

against ovarian cancer cell lines[58]. It was confirmed that treatments with both 4HPR and PG-4HPR decreased the expression of pre-angiogenic factor VEGF in SKOV3 tumors (Fig. 3)[59, 60]. *In-vivo*, PG-4HPR demonstrated significantly enhanced antitumor activities compared to 4HPR in both early treatment and later treatment protocols. Treatments with PG–4HPR suppressed the expression of VEGF and reduced blood flow into the tumor.

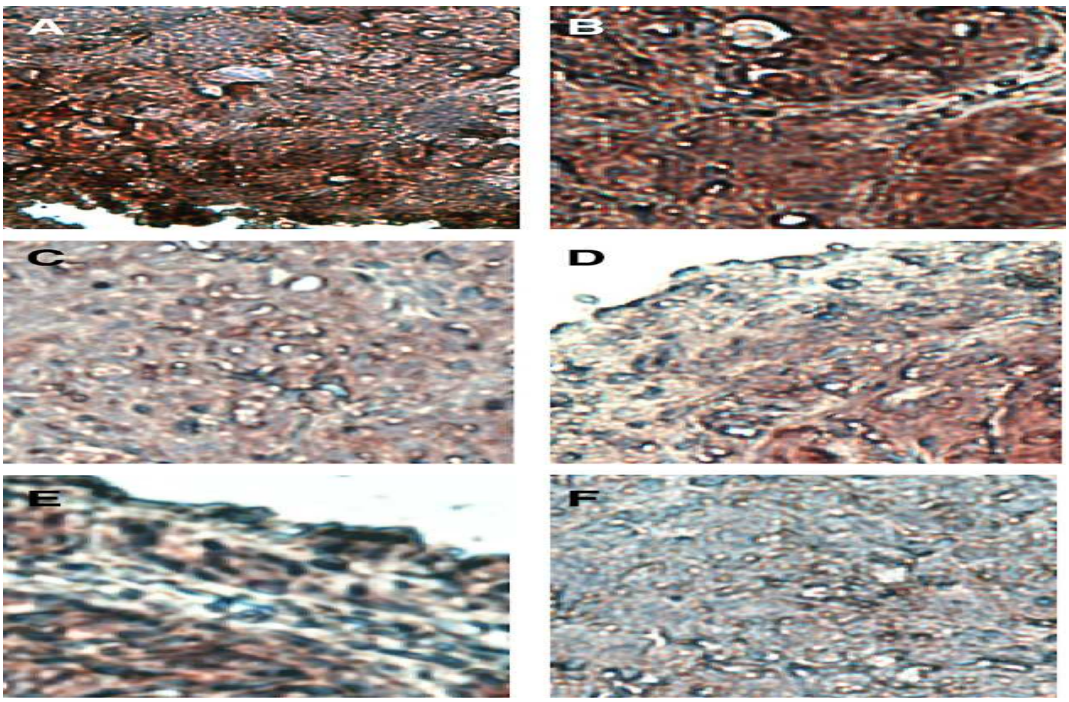

Fig. 3: Immunohistochemistry staining of VEGF in different treatment groups: (A) non-treatment control; (B) PG only; (C) 4HPR early treatment group; (D) 4HPR late treatment group; (E) PG–4HPR early treatment group; (F) 4HPR late treatment group. The expression of VEGF in SKOV3 tumors decreased in tumors of mice treated with 4HPR and PG–4HPR.

CONCLUSION

Compared to decades of anticancer drug research the progress has been marginal. So any research in this field will upgrade the present status of knowledge. Being at the 21st century, many people around the world died due to cancer. Work is going on to develop potential drugs without side effects. Glutamic acid and its derivatives will be more promising in its action against cancer with minimal side effects as they are endogenic in nature. Thus, glutamic acid analogs are of interest in search of antineoplastic agents.

Looking at the wide profile of activity, it has been proposed that though L-GA and glutamine were once considered nonessential for health, may now be considered as – 'conditionally essential' amino acids. While complete therapeutic role is yet to be elucidated, it may be anticipated that L-GA and glutamine may prove to be exciting molecules of interest to clinicians. The future research may therefore be directed at confirming the above activities and at investigating their role in other clinical conditions.

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