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EC PHARMACOLOGY AND TOXICOLOGY
Conceptual Paper

Radiolabelled Enzyme Inhibitors: Theranostic Radiopharmaceuticals for the Management of Prostate Cancer

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Received: August 07, 2017; **Published:** August 22, 2017

Discovery of new molecular medicines is an arduous task wherein a researcher picks a clue from one of the biochemical reactions involving cells to develop molecules that will target the disease at cellular level. In order to do this the researchers spent a large quantum of work designing the molecules, synthesizing and characterizing them with different techniques, testing them in suitable animal models to see the efficacy of the treatment. Despite very rational designs and well conducted experiments luck favours only a few to develop useful lead molecules. The lead molecules are taken by the drug companies and after elaborate clinical trials one of the molecules could become a drug. Majority of the researchers fail to achieve the final goal of drug discovery. These well conducted experiments often are not waste as the work done by those pioneers might emerge useful in another place. This conceptual paper illustrates how the work done by a group of drug researchers on the synthesis of inhibitors for the enzyme Carboxypeptidase (GCP II) have been utilized for developing nuclear medicine techniques for the diagnosis and therapy of prostate cancer [1].

Nuclear Medicine

Nuclear medicine is the branch of medicine which uses radioactive substances for diagnosis or therapy of different diseases. These radioactive medicines are called radiopharmaceuticals. Molecular imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) using radiopharmaceuticals are widely used for imaging several diseases such as organ dysfunctions, infection, inflammation and most importantly different types of cancers.

Fusion of SPECT and PET with computed tomography (CT) or magnetic resonance imaging (MRI) have helped to link the functional images obtained from SPECT and PET with the anatomical details derived from CT or MRI to pin point the exact location of the disease. PET-CT imaging is an integral part of cancer management for diagnosis, staging, prognosis, therapy planning, therapy monitoring and detection of cancer recurrence.

Radiopharmaceuticals are also used in nuclear medicine for the treatment of cancer as the radiation emitted from the decaying radioisotopes if properly targeted can be used to destroy cancer cells [2]. For therapy, beta (β-) or alpha (α) particle emitting isotopes are used as these particles deposit the energy within a short distance of its travel. Beta particle emitting Isotopes used for radionuclide therapy are ^{90}Y, ^{131}I, ^{153}Sm, ^{177}Lu and ^{188}Re. Alpha emitting radioisotopes such as ^{225}Ac and ^{213}Bi are used for alpha radionuclide therapy [3].

Development of cancer seeking theranostic agents

A diagnostic or therapeutic agent for cancer must show optimum ADME properties by selectively accumulating in the cancer cells and at the same time the activity accumulated in healthy organs and tissues must be cleared within a short time. In molecular imaging, such agents give very clear images of the cancer. In the case of molecular therapy, the above properties help in delivering the maximum radiation dose to the cancer and at the same time sparing healthy organs and tissues.

The most common vectors used for targeting the radioactive material to the cancer tissues are antibodies and peptides. The onset of cancer is marked by the elevated presence of certain antigens some of which are used as tumour markers. Monoclonal antibodies against such tumour antigens are used in immunotherapy of cancer. Similarly several receptor proteins are also over expressed on cancer cell surface. These receptors can be targeted by using peptides that specifically bind to them as is done in peptide therapy.

Anti CD-20 monoclonal antibodies radiolabelled with ^{131}I or ^{90}Y is used for radioimmunotherapy (RIT) of non-Hodgkin's Lymphoma (NHL) [4]. Likewise somatostatin receptor binding peptides are radiolabelled and used for the theranosis of neuroendocrine cancers [5].

Enzyme inhibitors in Medicine

Enzymes play a major role in the well being of our body such as digestion of food, assist in the process of providing cellular energy, support the brain functions and detoxification of blood. The enzyme catalyzes the reactions leading to the formation of essential products by degrading specific substrates. The excess bioavailability of enzymes also induces diseases. The enzyme substrate-reaction can be inhibited by using molecules which have chemical structures similar to the substrate. However, these molecules once bound to the enzyme are not converted to products and hence the enzyme activity is deregulated. Enzyme inhibitors have many important applications and are used as drugs, disinfectants, insecticides and herbicides.

GCPII enzyme inhibitors in medicine

Carboxypeptidase (GCP II) is an enzyme found in central and peripheral nerve tissues, small intestine, kidneys, parotid and lachrymal glands [6]. GCPII enzyme is also present in small quantities in the prostate; however, it gets significantly elevated during prostate cancer [7]. Hence, GCPII enzyme is more commonly referred as prostate specific membrane antigen (PSMA). The medical interest in GCP II or PSMA inhibitors has been for applications which involved glutamatergic transmission, since glutamate is the primary excitatory neurotransmitter in the human nervous system. A large number of GCPII enzyme inhibitors have been synthesized and evaluated for neuroprotection in experimental models of amyotrophic lateral sclerosis, traumatic brain injury and schizophrenia [6]. Despite the enormous amount of research in the development of PSMA enzyme inhibitors, their successful use as conventional medical drugs for the treatment of any known disease is yet to be established. However, the above discoveries of PSMA enzyme inhibitors have come very handy for the development of radiopharmaceuticals for molecular imaging and therapy of prostate cancer.

Theranosis of Prostate cancer with radiolabelled enzyme inhibitors

Prostate cancer is the major cancer affecting men and the incidence of which continues to increase. Targeting the PSMA enzyme over-expressed in prostate cancer cells by using radiolabelled enzyme inhibitors has been adapted for molecular imaging and therapy of prostate cancer.

Majority of the PSMA inhibitor molecule synthesized has structures similar to the enzyme substrate, N-acetyl-aspartyl-glutamate (NAAG). NAAG binds to an extracellular portion deep inside a ~20 Å tunnel leading to the NAAG binding pocket which contains two zinc cations that participate in the binding reaction [8]. Once the NAAG molecule binds to the enzyme, it is degraded to glutamate and N-acetyl-aspartate (Figure 1). The glutamic acid thus released is used in biochemical reactions.

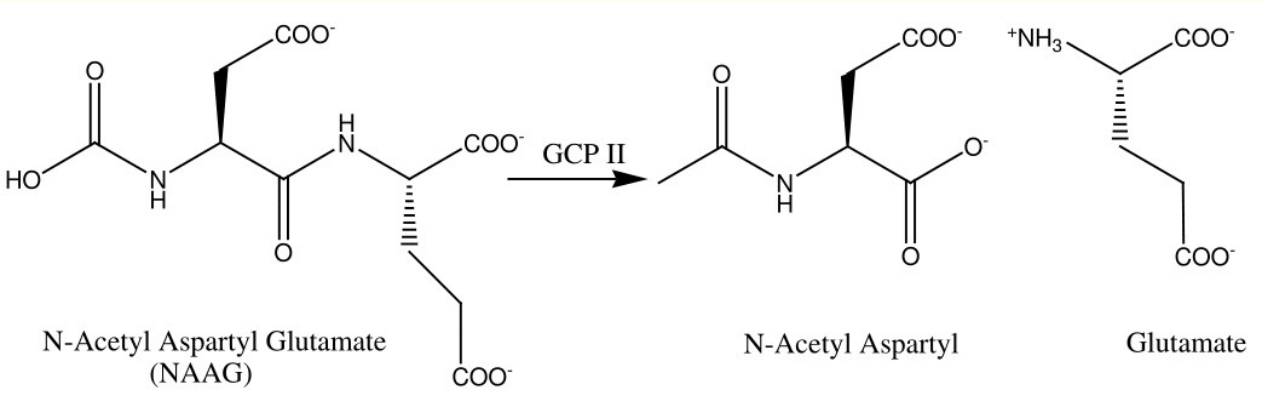

Figure 1: *Hydrolysis of N-acetyl-L-aspartyl-L-glutamate (NAAG) to N-acetyl-L-aspartate and -L- glutamate by GCPII or PSMA.*

Though synthesis of several PSMA inhibitors have been reported in literature, the successful ones used for the development of molecular imaging and therapy agents are urea based inhibitors in which one of the aminoacids is a glutamate [1]. The radioisotopes used for molecular imaging and therapy are metallic radionuclides that exit in +3 oxidation state. These include ^{68}Ga for PET-CT imaging and ^{177}Lu for targeted radionuclide therapy. Radiolabelling a dipeptide such as lysine-glutamate with a metallic radionuclide is accomplished by attaching a chelating agent that will hold the radionuclide firmly such that it is not released once injected inside the body. Attaching such chelating agents to the small dipeptides will prevent them to penetrate the ~20 Å tunnel on the enzyme surface to reach the NAAG binding pocket. This problem has been circumvented by adding a spacer made of aliphatic chain such that the dipeptide can still reach the NAAG binding pocket leaving the chelate and radionuclide on the surface. Yet another problem was that the metal chelate formed is hydrophilic, but a certain amount of lipophilicity is needed for cell binding and in order to achieve this the chemists worked around by introducing couple of phenyl groups as part of the chelate or as addendum to the spacer. Figure 2 depicts a urea based dipeptide, lysine-glutamate, modified by adding chelating agents through spacer molecules and used in theranosis of prostate cancer.

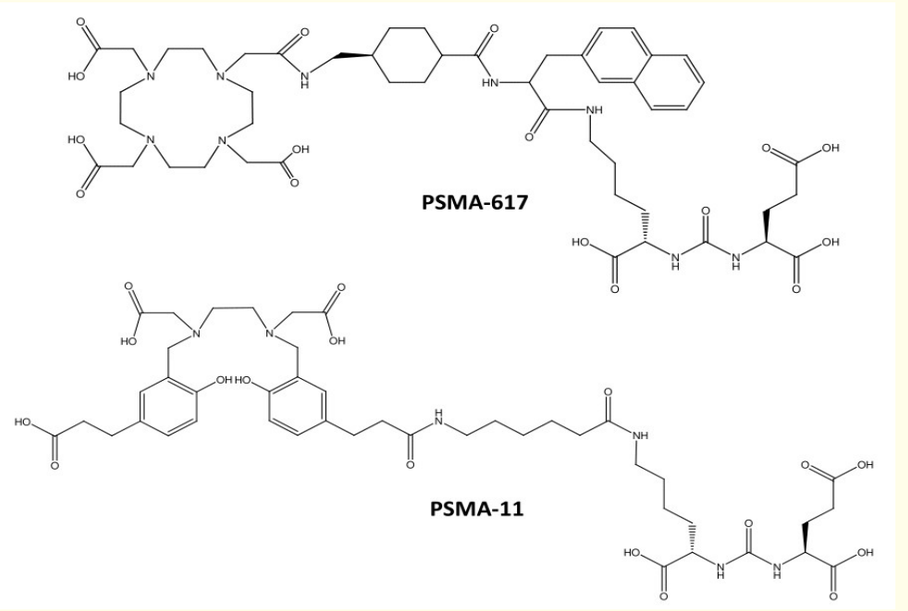

Figure 2: *Lysine-glutamate coupled to bifunctional chelating agents DOTA (PSMA-617) and HBED-CC (PSMA-11) through different spacer molecules.*

Molecular Imaging of prostate cancer

Gallium-68, having a half-life of 68 min is a positron emitting radionuclide available from a $^{68}Ge/^{68}Ga$ generator, is the most recent and widely used radionuclide in PET-CT imaging of different types of cancer [9]. Gallium forms highly stable complex (Log_{KML} = 35.6) with a chelating agent called HBED-CC (([N,N′-bis[2-hydroxybenzyl]ethylenediamine-N,N′-diacetic acid). HBED-CC chelate is coupled to lysine-glutamate through an aliphatic spacer (Figure 2 bottom). This molecule complexed with ^{68}Ga is now extensively used for PET-CT imaging of prostate cancer [10]. Figure 3 shows typical PET-CT images of patients suspected to be having prostate cancer. The tracer uptakes seen in patient 1 (Figure 3a) is biological distribution of the tracer, uptake is seen in the kidneys, parotid and lachrymal glands where as no uptake is seen in the prostate. Figure 3b shows a patient with disease just in the right side of the prostate whereas the patient shown in figure 3c has extensive metastasis in addition to disease in the prostate.

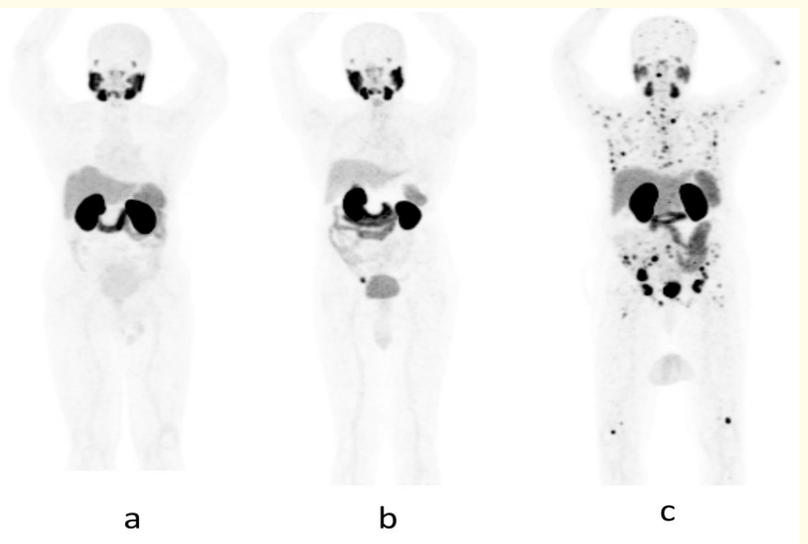

Figure 3: *PET-CT imaging with 68Ga-PSMA-11of patients suspected having prostate cancer. Normal biodistribution of the tracer with no disease seen in Figure 3a; whereas the patient in Figure 3b is at the onset of prostate cancer as focussed uptake is seen in the right prostate. Figure 3c gives the PET-CT images of a patient with extensive metastasis in addition to primary disease.*

Targeted radionuclide therapy of prostate cancer

Lutetium-177 is the most widely used therapeutic radionuclide for non-thyroid targeted radionuclide therapy [11]. Lutetium in +3 oxidation state forms very stable complex with the chelating agent DOTA [1,4,7,10-tetraazacyclododecane-1,4,7,10-triacetic acid]. For targeted therapy, a DOTA conjugated lysine glutamate is used. As Lu-DOTA complex is hydrophilic a biphenyl group is added in the spacer (PSMA-617, Figure 3 bottom). Multiple doses of ^{177}Lu-PSMA-617 of about 200 mCi (7.4 MBq) is given for therapy which is proving very effective for the treatment of prostate cancer.

Despite being introduced only couple of years back, Lu-PSMA-617 therapy is now practiced in several countries for the treatment of castration resistant hormone refractory prostate cancer [12]. Figure 4 shows the SPECT image of a patient administered with 177Lu-PSMA-11. The radioactivity uptake in primary as well as metastasis could be seen and is well correlated with the PET-CT images [13]. The activity accumulated in the cancer cells are not redistributed over long periods of time. The radionuclide decays completely within the tumour thereby depositing the entire energy to the tumour cells effectively killing them.

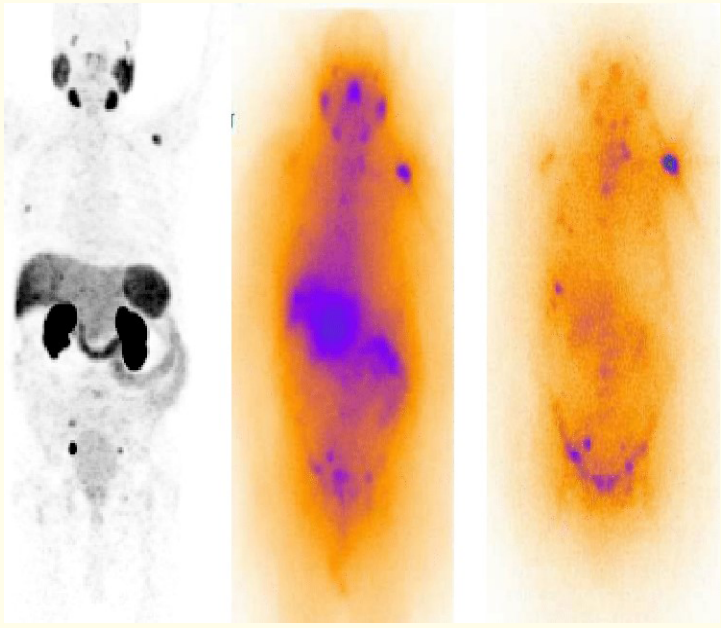

Figure 4: *PET-CT image (left) of a patient with prostate cancer; SPECT images at 24 hours (middle) and 7 days (right) post administration of a therapeutic dose of 177Lu-PSMA.*

Conclusion

Drug discovery is an ongoing important aspect of medical research and large number of scientists the world over are involved in this need based research. Most often such research studies do not find success leaving the researchers in despair. Nevertheless the results are documented in the form of publications. The clue picked from such publications by others often lead to important developments as illustrated in this conceptual paper. Thus no scientific work goes without use and hence there is a need to document even negative results.

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Volume 4 Issue 2 August 2017