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CARVEDILOL AS A POTENTIAL CHEMOTHERAPEUTIC AND CANCER PREVENTIVE AGENT THROUGH ITS INHIBITION OF PARP-1

McMurtray A.M.[2,3,4,*], Jain S.[1], Ekins S.[3], Tamrazian E.[2]

[1] Claremont McKenna College, Claremont, CA, USA
[2] Neurology Department, Harbor-UCLA Medical Center, Torrance, CA, USA
[3] Collaborations Pharmaceuticals, Inc., Fuquay-Varina, North Carolina, USA
[4] Neurology Department, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA

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Abstract

Carvedilol is an established anti-hypertensive medication used as one of the cornerstones of congestive heart failure. In addition to its cardiac remodeling properties, recent interest has been shown in the field of cancer mutagenesis. Inhibition of various proteins involved in proliferation pathways in cancer formation has shown potential therapeutic applications in protecting against apoptosis. Various clinical trials have recently been employed to study potential inhibitors of poly (ADP-ribose) polymerase 1, a well-known protein involved in cancer mutagenesis. Our study suggests a potential inhibitory interaction with polymerase 1and Carvedilol.

By using virtual screening target software, we hope to identify a potential pharmacological interaction between Carvedilol and various well known cancer genes, which can serve as possible therapeutic targets and potential chemo-preventative agents.

We used PyRx, which is a virtual screening and docking software program, to screen proteins known to be involved in DNA repair, cancer prevention, and programmed cell death pathways and measure their binding affinities with Carvedilol.

Among the various proteins tested with our virtual screening program, Carvedilol showed the highest binding affinity of -8.7 kcal/mol, to polymerase 1as well as with the receptor tyrosine-protein kinase erb-2, (HER2/neu), often found frequently in breast cancer (-8.3 kcal/mol) and also with the serine protein kinase ATM protein, involved in Ataxia Telangiectasia with a high binding affinity of -8.8 kcal/mol.

Although numerous anti-cancer mechanisms have been postulated and appropriate pharmaceutical agents have been designed which have decreased mortality, unwanted toxicity and cost has remained a limiting factor. Recent studies have focused on particular cancer mutagenesis pathways such as the role of polymerase 1 and proliferation in certain cancers. Our studies suggest a significant binding and inhibition of polymerase 1 with Carvedilol. This presents a novel and potential therapeutic role as a cost effective and readily available cancer treatment as well as possible chemo-preventative medication in certain cancers with little or no effective treatments.

Keywords: *cancer, carvedilol, inhibition, polymerase 1 (5ha9).*

Introduction

Cancer continues to be one of the leading causes of mortality in the US as well as worldwide. The prevalence of cancer has increased in the developed world as modern medicine has enabled interventions to increase the general lifespan as well as the increase of environmental pollutants in the developing countries. In 2015, cancer alone accounted for 8.8 million deaths – 1.69 million deaths from lung, 754,000 from stomach, and 571,000 from breast cancer. In the US alone, there were 1.6 million new cancers diagnosed and 600.000 consequent deaths [*WHO, 2017*]. Although great strides have been made in cancer therapies with cutting edge robotic surgeries to pa-

Address for Correspondence:
Aaron M. McMurtray, M.D., Ph.D, Associate Professor, Neurology Department, the David Geffen School of Medicine at UCLA, Bld N-25, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509, USA
Tel.: (310) 222-3897 Fax: (310) 533-8905
E-mail: amcmurtray@dhs.lacounty.gov

tient- specific immunotherapies, cancer rates continue to rise and the morbidity suffered from unwanted side effects of surgeries and chemo radiation therapies continue to remain a health burden on the patients as well as a financial burden in our healthcare system. Recent interest has been seen in developing low cost and preventative cancer agents [*Rao C, Reddy B, 2004*]. One such potential medication is Carvedilol.

Carvedilol is a non-selective beta blocker and a selective alpha-1 blocker. It is a well-established anti-hypertensive medication. In addition, it has shown to have beneficial remodeling of the heart, thus improving its ejection fraction [*Biaggioni M, Robertson D, 2009; Reis F et al., 2015*]. As a result of its anti-hypertensive properties, as well as its cardiac remodeling characteristics, it has been a cornerstone in managing heart failure [*Packer M et al., 1996; Packler M et al., 2002; Poole-Wilson P et al., 2003*]. In addition to its therapeutic role in cardiovascular disease, recent studies have shown great interest in Carvedilol as a potential chemotherapeutic and cancer prevention drug [*Lin C et al., 2015*]. Although the pathophysiological mechanisms responsible for the development of cancer are complex, multifactorial and tissue specific, one specific mechanism which Carvedilol appears to have a potentially therapeutic effect is through its inhibition of Poly (Adenosine diphosphate ribose) polymerase 1, more commonly known as PARP-1 or Poly (ADP-Ribose) polymerase-1. PARP-1 is a protein that is involved in repairing single stranded DNA nicks [*Hassa P, Hottiger M, 2008*]. If these nicks remain unrepaired, they can progress to double stranded nicks after replication and hence, the DNA will undergo death. Drugs that inhibit PARP-1 undergo double-stranded breaks and hence the cell dies [*McGlynn, P, Lloyd B, 2002*]. While this may appear as counter-intuitive, since inhibiting a repair mechanism should not lead to chemoprevention, the therapeutic application comes to light when taking into consideration the subsequent roles of mutated proteins. For example, with breast cancer patients, those with a defected Breast cancer type 1 (BRCA-1) susceptibility protein are not able to repair the tumor suppressor genes which they would otherwise be able to. If the PARP-1 is not inhibited, then the mutated products of the defected BRCA-1 gene would replicate without any further hindrance. However, in the presence of a PARP-1 inhibitor, the mutated BRCA-1 genes will develop DNA strand breaks, which will silence the replication of such mutated genes and hence potentiate a therapeutic response [*Kaelin W, 2005*].

As numerous clinical trials have recently emerged targeting PARP-1 with various drugs designed at inhibiting levels of PARP-1, more becomes known about the mechanisms responsible for its therapeutic effect. In a recent trial with BRCA-mutated ovarian cancer, a PARP inhibitor called Olaparib, showed that by inhibiting PARP-1, researchers were able to induce metaphase arrest and sister chromatid scattering, ultimately resulting in cell death [*O'Connor M, 2015*]. Similar interest has also been seen with PARP-1inhibition involving Ewing's Sarcoma, Small Cell Lung Cancer and Neuroblastoma [*Weaver A, Yang E, 2013, Sonnenblick A et al., 2015*]. Although the therapeutic role of PARP-1 inhibition relies on the presence of a mutation, such as the BRCA-1, studies have also suggested that Carvedilol also acts as an anti-cancer agent via other mechanisms such as inhibiting the Src activation, which is an essential component in leading to metastasis in breast cancer [*Ottenhoff-Kalff A et al., 1992; Dezong G et al., 2014*]. Although various studies have reported significant therapeutic results with inhibition of PARP-1, as well as with Carvedilol, the exact mechanism with which Carvedilol acts on PARP-1 has yet remained to be elucidated. We therefore postulate, that Carvedilol can act as a chemotherapeutic and a chemo-preventative drug through its inhibition of PARP-1.

Material and methods

A systematic literature search was first conducted using Pub Med to search for potential cancer associated genes. These included: tumor suppressor genes, oncogenes, DNA repair genes, telomerase reverse transcriptase, RET proto-oncogenes, as well as protein expression patterns in different forms of cancer. Specific genes that were searched included: BRCA1, TP53, HER2, ATM, PAXIP, RPA, RFC, XRCC1, PCNA, PARP1, ERCC1, MSH3, MEN2, ERBB2, EGFR (ErbB-1, HER1), MYC, KRAS, PIK3CA, PTEN, JUN, SOX2. Next, the proteins found were filtered to only include those that had known 3D structures

from the protein data bank (PDB). Only those proteins in the human genome were included. This yielded a list which included 7 total proteins. We then used PyRx, which is a Virtual Screening software for Computational Drug Discovery, to screen libraries of above-mentioned proteins against Carvedilol. The proteins were downloaded The Protein Data Bank: *https://www.rcsb.org/pdb/home/home.do*. The protein PARP-1, with the code 5ha9, from the protein data bank was screened against the ligand and we were able to see the 3-D image of where PARP-1 binds to carvedilol best. The sequence homology was analyzed to see what percentage is similar. For the majority of the proteins, whole proteins like 5ha9 were analyzed, rather than fragments. However, for a few, the fragments were noted in the information obtained from the protein data bank.

Results

Upon successful utilization of virtual screening and docking software with PyrX, the seven ligands mentioned above were compared with Carvedilol and its binding affinity measured. Of all the ligands measured, HER2/neu (3wsq), ATM (5np0), and PARP1 (5ha9) had the strongest binding affinities to Carvedilol with the following binding affinities; -8.3, -8.8 and -8.7 (*kcal/mol*), respectively. Among the top 3 ligands, HER2/neu which belongs to the receptor tyrosine-protein kinase erb-2 (HER2/neu) had the lowest binding affinity. The HER2/neu is a well-known proto-oncogene, involved in breast cancer [Rusnak D et al., 2001; *Mitri Z et al., 2012*]. The ligand moiety ATM, belonging to the Serine-protein kinase ATM, is also a protein whose mutation has been associated with Ataxia Telangiectasia, hence where its name is derived from [*Canman C, Lim D, 1998*]. In our experiment, the ATM ligand

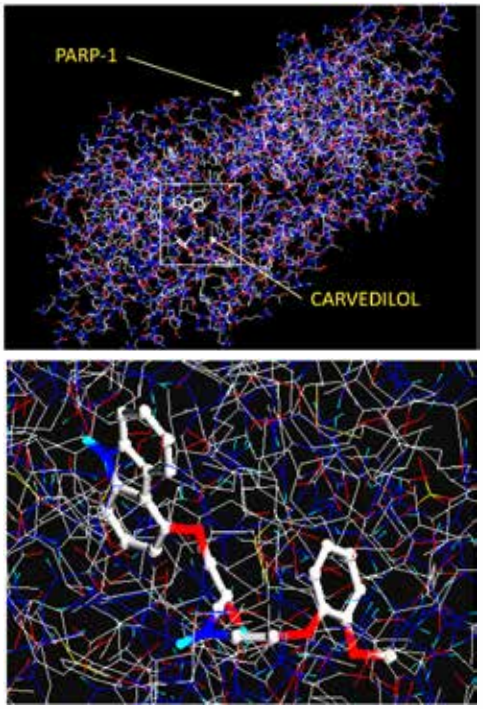

Figure. *Ligand and macromolrcule (Carvedilol and 5,* ***A.*** *Interaction of carvediol with PARP-1 (5ha9) binding site,* ***B****. Enlarged view binding site (5ha9)*

registered the strongest binding affinity. Of particular interest to our study is the binding affinity exhibited by the ligand PARP-1 corresponding to the protein PARP-1 (Table).

Carvedilol showed a high binding affinity to the PARP-1 ligand, thus confirming our hypothesis as a potential inhibitor. The pharmacological interaction of the PARP-1 ligand and its interaction can be seen in figure.

Discussion

After successful virtual screening and docking of Carvedilol with PARP-1, our hypothesis was thus confirmed as Carvedilol being a potential inhibitor of PARP-1 with a binding affinity of -8.7 *kcal/mol*. The therapeutic implications of such a

Table.

Value of binding affinity for several ligands.

Molecule	Protein	PDB Code	Binding Affinity (kcal/mol)
Breast cancer type 1 susceptibility Protein	BRCA1-BARD1	1jm7	-5.8
Breast cancer type 1 susceptibility protein	BRCT domain of BRCA1	1t2u	-6.4
Proto-oncogene serine/threonine-protein kinase Pim-1	PIM1	3jpv	-4.3
Receptor tyrosine-protein kinase erbB-2	HER2/neu	3wsq	-8.3
Poly [ADP-ribose] polymerase 1	PARP1	5ha9	-8.7
Serine-protein kinase ATM	ATM	5np0	-8.8

pharmacological interaction between these two molecules is quite significant. Inhibition of the PARP-1 molecule for therapeutic application is not recent and has been in research for the past 30 years. As stated earlier, the role of PARP-1 in cancer pathophysiology is complex and multifactorial, with various mechanisms being proposed for its therapeutic role. The fundamental role of PARP-1 has been its central role in DNA repair by binding to damaged DNA and facilitation the removal of the H-1 linker histone from the transcription initiation site, thus leading to chromatin de-condensation and allowing repair enzymes to attack the DNA sites [*Kraus W, Hottiger M, 2013*]. Furthermore, recent evidence has found a relationship between PARP-1 and NF-kb (nuclear factor kappa-light-chain-enhancer of activated B cells), where PARP-1 was found to co-regulate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) and increase the expression of pro-metastatic cytokines [*Dajee M et al., 2003*]. Furthermore, over-expression of PARP-1 has been found in patients with breast cancer, lung cancer as well as melanomas [*Nowsheen S et al., 2012*]. While abundant research has accumulated over the years in regards to the role PARP-1 inhibitors play in cancer physiology, its direct anti-tumor mechanisms have been attributed to the mechanisms elucidated with the interactions it poses with mutated BRCA-1 and BRCA-2 genes [*Farmer H et al., 2005; Malyuchenko N et al., 2015*]. In short, the PARP-1 inhibition renders double stranded DNA breaks which in the presence of a mutated gene, such as the BRCA-1, the mutated BRCA-1 and BRCA-2 are unable to replicate and hence the cell undergoes apoptosis. This mechanism has been the underlying feature for the development of the PARP-1 inhibitor Olaparib and its use in for BRCA-1 and BRCA-2 breast and ovarian cancer [*Fong P et al., 2009; Hutchinson L, 2010*]

Carvedilol ability to inhibit PARP-1 was first suggested in a study by Strosznajder R. and co-authors, where PARP-1 activity was noted to be reduced after administration of Carvedilol in the hippocampus of ischemia induced gerbils [*Strosznajder R et al., 2005*]. Carvedilol applications have recently been of a great interest as it has been shown to suppress apoptosis in reperfusion/ischemia models [*Usta E et al., 2010*], as well as to serve as a neuroprotective agent in amyloid induced neurotoxicity in N2A cells [*Liu J, Wang M, 2018*]. While much is known about the role of PARP-1 inhibition in cancer mutagenesis, the potential role Carvedilol may play in cancer chemoprevention and therapy has not yet been studied. As a fairly benign medication with limited side effect profile, carvedilol may play a role in chemoprevention in patients who also have underlying hypertension. While therapeutic response of Carvedilol appears to involve multiple mechanisms as its beneficial effects are seen in various pathological conditions, its role as a chemo-preventative and possibly a treatment modality via its PARP-1 inhibition is one which deserves larger multi-institutional trials, specifically with patients harboring a BRCA-1/2 mutation.

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