Journal of Pharmaceutical Sciences xxx (2015) 1–13

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Review

Heat-Shock Protein 90–Targeted Nano Anticancer Therapy

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
nanotechnology
nanoparticles
cancer
biomaterials
cancer chemotherapy
drug resistance

ABSTRACT

Suboptimal chemotherapy of anticancer drugs may be attributed to a variety of cellular mechanisms, which synergize to dodge the drug responses. Nearly 2 decades of heat-shock protein 90 (Hsp90)-targeted drug discovery has shown that the mono-therapy with Hsp90 inhibitors seems to be relatively ineffective compared with combination treatment due to several cellular dodging mechanisms. In this article, we have tried to analyze and review the Hsp90 and mammalian target of rapamycin (m-TOR)-mediated drug resistance mechanisms. By using this information we have discussed about the rationale behind use of drug combinations that includes both or any one of these inhibitors for cancer therapy. Currently, biodegradable nano vector (NV)-loaded novel drug delivery systems have shown to resolve the problems of poor bioavailability. NVs of drugs such as paclitaxel, doxorubicin, daunorubicin, and others have been successfully introduced for medicinal use. Hence, looking at the success of NVs, in this article we have also discussed the progress made in the delivery of biodegradable NV-loaded Hsp90 and m-TOR-targeted inhibitors in multiple drug combinations. We have also discussed the possible ways by which the market success of biodegradable NVs can positively impact the clinical trials of anti-Hsp90 and m-TOR combination strategy.

Introduction

Humankind's association with cancer is thought to be around 5000 years old.[1] Cancer is the second-largest disease in the record of Centre of Disease Control with a death toll of 574,738 in the United States during 1999-2011.[2] World Health Organization reports 8.2 million deaths worldwide in 2012.[3] Among various classical ways of cancer treatment, chemotherapy is the most routinely used method.[4] The era of chemotherapy started at Yale University in 1942 when a patient named "JD" was given the first intravenous (iv) dose of nitrogen mustard.[5] Furthermore, the work of showing anticancer activity of folate antagonists in 1948 by Sidney Farber marked the advent of modern chemotherapy and new drug discovery.[6] This discovery also provided a foundation for development of next-generation targeted nano particle (NP)-based drug delivery. Today, we have around 36 known Food and Drug Administration (FDA)-approved anticancer drugs on the market.[7,8] Looking at the direction of new drug discovery, National Cancer Institute has reported to have screened 80,000 of 400,000 compounds from their repository for anticancer activity since 1990.[9] However, their translation to clinical trials is a major concern due to the lack of *in vivo* efficacy and drug- or dose-limiting toxicity issues.

Identification of a new drug target is usually the first step for drug development. Ironically, cancer is a complex heterogeneous disease with many routes of survival.[10] Hence, there are a number of protein-, enzyme-, and gene-based targets for treatment of cancer.[11,12] National Cancer Institute reports having 21 promising anticancer agents, which target cancer pathways associated with activation of apoptosis, cell cycle control, cell signaling, angiogenesis, tumor invasion, metastasis, DNA synthesis, and immune functions.[10] They have also reported around 1500 clinical trials with mono and combination therapies for a variety of cancers.[10]

Heat-shock protein 90 (Hsp90) is one of such drug targets for which molecules have reached clinical trials. It is a molecular chaperone that is overexpressed in different types of cancers. Drugs targeting Hsp90 of cancer have been under development since 1999 and have shown potential as next-generation anticancer therapeutics.[13] Parallel to Hsp90-targeted drug development, molecules targeting phosphatidylinositol 3-kinase (PI3K) or Akt or mammalian target of rapamycin (m-TOR) signaling pathway, are also considered as a viable strategy for cancer therapy.[14] It is

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2015.10.007.

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http://dx.doi.org/10.1016/j.xphs.2015.10.007
0022-3549/

established that inhibition of m-TOR pathway potentiates Hsp90 inhibition.[15,16] Hence, exploring a combination of Hsp90 and m-TOR inhibitors is an obvious next step towards developing next-generation anticancer therapy. Perhaps adopting strategies of designing potent and target-specific molecules will help in overcoming drug resistance concern. Drug resistance perhaps is one of the causes responsible for cancer stem cell development.[17-19] Cancer drug resistance is a product of molecular pathways and formulation-related issues.[20-22]

Moreover, formulation of a potent drug affects its metabolism, elimination rate, patient acceptability, and dose limiting toxicity during clinical trial. Nanotechnology has shown promises in cancer-targeted drug delivery.[23] It also helped in providing solutions to off-target effects of chemotherapy by developing smart NPs. It has also shown promises in improving kinetics and drug release of known anticancer drugs.[24] Since the advent of an era of nanomedicine, we have around 24 accepted NP-based drugs for treatment of cancer.[25] There are around 554 anticancer drug–loaded NP or liposomes clinical trials, which have been registered at clinicaltrial.gov database for mono and combination therapy.[26] Most of the anticancer drugs including the pathway-specific molecules such as Hsp90 or m-TOR inhibitors are being administered through iv or oral routes of administration.[27] Hence, the present review will focus on the NP or nano vector (NV) strategies for the delivery of such specialized next generation anticancer molecules. Here, we will also confer the impact of chemistry of newly designed NVs on the pharmacokinetics and therapeutic efficacy of parent molecule by taking specific examples of Hsp90 and m-TOR inhibitors. We will also discuss the impact of fulfillment of all the 6 tenets of drug delivery on the development of smart and advanced chemotherapy.[28]

Chaperones as Anticancer Drug Target

Stress response is a universally paradoxical and a conserved phenomenon in unicellular and multicellular organisms.[29] It helps in providing cytoprotection from stress conditions such as hyperthermia, hypoxia, hyperoxia, chemotherapy, and other environmental perturbations, which alter cellular homeostasis.[29,30] Stress proteins are universally conserved across the living system and it is regarded as minimal stress proteome. HSP and stress proteins terms are mostly used interchangeably.[29] Most HSPs work as molecular chaperones and cover 5%-10% of the total cellular proteome under healthy conditions.[31] They help in homeostatic functions such as repair of unfolded or misfolded proteins; they help in maintaining protein structures, maintenance of mitochondria and cell wall lipoproteins, transport of proteins in cellular compartments, cell cycle control, signaling, and protection against stress and/or apoptosis.[32,33] HSPs get upregulated to cope with the damaging effects of stress on the functional proteins.[31] There are different types of HSPs such as Hsp10, Hsp40, Hsp70, Hsp90, Hsp101, and others, which help in managing the previously mentioned cellular functions.

Hsp90 as a Therapeutic Target

Hsp90 is one of the most studied molecular chaperones with evolutionary and therapeutic potentials. Like other HSPs, this is also a highly conserved molecular chaperone. Ferrucio Ritossa first discovered Hsp90 protein in 1962 from the salivary glands of Drosophila melanogaster.[34] Hsp90 is roughly a 90 KDa dimeric protein. It belongs to GHKL (gyrase, Hsp90, histidine kinase, MutL) class of adenosine triphosphatases (ATPases) or kinase superfamily.[35] Hsp90 has 4 functional domains, namely (a) N-terminal or ATP binding domain, (b) charged linker region with variable length, (c) middle domain, and (d) C-terminal domain which serves as dimerization and interaction site for cochaperones such as p23, FKBP52, Cyp40, Hop, and others.[36,37] Cytosolic Hsp90 exists in the isoforms as GRP94 (endoplasmic reticulum) and TRAP-1 (mitochondrial Hsp90). It works as a multichaperone complex for carrying out its cytoprotection function. There are >300 client proteins, which require Hsp90 for their maturity (www.picard.ch/downloads).[38] Of these clients, kinases form the largest group of interactors for Hsp90 followed by transcription factors.[38,39] As a part of protein folding pathway, unfolded or misfolded client proteins interact with the open conformation of multichaperone complex of Hsp90-Hsp70-Hsp40. In presence of cochaperones and ATP, Hsp90 attains a closed conformation state, required for the client folding. After completion of task of client protein folding, the closed conformation reverts back to open state due to the ATPase activity of Hsp90 and releases the functional client protein (as shown in Supplementary Fig. 1).[40] To deal with the cytosolic stress of cancer cells, there is an upregulation of Hsp90 in a variety of cancers including solid tumors and hematological malignancies.[41-45] It is also implicated that Hsp90 plays a critical role in epithelial mesenchymal transition, invasion, and motility of colorectal cancer.[46] This shows that inhibiting Hsp90 using small molecular inhibitors may serve as a potential chemotherapeutic strategy.

Looking into the role of ATP binding in Hsp90-driven protein folding pathway, newer molecular inhibitors are being designed that can fit into the N-terminal domain's ATP binding pocket as shown in Figure 1a.[37,47] These inhibitors are being considered as a viable strategy for cancer treatment. Because of their N-terminal interactions, Hsp90 loses its ATPase activity and multichaperone complex of client protein remains in the opened state, which prevents the folding of client proteins. This leads to activation of ubiquitin-dependent proteasomal degradation of unfolded Hsp90 clients (as shown in Supplementary Fig. 1).[40,48] This leads to loss of essential client proteins of cancer cells, ultimately leading to cell death.

Role of Hsp90 in Drug Resistance

Multiple drug resistance (MDR) has been a major problem for chemotherapeutic drugs belonging to anthracyclins, antimetabolites, epipodophyllotoxins, taxanes, vinca alkaloids, and other macrocycles (camptothecin-11, dactinomycin, and mitomycin C).[49,50] Hsp90 acts as a capacitor of morphological evolution which in turn helps in buffering the widespread variation which affects morphogenic pathways. Hence, by using Hsp90 inhibitors the buffering activity is compromised. This may cause drug resistance due to development of cryptic phenotypes, which when selected continues to develop even if the function of Hsp90 is restored. This phenomenon can add to an MDR problem in cancer by acquiring these resistance traits due to the alterations in buffering action of Hsp90.[51-53] Furthermore, chemotherapy is one of the factors responsible for inducing cellular stress response that causes over expression of Hsp90.[54,55] Cancer cells seem to smartly exploit this stress response for their survival benefit. It is also noticed that along with the chemotherapy, ionizing radiation–induced hyperthermia therapy also activates stress response via activations of transcription factors such as heat-shock factor protein 1 (HSF1).[56-58] This leads to upregulation of Hsp90 in cancer cells, which may cause suboptimal therapy and resistance development. Furthermore, Hsp90-targeted treatment is marked by upregulation of Hsp70, which plays an important role in cancer development by blocking intrinsic and extrinsic pathway of caspase-mediated programmed cell death.[53,59,60] On the other hand, *MDR*-1 gene coding P-glycoprotein (P-gp) involved in cancer drug resistance is