Advanced Drug Delivery Reviews 114 (2017) 143–160

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Nano-technology based carriers for nitrogen-containing bisphosphonates delivery as sensitisers of γδ T cells for anticancer immunotherapy☆

Naomi O. Hodgins, Julie Tzu-Wen Wang, Khuloud T. Al-Jamal *

Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK

ARTICLE INFO

Article history:
Received 1 April 2017
Received in revised form 29 June 2017
Accepted 5 July 2017
Available online 8 July 2017

Keywords:
Liposomes
Gamma delta T cells
Immunotherapy
Biphosphonate
Zoledronic acid
Alendronic acid

ABSTRACT

Nitrogen containing bisphosphonates (N-BPs) including zoledronate (ZOL) and alendronate (ALD) inhibit farnesyl diphosphate synthase, and have been shown to have a cytotoxic affect against cancer cells as a monotherapy and to also sensitise tumour cells to destruction by γδ T cells. γδ T cells are a subset of human T lymphocytes and have a diverse range of roles in the immune system including the recognition and destruction of cancer cells. This property of γδ T cells can be harnessed for use in cancer immunotherapy through *in vivo* expansion or the adoptive transfer of *ex vivo* activated γδ T cells. The use of N-BPs with γδ T cells has been shown to have a synergistic effect in *in vitro*, animal and clinical studies.

N-BPs have limited *in vivo* activity due to rapid clearance from the circulation. By encapsulating N-BPs in liposomes (L) it is possible to increase the levels of N-BPs at non-osseous tumour sites. L-ZOL and L-ALD have been shown to have different toxicological profiles than free ZOL or ALD. Both L-ALD and L-ZOL led to increased spleen weight, leucocytosis, neutrophilia and lymphocytopenia in mice after intravenous injection. L-ALD was shown to be better tolerated than L-ZOL in murine studies. Biodistribution studies have been performed in order to better understand the interaction of N-BPs and γδ T cells *in vivo*. Additionally, *in vivo* therapy studies have shown that mice treated with both L-ALD and γδ T cells had a significant reduction in tumour growth compared to mice treated with L-ALD or γδ T cells alone. The use of ligand-targeted liposomes may further increase the efficacy of this combinatory immunotherapy. Liposomes targeting the αvβ6 integrin receptor using the peptide A20FMDV2 had a greater ability than untargeted liposomes in sensitising cancer cells to destruction by γδ T cells in αvβ6 positive cancer cell lines.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Immuno-engineering".

* Corresponding author.
E-mail address: khuloud.al-jamal@kcl.ac.uk (K.T. Al-Jamal).

http://dx.doi.org/10.1016/j.addr.2017.07.003
0169-409X/

1. Nitrogen-containing bisphosphonates

1.1. Mechanism of action of N-BPs

While nitrogen-containing bisphosphonates (N-BPs) were originally used in the treatment of osteoporosis and other bone disorders [1], they have shown to have several clinical indications including cytotoxic activity as a monotherapy [2,3] and activation of γδ T cells [4]. Bisphosphonates (BPs) are chemically stable analogue of pyrophosphate compounds found in nature. All BPs in clinical use consist of a core structure made up of P-C-P bonds which is resistant to enzymatic hydrolysis (Fig. 1) [5].

Later generations of BPs led to in incorporation of nitrogen atoms into their structure, increasing their potency due to an additional mechanism of action [1]. N-BPs inhibits farnesyl diphosphate (FPP) synthase which is a key enzyme in the mevalonate biosynthetic pathway [7]. Inhibition of FPP synthase leads to the upstream accumulation of the triphosphoric acid 1-adenosin-5-yl ester 3-(3-methylbut-3-enyl) ester (Appp1) which then induces apoptosis *via* the inhibition of mitochondrial ADP/ATP translocase [8]. Inhibition of FPP synthase also prevents the prenylation of Guanosine triphosphate (GTP)-binding proteins such as Ras, Rho and Rac leading to cell growth inhibition and can activate the caspase dependant mechanism of apoptosis [9].

1.2. Anti-cancer activity of N-BPs

BPs have also found clinical applications in the treatment of cancer. Both BPs and N-BPs are use in the treatment of multiple myeloma [10] and bone metastases from breast, lung, prostate and other solid tumour cancers [11]. They have shown to be effective in reducing skeletal-related events associated with malignancies such as hypercalcaemia and increased bone destruction [12]. N-BPs have also shown to have anti-tumour activity in non-osseous tumours and have been shown to induce tumour apoptosis and inhibit tumour cell proliferation, migration and invasion. They also have anti-angiogenic effects and interfere with endothelial cell migration, proliferation and tube formation [6]. The use of ZOL and ALD as anti-cancer agents will be focused on in this review.

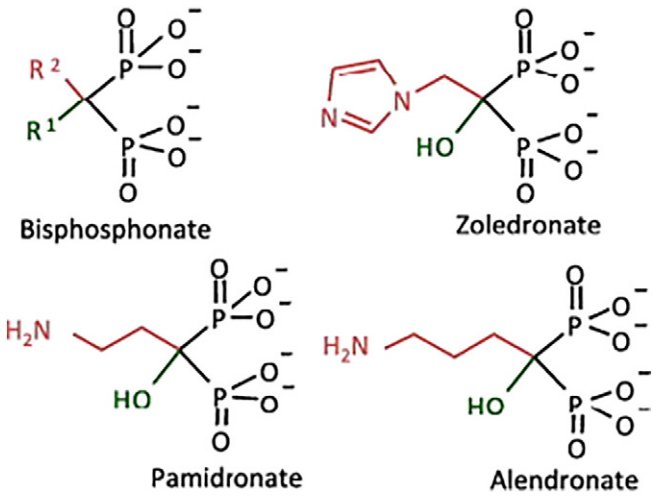

Fig. 1. Structure of various bisphosphonates. Template structure of bisphosphonates and structure of zoledronate and other nitrogen-containing bisphosphonates discussed. (Adapted from Stresing et al., 2007 [6]).

ZOL is the most potent of the clinically used BPs. due to the presence of two nitrogen atoms contained within a heterocyclic ring structure [13]. ZOL has direct effects on tumour cells *in vitro* and has been shown to decrease the viability and proliferation of several cancer cell lines as well as inducing apoptosis of cancer cells. After exposure of the prostate cancer cell lines PC-3, DU-145, LNCaP and CRW22Rv1 to 100 μM of ZOL, the autophagic and apoptotic proteins LC3-II and activated caspase-3 were detected showing that ZOL exposure resulted in cell death [14]. ZOL has also been shown to have a synergistic effect *in vitro* when used in combination with the cytotoxic agents; paclitaxel, etoposide and cisplatin [15].

ZOL has been shown to inhibit progression of established bone metastases and development of new bone metastases in two models of breast cancer in mice [16]. Nude mice bearing MDA-MB-231 tumours were subcutaneously injected with 0.2, 1.0 or 5.0 μg/day of ZOL for 10 consecutive days and were shown to have reductions in bone lesions of >80% compared to controls [16]. Treatment with 5 μg/day ZOL for seven days after injection of 4T1 murine mammary tumour cells were shown to decrease the formation of new bone metastases [16]. Another study in a murine model of multiple myeloma has shown a reduction in osteolysis, tumour burden and angiogenesis with a subsequent increase in survival time [17]. ZOL has also shown to be effective in the treatment of soft tissue tumours in extra-osseous sites. It has been shown to reduce the growth of cervical tumours and the progression of premalignant lesions in a transgenic mouse model [18]. ZOL was shown to inhibit expression of the pro-angiogenic protease, matrix metalloproteinase 9 (MMP-9) by tumour associated macrophages.

Clinically, ZOL has been used in several studies to determine its anti-cancer effects in humans. A phase II clinical study has shown that ZOL increases the clearance and reduces the number of disseminated tumour cells in the bone of patients with early breast cancer [2,3]. However, other studies have not shown the benefit of adjuvant ZOL treatment in early breast cancer [19]. A clinical trial of the use of ZOL in multiple myeloma, demonstrated 16% reduced mortality and an extended mean survival of 5.5 months when compared to the non nitrogen containing BP, clodronate [20]. A Cochrane meta-analysis of other clinical trials confirmed the therapeutic efficacy of ZOL in myeloma patients [21]. ZOL has also shown to be effective in the treatment of malignancy-related skeletal issues in acute myeloid leukaemia [22] and lymphoma [23]. Additionally, prostate cancer patients treated with ZOL were shown to improved prostate-specific antigen progression-free survival time [24]. Various trials in patients with bone metastasis from solid malignancies have shown the benefit of ZOL therapy [25].

ALD has also been shown to have anti-cancer activity in both *in vitro* and *in vivo* studies. When human umbilical vein endothelial cells (HUVEC) were treated with ALD, migration and formation of capillary-like structures were inhibited *in vitro* [26]. This is thought to be due to the inhibition of Rho geranylgeranylation. *In vitro* inhibition of migration of the prostate cancer cell lines PC-3 and Du-145 and the breast cancer cell lines MDA-MB-231 after treatment with ALD has also been observed [27]. ALD inhibited the proliferation of the Huh-7 hepatocellular carcinoma cell lines in a time and dose dependant manner at concentrations between 5 and 20 μM [28]. ALD has also been shown to have anti-proliferative effect on the epidermoid carcinoma cell line A431, as well as inhibiting cell invasion in a matrigel invasion assay [29]. ALD