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AP-5346

Polymer-Delivered Platinum Complex

EN: 320523

Abstract

AP-5346 is the newest member of a growing class of chemotherapeutic agents that utilize macromolecular carriers to enhance the delivery of drugs to tumors. AP-5346 consists of a cytotoxic diaminocyclohexane (DACH)-platinum moiety coupled to a 25-kDa water-soluble biocompatible hydroxypropylmethacrylamide copolymer via a pH-sensitive linker. It was designed to remain inactive in the systemic circulation, have a long half-life relative to small-molecule platinum-containing chemotherapeutic agents, and to increase delivery to tumors via a mechanism dependent on the enhanced permeability of tumor capillaries. AP-5346 demonstrated a superior therapeutic index in multiple preclinical tumor models. In the B16F10 melanoma model, it provided more than 12-fold greater delivery of platinum to the DNA of the tumor than the DACH-platinum agent oxaliplatin when both drugs were given at equitoxic doses. AP-5346 is about to enter phase II clinical testing.

Preparation

The random copolymer poly(HPMA-co-MA-Gly-Gly-ONp) (20-25 kDa) is produced in 80% yield according to methods described previously (8), where hydroxypropylmethacrylamide (HPMA) is copolymerized with the *p*-nitrophenyl ester (designated -ONp) of *N*-methacroylglycylglycine (designated MA-Gly-Gly). Boc-protected glycine is coupled to diethylaminomalonate, followed by deprotection (73% overall yield). This chelating group is then coupled to the polymer via replacement of the ONp groups and the polymer product is isolated (85% yield). Hydrolysis of the diethyl ester groups followed by complexation with 1*R*,2*R*-diaminocyclohexane (designated 1*R*,2*R*-DACH-Pt$(OH_2)_2$) results in the formation of the *O*,*O*'-amidomalonate chelate, which is quantitatively converted to the *N*,*O*-amidomalonate chelate in phosphate-buffered saline. Purification via tangential flow filtration yields pure poly(HPMA-co-MA-Gly-Gly-Gly-Ama=Pt-1*R*,2*R*-DACH *N*,*O*-chelate), as confirmed by ^{195}Pt NMR spectroscopy.

Polymer therapeutics such as AP-5346 present a unique pharmaceutical manufacturing challenge. Most therapeutic agents typically possess a single discrete chemical structure whose identity, purity and impurity profile can be precisely defined. This is not the case for polymer therapeutics, which are typically polydisperse; that is, the population of drug molecules contains individual polymer strands that have a range of different molecular weights. Furthermore, the amidomalonato chelating groups of AP-5346, typically 8-10 per polymer strand, are randomly located on each strand. Given this variability in the polymer carrier, it is important that the DACH-platinum is bound to the polymer in a well-controlled manner, so that there is consistent release of platinum *in vivo*. As outlined above, DACH-platinum is bound to the polymer in the final step of drug substance manufacture. Size-exclusion chromatography and NMR spectroscopy, particularly ^{195}Pt NMR spectroscopy, play a key role in product characterization and batch-to-batch quality control. The purity of AP-5346, as measured by the percentage of platinum bound as the *N*,*O*-chelate, is routinely > 95%.

Introduction

The platinum-containing chemotherapeutic agents cisplatin and carboplatin are widely employed in the treatment of lung, head and neck, ovarian and testicular cancers (1, 2). A third agent, oxaliplatin (Eloxatin^{TM}), in which the platinum is complexed with a diaminocyclohexane (DACH) ligand, is also in clinical use and has activity in colorectal cancer, particularly in combination with other agents (3).

The conventional platinum agents have pronounced toxic effects which limit the doses and schedules that can be administered, and compromise therapeutic effectiveness. The major adverse events for the platinum drugs include myelosuppression, nephrotoxicity and both sensory and motor peripheral neuropathies. The therapeutic index of the conventional agents is limited by their relatively rapid elimination and extensive distribution to normal as well as malignant tissues. One approach to improving the therapeutic index is to link them to carriers that modify the transport of the cytotoxic platinum moiety

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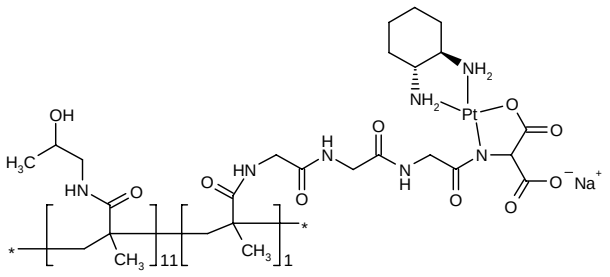

Fig. 1. Partial structure of AP-5346.

to increase the amount delivered to the tumor while also decreasing toxicity to normal tissues (4, 5).

AP-5346 was designed to utilize a water-soluble, biocompatible polymer carrier to enhance the delivery of a cytotoxic DACH-platinum moiety to solid tumors. This enhanced delivery is achieved in part by capitalizing on biological differences in the permeability of the neovascular endothelium of certain rapidly growing tumors relative to the mature vasculature found in normal tissues. This process is termed the "enhanced permeability and retention" (EPR) effect (6). Enhanced delivery is also attained by virtue of the fact that linkage of the DACH-platinum moiety to the polymer prolongs the half-life and increases the duration of exposure for the tumor. The polymer portion of AP-5346 consists of a random copolymer of hydroxypropylmethacrylamide monomer (HPMA) and a second methacrylamide monomer substituted with a triglycine linker group (Gly-Gly-Gly), in a ratio of 11:1. The linker chain ends in an amidomalonate group, to which the DACH-platinum group is complexed as an *N*,*O*-Pt chelate. The structure of a segment of the AP-5346 molecule is shown in Figure 1. The weighed-average molecular weight (Mw) of AP-5346 is 25 kDa and it contains *ca.* 10% platinum by weight. The molecular weight of the polymer carrier was selected to ensure that the macromolecule was below the renal threshold for eventual clearance via glomerular filtration, yet large enough to accumulate in tumors via the EPR effect.

Current evidence indicates that the DACH-platinum complex remains biologically inert while being transported by the polymer in the plasma, and that it is not capable of damaging cells until it is released from the carrier. The rate at which the DACH-platinum moiety is released from the polymer is highly dependent on pH. At plasma pH, the rate of release is very slow; however, endocytosis by tumor cells is expected to deliver AP-5346 to lysosomes, the pH of which is 2 orders of magnitude lower (7). Once released from the polymer, the active moiety is able to escape the lysosome and interact with key intracellular targets, including DNA. Since in some tumors the pH of the tumor extracellular fluid compartment is already much lower than that of plasma, it is possible that some fraction of the DACH-platinum moiety is released extracellularly in the tumor and enters tumor cells via the same transporters that transport the free platinum drugs.

Pharmacological Actions

The antitumor activity of AP-5346 and its superiority relative to that of oxaliplatin (conventional DACH-platinum agent) has been demonstrated in a variety of murine and human tumor xenograft models. In these studies, the activity of each agent was assessed using doses that closely approached the maximum tolerated dose (MTD) in the model under study. The MTD was defined as the dose that reproducibly induced 10-15% mean maximum body weight loss and produced no more than 10% early toxic deaths. Comparing the activities of both agents on an "equitoxic" basis in this way mimics the manner in which chemotherapeutic agents are utilized in the clinic.

An example of one such study is presented in Figure 2, which shows the effect of AP-5346 and oxaliplatin on the growth of subcutaneous murine B16F10 melanoma. A single i.p. dose of AP-5346 resulted in a substantially greater degree of tumor growth inhibition than a single dose of oxaliplatin, while producing equivalent systemic toxicity.

A second illustration of the superior activity of AP-5346 is presented in Figure 3, which compares the effect of AP-5346 to that of oxaliplatin and carboplatin in the 2008 human ovarian tumor xenograft model. All drugs were given as single equitoxic doses by the i.p. route. In this model, oxaliplatin had little activity and carboplatin had moderate antitumor activity. However, AP-5346 produced a much greater suppression of tumor growth, and since all three drugs were given at their respective MTD, the results indicate that coupling the DACH-platinum moiety to the HPMA polymer substantially improved the therapeutic index.

Pharmacokinetics

The molecular mechanism that serves as the basis for the enhanced activity of AP-5346 has been investigated by examining the delivery of platinum to the genomic DNA of B16 melanoma tumors. The reaction of platinum species with tumor cell DNA to form platinum-DNA adducts is believed to be the primary mechanism by which these drugs trigger apoptosis in malignant cells (9). The relative ability of AP-5346 and free oxaliplatin to deliver platinum to tumor DNA, as opposed to just the whole tumor, was studied in this model. Tumor-bearing mice were given a single MTD bolus dose of each drug by the i.v. route. Groups of mice were sacrificed at various time points, and DNA was isolated from groups of 5 tumors per time point. The amount of platinum in the tumor DNA was quantified by inductively coupled plasma mass spectrometry. Figure 4 shows a plot of the platinum content of the tumor DNA as a function of time after drug injection. AP-5346 produced markedly higher levels of DNA adducts than oxaliplatin. Following administration of oxaliplatin, the peak tumor DNA platinum level occurred at 8 h and decreased progressively thereafter. In marked contrast, following injection of AP-5346 there was pro-

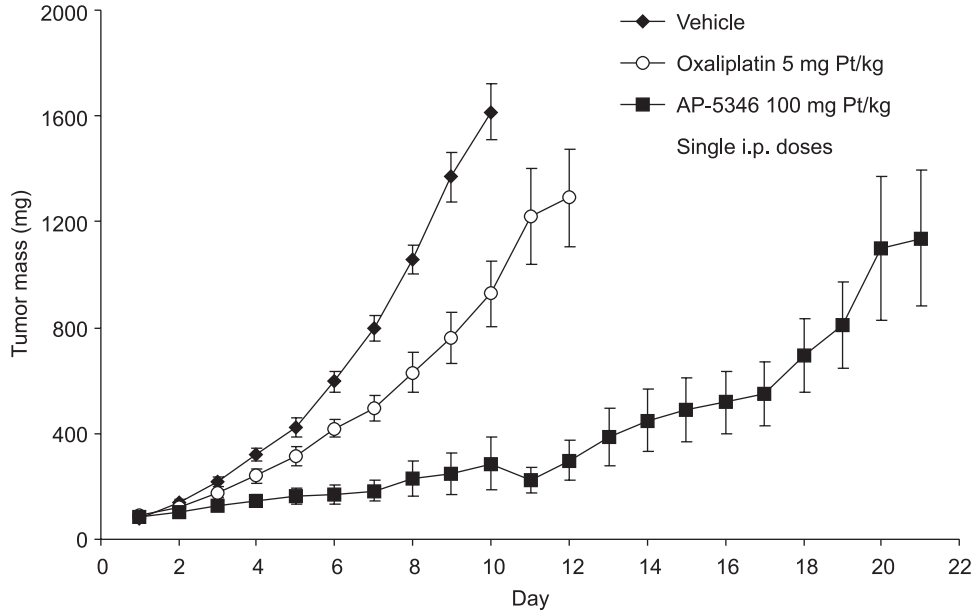

Fig. 2. Inhibition of B16 melanoma tumor growth by equitoxic single doses of oxalipatin or AP-5346. Data represent the mean ± SEM of estimated tumor mass in groups of 10 mice per group, each receiving single i.p. doses. Diamonds: vehicle control; open circles: oxaliplatin; squares: AP-5346.

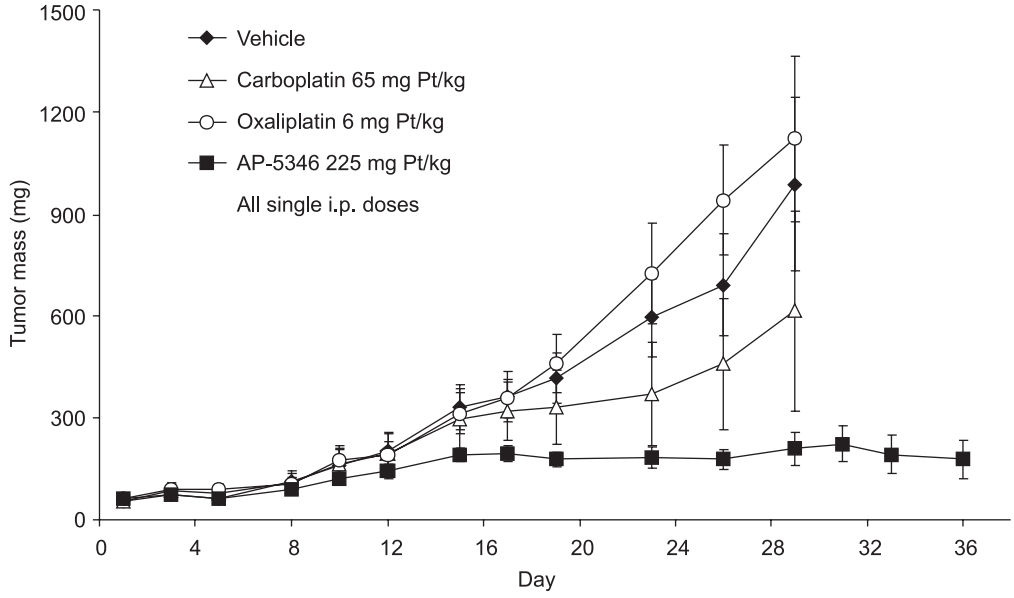

Fig. 3. Response of human ovarian 2008 tumor xenografts to equitoxic single doses of oxaliplatin, carboplatin or AP-5346. Data represent the mean ± SEM of tumor mass in groups of 6-10 mice per group, each receiving a single i.p. dose. Diamonds: vehicle control; open triangles: carboplatin; open circles: oxaliplatin; squares: AP-5346.

gressive accumulation of DNA adducts over the entire 168-h (7-day) period of sampling. At 168 h, the DNA content reached 70.2 pg/μg of DNA, a 10.8-fold increase over the peak reached by oxaliplatin treatment at 168 h. The area under the DNA platinum level-time curve was 13.6-fold greater following administration of AP-5346 than oxaliplatin. Even after 7 days, the tumor DNA platinum level had not yet started to decline in tumors treated with AP-5346, likely reflecting a prolonged phase of continued delivery of new AP-5346 molecules to the tumor.

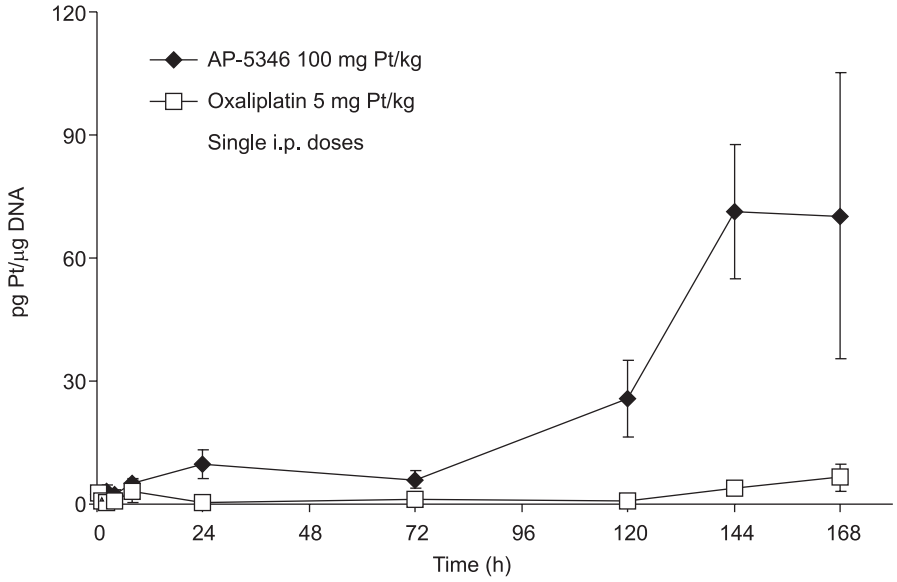

Fig. 4. Platinum-DNA adducts in the tumor tissue of mice bearing B16 melanoma tumors as a function of time following a single i.p. injection of AP-5346 at 100 mg Pt/kg (top curve; diamonds) or oxaliplatin at 5 mg Pt/kg (bottom curve; open squares). Data represent the mean ± SEM of 5 tumor samples per time point.

Toxicity

In preclinical rodent studies, the MTD of AP-5346 was found to be 20-fold greater than that of oxaliplatin. Such a high dose of cytotoxic agent is achieved because AP-5346 remains largely in an inactive form in the circulation, thereby protecting normal tissue, a design goal for this drug delivery system. Toxicology studies in rodents revealed that AP-5346 produced dose-limiting toxicities similar to those of other platinum agents, including impairment of renal function and myleosuppression. No adverse effects were noted other than those already known for platinum cytotoxic agents. Significantly, there was no evidence of the neurotoxicity seen with oxaliplatin.

Clinical Studies

On the basis of the activity in preclinical models and the benign results of the toxicology studies, AP-5346 entered phase I clinical testing at two major cancer institutes. At the time of writing, this trial, which is focused on defining the MTD and the dose-limiting toxicity when AP-5346 is given once weekly for 3 of every 4 weeks, is approaching its endpoint. Phase II studies are expected to commence in 2004.

Conclusions

Preclinical studies indicate that the design objectives for AP-5346 have largely been achieved. The drug has a prolonged plasma half-life, remains largely in an inactive form in plasma, but delivers much more platinum to the tumor and to tumor DNA than does oxaliplatin when both agents are administered at equitoxic doses. The preclinical toxicology studies did not reveal any types of toxicities not already associated with platinum agents, and the polymer delivery approach of AP-5346 appears to circumvent the neurotoxicity associated with its small-molecule analogue oxaliplatin. AP-5346 is a compelling candidate for continued clinical development, in which the key question to be addressed is whether the promise exhibited in murine tumor models, improved efficacy compared with existing platinum agents and superior therapeutic index, will also be seen in man.

Source

Access Pharmaceuticals, Inc. (US).

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