The Use of AFP-Complexes to Induce Apoptosis in Cancer Cells

V. Pak*

Constat Pharmaceutical Inc., MaRS Centre, Toronto, Canada

Abstract: An anticancer “magic bullet” should have both efficacy and specificity parts. We have used an effective Apoptosis Inducer to trigger the apoptosis. Alpha-fetoprotein was used to deliver An apoptosis Inducers specifically to cancer cells. The AFP-AI complex inhibited tumor growth in mice, enlarged mice life survival and has shown a 50% response in patients with metastatic colorectal cancer.

Keywords: Cancer, apoptosis inducer, targeted delivery, alpha-fetoprotein, multi drug resistance.

INTRODUCTION

Cancer is the proliferation/apoptosis overbalance. Proliferation and apoptosis are different systems and a re us ed completely dif fering ly: ke t he c ar g a s a nd bra ke s ystems (Fig. 1). Traditional anticancer drugs mainly aim to suppress proliferation by any mean (more than 700 molecular targeted agents are cu rently in trials), but directly activating apoptosis is a better idea (less than 30 agents in trials) [1, 2]. Apoptosis is responsible for recycling billions of cells everyday in the body. Cell accumulation mutations in both proliferation and apoptosis systems, but crucial mutations in the apoptosis system help them to avoid the natural suicidal pathway. To overcome the broken apoptosis elements, the drug should act “downstream” of mutated apoptosis cascade elements (Fig. 1). To trigger the apoptosis, a drug needs to: 1. be specific to cancer cell, 2. provide direct Apoptosis Inducer (AI) internalization, 3. Use AI acting “downstream” of the broken apoptosis cascade element. For those purposes, several combinations can be obtained with nanoparticles, immunoliposomes, cell-penetrating peptides, small molecule AIs, etc. We have prepared a non-covalent complex of alpha-fetoprotein (AFP) that can specifically deliver AI to cancer cells and act on functional executive apoptosis elements.

EFFECTIVE KILLING: DON’T REINVENT THE WHEEL – FIX THE BRAKES

Many cancer drugs are not optimal and often ineffective since they aim at the targets within the proliferation system (for example, DNA) which might not lead to the cancer cell death due to a frequent mutation of the p53 protein or other mutations in apoptosis pathways (Fig. 1) [3].

The problem of specific and effective elimination of defective cells in the body is already solved in Nature. Cytotoxic T lymphocytes (CTL), for example, use this mechanism. They first recognize wrong cell by membrane antigens and then use perforin for the internalization of granzyme B that activates caspasases – the final enzymes within the apoptosis cascade (Fig. 1) [4]. Granzyme B is a direct apoptosis inducer and is not dependent on “upstream” cancer cell mutations such as p53 and others. For this reason, CTL can “fix the brakes” meaning they are using undamaged apoptosis elements in side cancer cells (Fig. 1). Traditional chemotherapy targeting proliferation system elements (for example, doxorubicin: DNA) can be a tributed to the direct apoptosis inducers because it can lead to the following next activation of a apoptosis. Non-repairable DNA mutations caused by doxorubicin lead to p53-dependant apoptosis in normal cells. Direct apoptosis inducers on the opposite, trigger apoptosis themselves. The closer direct apoptosis inducer can act on a late apoptosis event less chances are that cancer cell will prevent its action. The ideal situation is that caspase activators such as P AC-1 molecule, b eing the last target in the cascade, will activate p rocaspe 3 and triggering aspase 3 the oncogenesis an effective anticancer action [5].

We tried to copy the effective natural CTL “know-how” to find and kill cancer cells by combining our lessons learned from CTL and using the known biological tools.

A mem brane is considered being the edge between life and death. Damage in the membrane of mitochondria, endoplasmic reticulum, lysosome or peroxisome can lead to the whole eukaryotic cell death. The membrane of mitochondria was chosen as a target because mitochondrion is a “point of no return” in the trinsic apoptosis pathway [6]. We have used prove n a n apoptosis inducer c ighting on m itochondrion such as a tracyloside, beutin acid [7, 8] and an endoplasmic reticulum apoptosis inducer thapsigargin [9]. Within the intrinsic apoptosis pathway, the mitochondrial a ctivates downstream of the p53 protein. P53 is inactive in more that 50% of cancer cells and often lead re sistance (MDR) [10]. Atractyloside was shown to induce apoptosis in p53-unfunctional Huh-7 cancer cells [11].

Since many of the agents do not cross the plasma membrane, w e had to use agents combined to a special delivery system in order to induce their internalization of those agents with a special delivery system. Moreover, this delivery system should have the ability to select cancer cells and avoid healthy tissues.

SPECIFIC DELIVERY

AFP may be responsible for embryotoxic and teratogenic action of same drugs, pollutants and chemicals. One of the...
Explanations of this phenomenon is that AFP has the ability to take toxins from mother’s blood and bring it to the embryo [12]. In such case, AFP should compete with the mother’s albumin for binding to the toxin. Among many other substrates, AFP has the strongest binding affinity to polyunsaturated fatty acids (PUFA). After the AFP-PUFA complex endocytosis by embryo cells, the PUFA is internalized [13]. We have substituted the PUFA in the porcine AFP-PUFA complex for atractyloside and have obtained the natural effective delivery system for the powerful direct AI.

A lot of AFP conjugates were used for the targeted delivery of different toxins to cancer cells, for example, AFP-esperamicin A [14].

We use the non-covalent complex that is closer to natural protein form and will not provoke immune attack as AFP conjugates can possibly do. In this case we can rely on the pharmacokinetic data obtained for natural human AFP in injectable form [15]. Pre-binding of AFP (MW=70 kDa) to atractyloside (MW=0.8 kDa) in 1:1-2 molar ratio possibly does not change pure AFP pharmacokinetic profile.

**DISCUSSION**

AFP is a known oncology marker [16, 17, 18]. For the addressed delivery of the AI, we have used AFP which has highly specific receptors re-expressed on the majority of cancer cells [19] and is up taken by them [20].

AFP-mediated delivery of di oxin to cancer cells having AFP receptor (AFP R) was 200-1400 times more effective than di oxin alone [12]. The environment tolerates atractyloside (AFPR) as I s a absorbed from rectal and is effective to suppress replication of hepatic viruses in the livers of children with hepatitis B and C [28]. The oil added to the drug formulation probably helped prevent degradation ofAFP drug conjugates testing [14].

AFP is known to be resistant to proteolytic attack from enzymes such as trypsin [26]. The oral route for peptide and protein drug delivery for several drugs was shown before [27]. The protein of the similar size as AFP - recombinant interferon - I s a absorbed from rectal and is effective to suppress replication of hepatic viruses in the livers of children with hepatitis B and C [28]. The oil added to the drug formulation probably helped prevent degradation of AFP drug conjugates testing [14].

We non-covally bound AFP to a proven direct AI - atractyloside which is toxic to the mitochondria. The AFP-AI complex (Aimpila™) was prepared as described in [25]. Briefly, the porcine embryo blood and urinary fluid were collected, purified and concentrated with 50 kDa MWCO ultrafiltration module, AFP was extracted by butanol, diafiltered, complexed with atractyloside in 1:1-2 molar ratios and lyophilized.

The Aimpila™ (0.02 mg in 0.2 ml of oil/day) was given orally and shown to inhibit tumor growth in the mice p388 leucosis model by 85% within 25 days and enlarged mouse life survival by 36% [25].

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An encapsulated form of Aimpila™ (0.6 mg/day) was used as monotherapy in pancreatic and colorectal cancer patients in a phase I/II trial [26]. The oral route for peptide and protein drug delivery for several drugs was shown before [27]. The protein of the similar size as AFP - recombinant interferon - is a absorbed from rectal and is effective to suppress replication of hepatic viruses in the liver of children with hepatitis B and C [28]. The oil added to the drug formulation probably helped prevent degradation of Aimpila™ in the intestine.

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three (3) patients were stabilized. No significant side effects, quick growth inhibition and tumor reduction were reported in that human study. This could be attributed to the way metastases were treated by us in a direct a apoptosis mode of Aimpila™ action.

Breast, liver, lung, ovarian, stomach, prostate and other cancers are AF PR-positive [19] and could potentially be treated with Aimpila™.

The data provided here support the idea of the necessity of the right combination of specific and effective parts to form an anticancer “magic bullet”. This means a factor which inevitably induces apoptosis in cancer cells and avoids potential mutation pathways. In a mixture, a second feature should be added to deliver the drug only to cancer cells and internalize it for optimal results.

CONCLUSION

Aimpila™ meets the necessary and sufficient conditions to be considered as an anticancer “magic bullet” because of its high specificity to A FP-positive cancer cells and its high efficacy in killing cancer cells due to direct apoptosis inducer action.

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REFERENCES


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