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Inhibition of Poly(ADP-ribose) polymerase – PARP

Truncated title – PARP inhibition

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Summary of recent advances

Inhibition of the DNA repair enzyme PARP-1 has been extensively investigated in the pre-clinical setting as a strategy for chemo- or radio-potentiation. Recent evidence has suggested that PARP inhibitors may be active as single agents in certain rare inherited cancers which carry DNA repair defects. Potent PARP-1 inhibitors have entered early clinical trials in cancer patients in the last 3 years and the final results of these trials are awaited.

Introduction

There has been considerable focus in recent years on the pathways of DNA repair as potential targets for improving cancer treatments, and inhibitors of some of these pathways are in early clinical development[1]. There are 5 major DNA repair pathways which preserve the integrity of the genome in normal cells (reviewed in [2-4]). Cellular DNA is repeatedly exposed to exogenous and endogenous toxins, it is estimated that the average rate of damage is about 10^4 events per cell per day [5] and these repair pathways are vital, with a number of inherited diseases being caused by mutations within them [2, 6]. However, in cancer cells, the protective effect of these pathways can be a disadvantage, acting as a cause of treatment resistance by

overcoming the damage caused to tumour cells by cytotoxic agents and reducing cancer cell kill.

Ionising radiation causes single and double strand breaks which are repaired by the Base Excision Repair (BER) and recombinational repair pathways respectively. Recombinational repair has two pathways, the error-free homologous repair (HRR) in dividing cells and error-prone non-homologous end joining active in G1 (NHEJ). These two pathways repair much of the damage caused by ionising radiation and chemotherapeutic agents such as cisplatin and mitomycin C. Nucleotide excision repair (NER) is involved in the repair of UV damage and removal of bulky DNA adducts such as those caused by cross-linking agents. The mismatch repair pathway (MMR) repairs replication errors and is frequently mutated in cancer cells allowing tolerance of such lesions [7-9]. BER is involved in the repair of single strand breaks, contributing to resistance to ionising radiation and alkylating agents. O^6-alkylguanine-DNA alkyltransferase (MGMT, OGAT, ATase) is the main component of the Direct Repair Pathway, an efficient mechanism of DNA repair where the altered base is corrected without removal or disruption of the phosphodiester backbone. Over expression of ATase in mammalian cells confers resistance to DNA alkylating agents (reviewed in [10]), and is a major factor in tumour resistance to these drugs.

Poly (ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) is the most abundant member of a family of highly conserved enzymes discovered 4 decades ago [11]. PARP-1 is a nuclear enzyme known to be involved in BER. Its role as a "molecular nick-sensor" was elegantly described by de Murcia in 1994 [12]. Briefly PARP-1 has three functional domains; the DNA biding fragment (DBD) which contains two zinc finger motifs involved in DNA strand break recognition and a nuclear location signal, the central auto-modification domain which includes a BRCA-1 C-terminal domain (BRCT) and the C-terminal catalytic fragment which binds NAD^+. The enzyme binds to, and is activated by, DNA strand breaks, forming long and branched polymers of poly(ADP-ribose) from NAD+. The negatively charged polymers are formed on various acceptor proteins, including PARP itself, histones and p53. X-ray crystallography has shown that the negative charge helps open up the damaged DNA to allow access to other components of the repair process[12]. The polymer is rapidly

removed by poly(ADP-ribose) glycohydrolase[13], allowing release and inactivation of PARP-1 to "search and signal" further DNA damage.

The many *in vitro* experiments demonstrating that PARP-1 inhibitors potentiate the cytotoxicity of anti-cancer drugs and ionising radiation, and the fact that *in vivo* PARP-1 knock out mice show increased sensitivity to these agents has stimulated the development of specific PARP-1 inhibitors as potential chemo- and radiosensitisers (reviewed in [14]). This article will focus on this area of therapeutic potential.

Development of PARP inhibitors

PARP-1 inhibition is an attractive method for studying the significance of this enzyme in biological systems and the early inhibitors were designed with this aim. Mainly inhibitors have been designed around nicotinamide, which is a by-product of PARP-mediated NAD^+ cleavage, and is itself a weak PARP-1 inhibitor. The first nicotinamide analogues were the benzamides. Inhibition of PARP by 3-aminobenzamide (3-AB) was reported over two decades ago by Whish with an inhibitory constant (K_i) of 1.8 ± 0.2 µM in L1210 cells [15]. The newer more potent PARP inhibitors have been designed based on interaction and inhibition at the NAD^+ binding site with X-ray crystallography of inhibitors in the binding site complementing structure activity relationships and allowing the identification of three critical hydrogen bonds [14, 16, 17].

As previously mentioned, part of the drive to develop potent, specific PARP inhibitors has been based on the observations that inhibition of PARP can potentiate the cytotoxicity of anticancer drugs. Durkacz et al first reported the ADP-ribosylation was involved in DNA excision repair and the inhibition of the enzyme enhanced the cytotoxicity of demethyl sulphate, a DNA alkylating agent, in 1980. At the end of this seminal paper the authors suggested that this "potentiation of cell killing by alkylating agents and PARP inhibitors may be of use in the treatment of human leukaemia" [18].

The Drug Development Programme, Northern Institute for Cancer Research, University of Newcastle Upon Tyne has identified, using rational drug design,

quinazolin-4-[3H]one (e.g. NU1025) and benzimidazole-4-carboxamide(e.g. NU1085) derivatives which are potent inhibitors of PARP-1 [16]. NU1025 and NU1085 have been shown to potentiate the cytotoxicity of alkylating agents, bleomycin (a free-radical producing glycopeptide which causes single and double strand breaks) and ionising radiation in a murine leukaemia cell line (L1210) ([19]; [20] but not the thymidylate synthase inhibitor nolatrexed or gemcitabline (a nucleoside analogue). Potentiation of camptothecan (a topoisomerase I poison) but not etoposide (a topoisomerase II poison) was reported in the same experimental system [21]. Camptothecan forms both protein-associated and non-protein associated single strand breaks which would bind and activate PARP-1. Etoposide is causes the formation of protein-associated double strand breaks which would not be a stimulus to PARP-1. The enhancement of both temozolomide and topotecan (a clinically active topoisomerase I poison) cytotoxicity has been confirmed in a panel of human common tumour cell lines independent of p53 status and tissue of origin [22]. Chemopotentiation of temozolomide, cisplatin and irinotecane has been demonstrated by the PARP-1 and PARP-2 inhibitor CEP-6800 (a 3-aminomethyl carbazole imide) both in tumour xenografts and cell lines [23]. Potentiation of temozolomide has also been reported with GPI 15427 [24] and Ino-1001 [25].

There is now considerable excitement over the potential of these agents in a wide variety of medical disciplines, as chemo- or radio-potentiators, as single agents in those cancers with other DNA repair defects and as protective agents against necrotic cell death in non-malignant conditions. The oncological indications will be discussed below and the non-oncological potential summarised. There are at least 3 PARP inhibitors in early clinical trials, AG014699 (Pfizer GRD) in combination with temozolomide, INO -1001 (Inotek) which is in phase II development in cardiovascular indication but also phase I studies with temozolomide in malignant glioma, and an oral compound from KuDOS which is in phase I studies as a single agent in cancer patients.

PARP inhibition in cancer therapy as chemopotentiation

There is a wealth of preclinical data that co-administration of a PARP-1 inhibitor with cytotoxic drugs which cause single and double strand DNA breaks potentiates the

activity of these agents and causes persistent DNA single strand breaks[19, 22, 23, 26-28]. Much of the pre-clinical work has focussed on potentiation of temozolomide, with enhanced activity seen in models inherently resistant to this agent [29-31] and it is in combination with this cytotoxic that PARP-1 inhibitors were taken into the clinic in cancer patients in 2003.

AG014699 is a potent tricyclic indole PARP inhibitor which has completed both phase I and II studies in combination with temozolomide. The phase I study was carried out in 2 stages in a collaboration between Cancer Research UK and Pfizer GRD. A PARP inhibitory dose (PID) of AG014699 of 12 mg/m^2/day was established by treating patients with advanced solid tumours with escalating doses of the PARP inhibitor in combination with 100 mg/m^2/day temozolomide (50% of the recommended dose) on a daily x5 schedule every 4 weeks. PARP inhibition was measured after dosing using a validated quantifiable immunoblot in peripheral blood lymphocytes. At the PID profound inhibition of the enzyme was observed for over 24 hours after a single dose. In the second stage of the study patients with advanced metastatic melanoma were treated with the PID plus escalating doses of temozolomide to establish the maximum tolerated dose of the combination. All patients agreed to paired tumour biopsies to allow target tissue PARP-1 inhibition to the explored. It proved possible to administer full dose (200 mg/m2/day) temozolomide in combination with this dose, achieving ~90% tumour PARP inhibition 5 hours after drug administration. No toxicity specific to the PARP inhibitor was observed, although increasing the dose of AG014699 above 12 mg/m^2/day caused enhancement of the myelotoxicity of temozolomide. The phase II recommended dose was AG014699 12 mg/m^2/day with temozolomide 200 mg/m^2/day on the above schedule and encouraging evidence of activity was seen (Plummer et al, paper in preparation, abstract in Proc Am Soc Clin Oncol 2005,). A phase II study as first line therapy in metastatic malignant melanoma has just completed recruitment and the results will be reported later this year.

There are no published data so far from the on-going trials of Ino-1001 in combination with temozolomide in patients with malignant glioma.

PARP inhibitors as radiopotentiators

An intriguing area of use of these agents is to potentiate radiotherapy. Treatment with ionising radiation is the most widely used anticancer intervention after surgery. Radiotherapy damages cells by causing both single and double strand breaks and inducing apoptotic cell death. DNA repair mechanisms within the cancer cells attempt to minimise this damage and are therefore a cause of resistance. Once again, there are encouraging pre-clinical data to support this use both in cell lines [27, 32, 33] and xenograft models [33]. This data is reinforced by the fact that PARP knock-out animals are hypersensitive to radiation [34, 35].

Trials of PARP inhibitors with radiotherapy will be challenging in terms of defining endpoints to establish benefit. Much radiotherapy is used to improve local control rates in the adjuvant setting, here the concerns over potentiation of local toxicity means dose reductions of established regimens might need to be investigated and the long follow-up needed are challenges to trial design.
Radiotherapy is also used in a palliative setting where it is often very effective in a wide range of lesions making standardisation of response very difficult. The increasing use of chemo-radiotherapy introduces another level of complexity into any PARP inhibitor combination studies.

It is for the reasons discussed above that these novel agents have first been taken into the clinic in combination with chemotherapy, and trials with radiotherapy remain an interesting possibility at the design stage.

Potential use as single agents in cancer therapy

In the last year very exciting data have emerged suggesting that PARP inhibitors might be beneficial in cancer treatment as a single agent. This would certainly have an advantage in terms of toxicity, as one of the concerns of chemotherapy combination studies is that, as well as enhancing anticancer effect, systemic PARP inhibition during exposure to a cytotoxic might increase myelosuppression and other organ toxicities.

Paired papers were published in Nature last spring [36, 37] demonstrating hypersensitivity of both BRCA-1 and BRCA-2 homozygous mutant cell lines and xenografts to a PARP inhibitor alone. The matched heterozygote and wild-type cell lines were not sensitive. The proposed model for this effect is that in the dividing wild type and BRCA heterozygous cells any endogenous single strand breaks are normally rapidly repaired by BER, but when PARP is inhibited the replication fork will encounter persisting single strand breaks and a double strand break will be formed. Despite the lack of effective BER, recombinational repair will efficiently correct the defect and replication continues. However, in BRCA homozygous mutant cells, the lack of effective recombination repair in the presence of PARP inhibition would lead to a collapse of the replication fork and the induction of apoptosis[38, 39].

It is these intriguing data that have led to the investigation of the KuDOS orally available PARP-1 inhibitor as a single anti-cancer agent, and a Phase I study is on-going. In addition, a phase II study of the PID of AG014699 in metastatic breast and ovarian cancer in proven carriers of a BRCA-1 or BRCA-2 mutation is in development, sponsored by Cancer Research UK.

PARP inhibitors in other clinical indications

One fascinating aspect of these novel agents is that there are myriad potential applications outside the area of cancer treatment. In cancer medicine, the potential therapeutic effect is caused by inhibition of base excision repair maximising DNA damage and triggering the apoptotic pathway. However, in the face of massive DNA damage, such as after an ischaemic episode, the activation of PARP-1 can deplete the cell of NAD^+, causing acidosis and triggering necrotic cell death. There are many pre-clinical models, utilising knock-out animals or PARP inhibitors, demonstrating that reduction in PARP activity in this situation is protective and minimises the damaged area. Full discussion of this area is beyond the scope of this review, but excellent recent publications are available [32, 40-43].

Conclusions

PARP-1 inhibition as a clinical intervention for a range of diseases has been proposed for many years. In the last two years potent inhibitors have entered the clinic and data

published so far suggest that these agents can be highly active against the target enzyme without inhibitor specific toxicity. It will be a number of years before the true potential of this class of agents is fully known. They may have an important niche outside the field of cancer; this is a very exciting drug development area to watch.

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