Poly(ADP-ribose) polymerase inhibitors as potential chemotherapeutic agents

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Abstract

PARP [poly(ADP-ribose) polymerase] activity is up-regulated by binding to DNA strand breaks and its association with DNA repair is well documented. Many anticancer therapies work by inducing breaks in DNA, if unrepaired these can lead to cell death. As PARP promotes DNA repair there is a strong rational to suggest that its inhibition may increase the efficiency of certain cytotoxic treatments. This review discusses the advances made in PARP inhibitor design and the mechanism by which they enhance anti-tumour therapies.

The enzyme PARP [poly(ADP-ribose) polymerase]

The abundant nuclear protein PARP is the most well-characterized member of a family of enzymes, which cleave NAD\textsuperscript+ to nicotinamide and ADP-ribose, forming negatively charged long-branched (ADP-ribose) polymers on glutamic residues of itself and acceptor proteins. Addition of the negative charge can drastically affect the activity of acceptor proteins and poly(ADP-ribosyl)ation has been reported to regulate many cellular processes such as DNA repair, genomic stability, cell-cycle progression, cell death and gene transcription (reviewed in [1]).

History of PARP inhibitors

Almost all PARP inhibitors are competitive inhibitors of NAD\textsuperscript+. The first were analogues of nicotinamide (e.g. 3-aminobenzamide). These were useful for in vitro studies of PARP activity [2] and, along with molecular genetic studies [3], indicated that PARP inhibition could indeed enhance the cytotoxicity of DNA damaging agents. However, they were of low potency, low solubility and lacked specificity and therefore were of less clinical use. The next generation of rationally designed inhibitors, benzimidazole-carboxamides, quinazolin-4-[3H]-ones and isoquinoline derivatives [e.g. 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide (NU1085), 8-hydroxy-2-methylquinazolin-4-(3H)-one (NU1025) and dihydroisoquinoline (PD128763)], were vastly more potent and were used to demonstrate striking increases in radio and chemosensitization in vitro (reviewed in [4]). For example, NU1025 enhanced the cytotoxicity of the monofunctional DNA-alkylating agent temozolomide and the Topo I (topoisomerase I) inhibitor camptothecin in 12 human tumour cell lines [5]. Unfortunately, these were still not potent or specific enough for extensive preclinical trials. Recently, a PARP inhibitor more than 1000 times as potent as 3-aminobenzamide has been developed. AG14361 has been used in vivo at non-toxic doses to augment the effect of the DNA-damaging agents irinotecan, γ-irradiation or temozolomide [6]. Combination therapy resulted in 2–3-fold enhancement in the delay of LoVo xenograft growth when compared with DNA-damaging agent alone. In addition, the combination of AG14361 and temozolomide caused complete remission of SW620 xenograft tumours. Another important potential application of PARP inhibitors is in the treatment of cells deficient in MMR (mismatch repair), where they have been shown to sensitize cells resistant to temozolomide [4,7].

PARP inhibition in enhancing cytotoxicity of alkylating agents and ionizing radiation

PARP binds to DNA SSBs (single-strand breaks) formed during BER (base excision repair) of alkylating agent-induced small DNA adducts or produced directly by ionizing radiation [8]. Binding activates PARP; the addition of negative charge through autopoly(ADP-ribosyl)ation attracts other repair proteins and will eventually cause PARP to be repelled from DNA allowing access to further repair proteins and completion of the process [9]. Thus the hypersensitivity of PARP depleted or inhibited cells to agents which induce lesions normally repaired by BER is a reflection of a reduced ability to attract the proteins required for complete BER. As PARP is not required for the process of BER itself [10], the presence of enzymically inactive PARP-bound DNA will also prevent DNA repair proteins from accessing DNA and further increase the toxicity of chemotherapeutic agents.

PARP inhibition in enhancing cytotoxicity of Topo I inhibitors

PARP inhibitors can also enhance the cytotoxicity of Topo I inhibitors [4–6]. During replication, transcription,
**Figure 1 | Mechanism for enhanced toxicity by inhibition of PARP**

(A) Many cytostatic drugs induce SSBs either directly (e.g. ionizing radiation or Topo I inhibitors) or indirectly (e.g. alkylating agents). (B) In the presence of PARP, these SSBs are efficiently repaired and replication is mostly unaffected. (C) When PARP is inhibited, SSBs may persist and be converted into DSBs due to inefficient repair. These collapsed forks are probably the cytotoxic lesion causing death. (D) HR is critical in the repair of these collapsed replication forks and promotes survival [12].

Recombination and chromatin remodelling DNA Topo I relaxes DNA relieving the torsional stresses by introducing a transient SSB. Topo I inhibitors interfere with the rescaling activity of the enzyme and leave unrepaired SSBs in the DNA. Most Topo I inhibitors exert their cytotoxic effect predominantly during the S-phase of the cell cycle. It has been shown that the cytotoxic lesion is a DNA DSB (double-strand break), which is formed when the replication fork encounters the SSB (Figure 1) [11]. PARP normally functions in SSB repair and, thus, inhibition of its enzyme activity will result in more SSBs and presumably more toxic DSBs (Figure 1). HR (homologous recombination) has an important role in the repair of replication-associated DSBs formed after treatment with Topo I inhibitors [12]. The formation of toxic DSBs at replication forks is a probable mechanism to explain why combined treatments with Topo I and PARP inhibitors are so effective [11,12].

**PARP inhibition and MMR-deficient cells**

Defects in genes involved in MMR or in their expression result in increased risk of tumour development, and in increased resistance to many anticancer therapies [13]. PARP inhibitors have been shown to sensitize resistant cells to alkylating agents [2,4]. In one study [7], a PARP inhibitor (AG14361) enhanced temozolomide activity in MMR-proficient cells but was more effective in MMR-deficient cells, where it overcame their resistance to temozolomide. As the PARP inhibitor did not enhance the level of DSBs nor potentiate cisplatin activity in resistant MMR-deficient cells it was supposed that PARP did not have a direct role in MMR-mediated repair of DNA lesions. It is more probable that in MMR-deficient cells there is a switch in toxic lesion and that this new lesion is repaired in a PARP-dependent way. Temozolomide causes a mixture of lesions [14]. In MMR-proficient cells, O^6^-methylguanine is highly toxic. However, in MMR-deficient cells these lesions are tolerated and the primary cytotoxic lesion becomes N^7^-methylguanine and N^3^-methyladenine adducts. These two lesions do not cause mispairing and can be repaired by BER. As PARP enhances BER, PARP inhibitors would be expected to enhance specifically the cytotoxicity of temozolomide in MMR-deficient cells.

**PARP inhibitors and HR**

PARP-1 appears to be important for HR levels; however, it is not directly involved in the process itself [3,15], more probably the increase in HR seen in PARP-deficient cells is due to an accumulation of recombinogenic substrates in MMR-deficient cells. Thus HR may have a particularly important role in repairing lesions in these cells and HR-deficient cells may be especially sensitive to inhibition of PARP-1. Recently, we tested this hypothesis and found that HR-deficient cells (including BRCA2-deficient cells) were indeed sensitive to PARP inhibition (H.E. Bryant, N. Schultz, H.D. Thomas, D.R. Flower, E. Lopez, S. Kyle, M. Meuth, N.J. Curtin and T. Helleday, unpublished work). This may be of particular relevance as BRCA2 mutation is commonly associated with breast or ovarian cancer. It is important to note that this treatment differs from previous therapies in that no additional genotoxic drug was needed to cause cell death and in this respect we would expect the treatment to have few side effects, especially as wild-type cells showed very low toxicity to PARP inhibition.

**Summary**

The potential use of PARP inhibitors to enhance the activity of DNA-damaging agents has been known for some time; however, it is only now with the design of soluble, stable, specific, high potency inhibitors that this potential can begin to be evaluated in human models. As PARP inhibitors alone appear to be non-toxic at radioactivating doses, there may be an attractive alternative to other radiosensitizing methods such as inactivation or inhibition of epidermal growth factor and the use of bioreductively activated drugs. Their ability to sensitize both MMR-proficient and -deficient cells to temozolomide makes them more versatile than other methods tried, e.g. inhibition of the repair protein O^6^-alkylguanine DNA alkyltransferase. In addition, this ability to overcome drug resistance in MMR-deficient cells may be of particular clinical significance as loss of MMR genes can occur during anticancer therapy and has in certain cases been associated with a poor prognosis, especially as MMR defects have not been reported in normal tissues. We have already seen the first PARP inhibitor used in combination with temozolomide to treat successfully a human colon tumour xenograft model [6]; hence, the possibility of a clinical trial would now be an exciting prospect.

In conclusion, inhibition of PARP potentiates the cytotoxicity of many anticancer drugs presumably by inhibiting its normal role in DNA repair. An exciting new opportunity for chemotherapy may be emerging, as PARP inhibitors alone
can specifically kill HR-deficient cells, the exploitation of the genetic defect causing the cancer itself may not be too far away.

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References

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