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Commentary

Oxidative activation of indole-3-acetic acids to cytotoxic species—a potential new role for plant auxins in cancer therapy

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Abstract

Indole-3-acetic acid (IAA) and some derivatives can be oxidised by horseradish peroxidase (HRP) to cytotoxic species. Upon treatment with IAA/HRP, liposomes undergo lipid peroxidation, strand breaks and adducts are formed in supercoiled plasmid DNA, and mammalian cells in culture lose colony-forming ability. IAA is only toxic after oxidative decarboxylation; no effects are seen when IAA or HRP is incubated independently in these systems at equivalent concentrations. Toxicity is similar in both hamster fibroblasts and some human tumour cells. The effect of IAA/HRP is thought to be due in part to the formation of 3-methylene-2-oxindole, which may conjugate with DNA bases and protein thiols. Our hypothesis is that IAA/HRP could be used as the basis for targeted cancer therapy involving antibody-, polymer-, or gene-directed approaches. HRP can thus be targeted to a tumour allowing non-toxic IAA delivered systemically to be activated only in the tumour. Exposure to newly synthesised analogues of IAA shows a range of four orders of magnitude difference in cellular toxicity but no structure–activity relationships are apparent, in contrast to well-defined redox dependencies of oxidation by HRP intermediates or rates of decarboxylation of radical-cation intermediates.

Keywords: Indole-3-acetic acid; Horseradish peroxidase; 3-Methylene-2-oxindole; Targeted cancer therapy (ADEPT; PDEPT; GDEPT)

1. Introduction

Recent research suggests that IAA, a plant growth hormone, when oxidised with HRP produces a toxic species that could be used as the basis for a novel cancer therapy [1]. IAA is well tolerated in humans [2] and is not readily oxidised by mammalian peroxidases, so that targeting of HRP to a tumour would allow production of the toxic IAA metabolite in the tumour alone, avoiding damage to normal tissues. Exploratory experiments have been performed investigating the use of antibodies, polymers, and genes as carriers for HRP tumour targeting.

IAA is a naturally occurring plant growth phytohormone. It has been studied intensively for many years by plant biologists, but questions remain as to how it carries out its role. Existing in picomolar levels in plants, it affects many different growth properties: cell enlargement, division and differentiation, as well as, in some instances, senescence and abscission of leaves [3]. IAA activity is regulated by the control of metabolism through irreversible removal via two different pathways: oxidation by peroxidases leading to decarboxylation, and non-decarboxylation reactions forming non-reactive conjugates [4]. The decarboxylation pathway catalysed by HRP has been investigated extensively over the years, and is known to be complex.

HRP is a widely studied heme-containing peroxidase enzyme, existing in its native state in the ferric form. It can oxidise a wide variety of substrates in the presence of hydrogen peroxide, catalysing one electron oxidation reactions through its compound I and II forms [5] (Fig. 1). The reaction between IAA and HRP has been studied primarily because of interests in plant biochemistry, but the mechanism is extremely complex and still not fully elucidated. One key feature in the reaction between IAA and HRP is that hydrogen peroxide is not required to oxidise native HRP to compound I, unlike reaction with many other substrates for the enzyme (e.g. phenols).

HRP compound I and II will oxidise IAA (Fig. 1, **1**) at neutral pH to an indolyl radical cation (Fig. 1, **2**). This cation dissociates to form an indolyl radical (Fig. 1, **3**) with

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Abbreviations: IAA, indole-3-acetic acid; HRP, horseradish peroxidase; TBA, thiobarbituric acid; MPO, myeloperoxidase; MOI, 3-methylene-2-oxindole; BSO, buthionine sulphoximine; GSH, glutathione; CEA, carcinoembryonic antigen; ADEPT, antibody directed enzyme prodrug therapy; PDEPT, polymer directed enzyme prodrug therapy; GDEPT, gene directed enzyme prodrug therapy; and PEG, polyethylene glycol.

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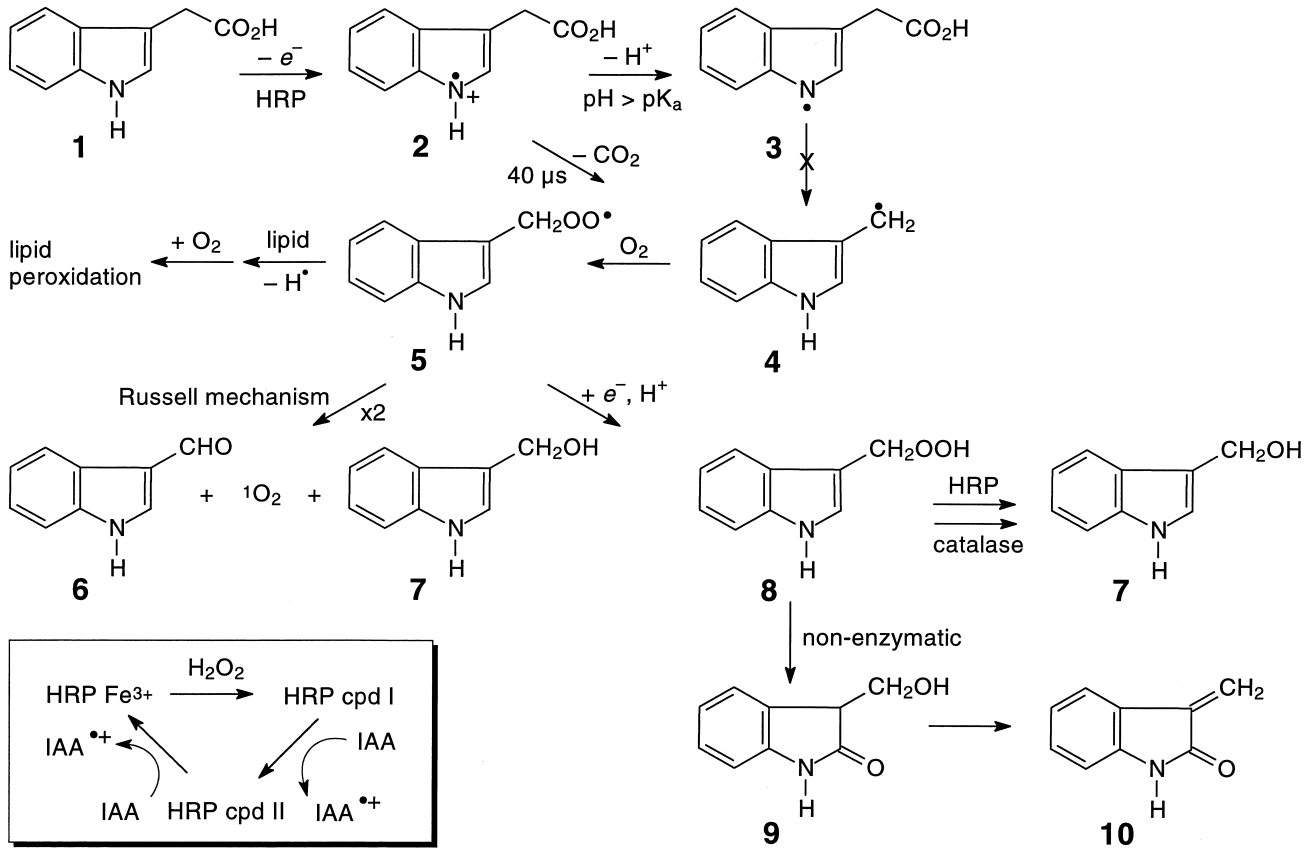

Fig. 1. Main reaction pathways involved in oxidative activation of IAA to toxic species.

a radical pK_a of 5.1 for dissociation of the indole N-H group. The radical cation (Fig. 1, **2**) but not the dissociated radical (Fig. 1, **3**) decarboxylates in approximately 40 μsec to form a skatoyl radical (Fig. 1, **4**) [6]. This carbon-centered radical is very reactive towards oxygen, rapidly forming a peroxyl radical (Fig. 1, **5**). The peroxyl radical can decay in two ways. Reduction and protonation form skatole hydroperoxide (Fig. 1, **8**), which reacts further with HRP compound I to form indole-3-carbinol (Fig. 1, **7**) [7]. The hydroperoxide can also decompose non-enzymatically to oxindole-3-carbinol (Fig. 1, **9**) and MOI (Fig. 1, **10**) (see below). In addition, combination and elimination by the Russell mechanism, in which two peroxyl radicals combine, form indole-3-aldehyde (Fig. 1, **6**), indole-3 carbinol (Fig. 1, **7**), and singlet oxygen [8], although this may not occur at physiological pH [9]. The reactivity of various indoles with HRP compound I has been shown to be closely related to the reduction potentials of the indolyl radical [10] with an increase in rate constant of approximately 300-fold for a decrease in reduction potential (radical/ground state) of only 0.1 V.

These free radical based mechanisms of the IAA/HRP combination were the basis of our original hypothesis [11] of possible elevated peroxidase levels in human tumours [12] metabolising IAAs for use as a chemotherapeutic strategy.

2. Exploitation of radical formation to induce lipid peroxidation

The formation of peroxyl radicals from the oxidation of IAA with HRP can induce lipid peroxidation in liposomes [13]. The peroxyl radical is repaired by abstraction of a hydrogen atom from an unsaturated fatty acid. The resulting lipid radical then reacts with oxygen producing a lipid peroxyl radical that abstracts another hydrogen atom, causing a chain reaction. The resulting lipid peroxidation can be measured by the TBA test and by the loss in fluorescence of incorporated *cis*-parinaric acid. The extent of lipid peroxidation shows some correlation with HRP compound I reactivity rates. Incorporation of antioxidants, including ascorbate, GSH, Trolox (a water-soluble form of vitamin E), α-tocopherol, and β-carotene, into the liposome system was shown to prevent HRP-induced lipid peroxidation [14]. These antioxidants could be protecting by either repairing the lipid radicals or initiating IAA peroxyl radicals.

Peroxidative damage in liposome models induced by the IAA/HRP system is easy to measure, with different ana-

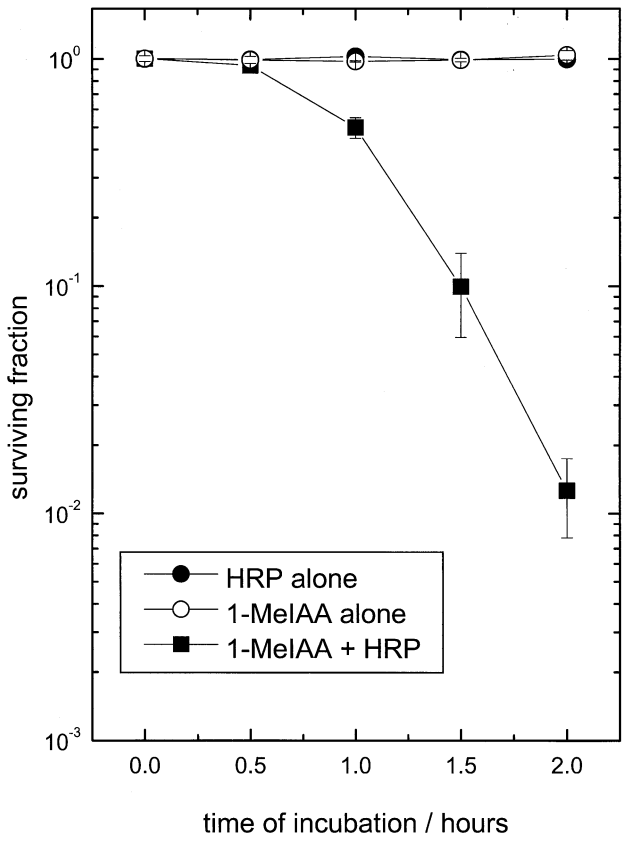

Fig. 2. Surviving fraction of V79 cells treated with 0.1 mM 1-methyl-IAA and 1.2 μg/mL of HRP in phenol red free Hanks' balanced salt solution. Values are means ± SEM of 3 experiments.

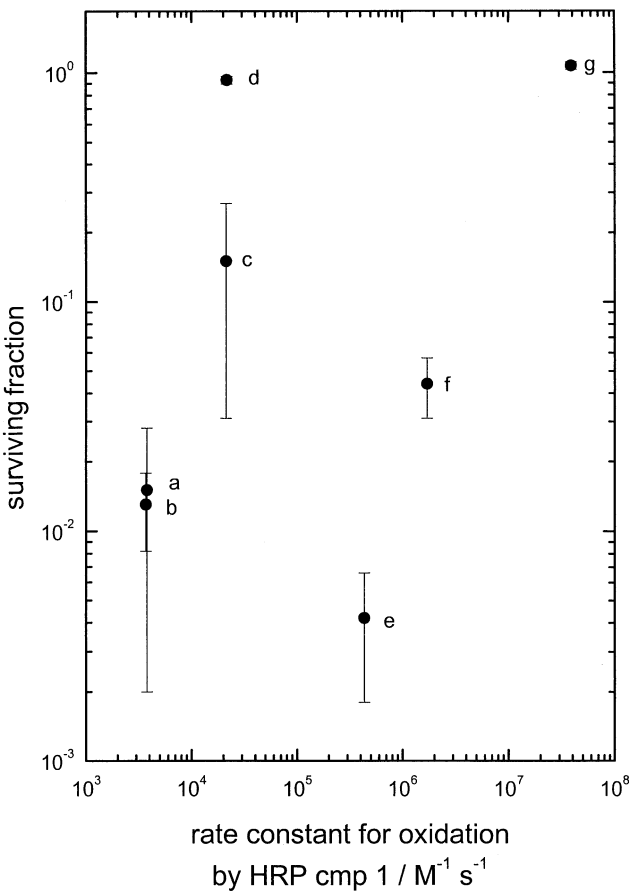

Fig. 3. Surviving fractions of V79 cells following 2-hr incubations with 100 μM indole derivatives and 1.2 μg/mL of HRP in Hanks' balanced salt solution compared with rate constants for reaction with 0.5 μM HRP compound I at pH 7. (a) IAA, (b) 1-methyl-IAA, (c) 5-benzyloxy-IAA, (d) 5-methoxy-IAA, (e) 2-methyl-IAA, (f) 2-methyl-5-methoxy-IAA, and (g) 2-methyl-5,6-dimethoxy-IAA. Values are means ± SEM of 3 experiments.

logues giving different degrees of lipid peroxidation, damage generally correlating with HRP compound I rates [13]. Our initial hypothesis was that such damage could be induced in tumour cells and lead to cytotoxicity [11]. However, subsequent studies with cellular systems suggested that lipid peroxidation was not an important component of IAA/HRP-induced cytotoxicity.

3. Cytotoxicity of IAA and HRP

HRP is able to oxidise IAA with only a trace of organic peroxide (a common impurity in biological media) required to initiate the reaction, with the production of the hydroperoxide throughout the reaction allowing the enzyme to recycle [15]. Treatment of hamster fibroblast V79 cells with 1-methyl-IAA (0.1 mM) and HRP (1.2 μg/mL) for 2 hr in the absence of added peroxide was shown to prevent colony formation with a surviving fraction of 0.013 (Fig. 2) [1]. Other variations in the IAA substituents were found to change the cytotoxicity of the indoles [1], but plotting surviving fractions against rates of reaction with HRP compound I showed no structure–activity relationships (Fig. 3) despite a difference of four orders of magnitude in toxicity for some of them.[1] Toxicity for one of the analogues was seen at equivalent doses in two human lines, MCF7 breast carcinoma and HT29 colon carcinoma cells [1]. Further, lipid peroxidation in whole cells could not be detected in any of the treatments tried. This led us to question our original hypothesis for the toxicity of the IAA/HRP combination in oxygenated cells. Even in anoxic cells toxicity can be measured,[2] which is likely to be free-radical based. The formation of the skatoyl radical (Fig. 1, **4**) still occurs in anoxia, but reaction with oxygen cannot; the carbon-centered radical can abstract a hydrogen atom from other sources (for example DNA [16]) or lipid, causing cell damage by the formation of radicals.

IAA (1 mM) is moderately toxic to cultured rat neutrophils (40% loss of viability after 24 hr) [17,18]. Cell death in these systems has been linked to endogenous peroxidase (MPO) activity, leading to ultrastructural changes and in-

[1] Folkes LK, unpublished results.
[2] Greco O, Unpublished results.

creased production of hydrogen peroxide and superoxide radicals. Lymphocytes, which have 10-fold less peroxidase activity than neutrophils, were not found to be sensitive to IAA unless exogenous HRP was added [18]. We have shown that cultured HL60 cells (human pro-myelocytic leukaemia lymphocytes) lose approximately 25% viable cells 72 hr after a 1-hr exposure to IAA (10 mM) in the absence of added peroxidase [16]. No cytotoxicity was seen for HL60 cells exposed to IAA and HRP in the same conditions used for the V79 cell incubations. Using a spin trap and EPR detection, endogenous MPO in HL60 cells was shown to oxidise IAA to the corresponding skatolyl radicals [16]. However, monitoring IAA turnover with purified MPO showed that the reaction was inhibited after only a few minutes. It appears that lymphocytes may have a protective mechanism against IAA damage. This might be expected as they are exposed to high levels of peroxidative stress in normal conditions, or possibly MPO in lymphocytes is not able to oxidise IAA sufficiently rapidly to achieve a toxic response. IAA is not the usual substrate of MPO (chloride ions are oxidised to hypochlorous acid in the presence of hydrogen peroxide in its normal function), and therefore ideal catalytic conditions may not be achievable.

4. Non-enzymatic oxidation of IAA to generate cytotoxic products

Radiation can be used to simulate HRP oxidation reactions. $Br_2^{\bullet-}$ produced by γ-radiolysis will oxidise IAA to the radical cation, thus producing the same oxidation products as HRP. IAA oxidised in this way and added to cells gives a toxic response, proving that cellular toxicity is not due directly to a transient peroxyl radical. A stable product must be formed, which is toxic to cells [1]. Further evidence for the formation of a stable cytotoxin was provided by the oxidation of IAA by HRP in the absence of cells. Toxicity was still seen after removing the enzyme by filtering through a high molecular weight cut-off filter followed by exposure of cells to the filtrate [16].

Photoactivation of riboflavin to its triplet excited state, which can also oxidise IAA to its radical cation, provides further evidence for the importance of the radical cation decomposition products in cytotoxicity (see below).

5. Identification of a possible cytotoxic product(s)

Monitoring the oxidation reaction of IAA by high performance liquid chromatography with mass spectrometric detection allows the identification of the major products as indole-3-carbinol (Fig. 1, **7**), oxindole-3-carbinol (Fig. 1, **9**) and MOI (Fig. 1, **10**). Indole-3-carbinol at likely concentrations with or without peroxidase was not toxic to V79 cells. This led us to consider the other products. MOI (Fig. 1, **10**) was first identified as a product of IAA oxidation in 1961 [19], being a breakdown product of oxindole-3-carbinol (Fig. 1, **9**). It is very reactive towards nucleophiles (for example free thiols, protein thiols, or DNA bases), which could lead to toxic responses in cells. Early research into IAA action in plants suggested that MOI is toxic to *Escherichia coli* and some plants [20,21]. The photoxidation of IAA in the presence of riboflavin causes cell death in *E. coli*, which was thought to be due to the production of MOI [20,21]. This photo-sensitised oxidation was suggested to be a possible reason why plants grow towards light since photolysed cells are prevented from growing in the presence of IAA.

We have shown that MOI produced by radiolytic oxidation reacts with GSH, but a rate constant is yet to be determined. Interestingly, when the products of radiolytic oxidation are mixed with excess GSH and then added to cells, toxicity is no longer seen. However, depletion of GSH in mammalian cells with BSO treatment does not increase toxicity. The presence in a cell of other protective nucleophiles (for example, protein thiols that could also react with MOI) could explain the lack of effect on the cytotoxicity of IAA/HRP after GSH depletion. IAA/HRP treatment of V79 cells results in the loss of approximately 50% of cellular GSH[3], although the same loss is seen with HRP alone (GSH is a substrate for HRP [22]) and no cytotoxicity is seen.

Supercoiled plasmid DNA in the presence of indoles oxidised by HRP undergoes strand scission to yield circular and linear DNA, with covalent binding to supercoiled DNA also occurring, increasing its molecular weight and decreasing its movement through a gel [1]. DNA strand breaks could conceivably be formed following hydrogen abstraction by IAA peroxyl radicals. However, DNA strand breaks are unlikely to be the cause of cell death as activation of IAA occurs extracellularly and short-lived free radicals are unlikely to be stable enough to transfer to the nucleus. A stable product, however, could diffuse from the extracellular space to the cell nucleus and bind with DNA, causing toxicity. Studies with [5-^{3}H]IAA showed that an oxidation product of IAA binds to cells treated with IAA/HRP. A 14-fold increase in radioactivity was seen in whole cells that had been treated with IAA and HRP compared to whole cells treated with IAA alone [1]. Analysis of cell fractions indicated that the radioactivity was 40-fold higher in the nuclear fraction in treated cells than in control cells.[3] It has been suggested that the binding of MOI to sulphydryl regions of histone DNA [23] or RNA [24] may be involved in a regulatory process for IAA in plants.

The conclusion from the available data is that IAA oxidised in the presence of HRP causes cell death due to the formation of a highly reactive metabolite, very probably MOI. However, proof is still required as pure MOI is yet to be obtained. Attempts to isolate MOI from oxidised IAA have also been unsuccessful. Note, however, that the for-

[3] Folkes LK, unpublished results

mation of MOI does not explain the high toxicity of 2-methyl-IAA and 2-methyl-5-methoxy-IAA to V79 cells [16]. Methylation at the indole 2-position should prevent the formation of a carbonyl group.

A major difficulty with mechanistic studies into IAA/HRP activity in respect to cell toxicity is the low turnover of drug resulting from the amount of enzyme (1.2 μg/mL) used to cause cell death. It is difficult to measure and identify the IAA products at these low concentrations. Monitoring at higher concentrations is easier but may not correspond to the reactions occurring in cell incubation experiments. HRP can act both as a peroxidase and as an oxidase; ratios of HRP to IAA, as well as their actual concentrations, are thought to affect the route by which IAA is activated [15]. Different products at different ratios may be formed from the different cycles [25]. Another factor to consider is that the formation of MOI and its subsequent reaction with cellular nucleophiles may affect the catalytic cycle of HRP. The ratios of the products may be changed if one of the products is being removed by reaction with cellular targets. This is not something that we have been able to investigate to date, as extraction and identification of any bound products in cells have been inconclusive.

Regardless of the detailed mechanism of cytotoxicity, it appears clear that oxidative activation of IAA and some analogues produces a potent cytotoxic response. We are seeking to ascertain whether this could be exploited to produce a novel chemotherapeutic effect.

6. Targeting of HRP for therapy

For IAA/HRP to elicit a specific anti-tumour effect, either enzyme or prodrug has to be targeted specifically to the tumour. If HRP was specifically targeted, circulatory non-toxic IAA would be activated only in the tumour, preventing cell damage in normal tissues. Although HRP oxidation of IAA is more efficient at lower pH, it is still active at physiological pH. The extracellular pH of tumours is reported to be less than that of normal tissue [26], indicating that HRP may have a higher activity in a tumour. HRP can oxidise many different substrates including physiological compounds, e.g. tyrosine. However, a steady supply of hydrogen peroxide is required to oxidise these substrates, unlike the indoles, allowing specific activation of IAA in a tumour. IAA is also a poor substrate for mammalian peroxidases in the absence of hydrogen peroxide [8, 27], and the suggested toxin MOI is very reactive towards cellular nucleophiles, reducing the chance of diffusion to and damage to normal tissue. The oxidation of IAA by HRP can also occur at low oxygen tensions,[4] allowing targeting to hypoxic regions of a tumour. These factors lend further support to the possibility of IAA/HRP being used as a targeted therapy.

We have explored three concepts as the basis for targeting of HRP to tumours (Fig. 4).

7. ADEPT

ADEPT [28,29] is currently in phase II clinical trials. Conjugation of an enzyme to an antibody or antibody fragment specific to an extracellular tumour antigen (e.g. CEA binds strongly to many epithelial carcinomas including colon tumours) allows it to be targeted selectively to the tumour after intravenous administration. When a non-toxic prodrug is added after sufficient time for clearance of the unbound antibody, activation to the drug occurs preferentially in the tumour with one molecule of conjugate able to activate many molecules of prodrug. The concept relies upon adequate uptake of the enzyme and prodrug into the tumour and sufficient activity of the enzyme once bound to the antibody to induce a toxic response, with bystander effects occurring such that neighbouring cells unattached to the antibody–enzyme complex will also be killed. Ideally, the drug should be active towards both dividing and quiescent cells, but prodrugs also have to be designed so that mammalian enzymes cannot activate them to induce normal cell death; a high differential in cytotoxicity between the prodrug and drug must exist. The targeted antigen needs to be specific to the tumour or expressed at a much higher density in the tumour compared with normal tissue; antigens present in the circulation will also compete with tumour sites for antibody binding. Lysis of tumour cells following drug-induced death will release the antigen into the circulation, although this can be allowed to clear before repeat administration of the prodrug. ADEPT can cause immunogenic problems from the addition of foreign proteins, although these responses can be reduced by the administration of immunosuppressive drugs during treatment, allowing more than one course to be given. Conjugating the antibody to a polymer like PEG [30] can also reduce immunogenic responses, as well as increase the circulatory time of the enzyme and therefore its potential to reach the tumour. This could lead to a delay in administration of the prodrug, however, as the enzyme needs to be cleared from the circulation first.

HRP has been successfully conjugated to anti-CEA antibody,[5] resulting in a retention of 50% of activity of the enzyme. Targeting to a human colorectal xenograft LS174T model occurs; however, tumour to normal tissue ratios are lower than those achieved with antibody alone. Further work is in progress to increase tumour uptake.

8. PDEPT

PDEPT is a variation on ADEPT [31]. Polymer binding has been used to administer cytotoxic drugs (e.g. doxoru-

[4] Greco O, unpublished results.

[5] Pedley RB, Royal Free Hospital, London, unpublished results.

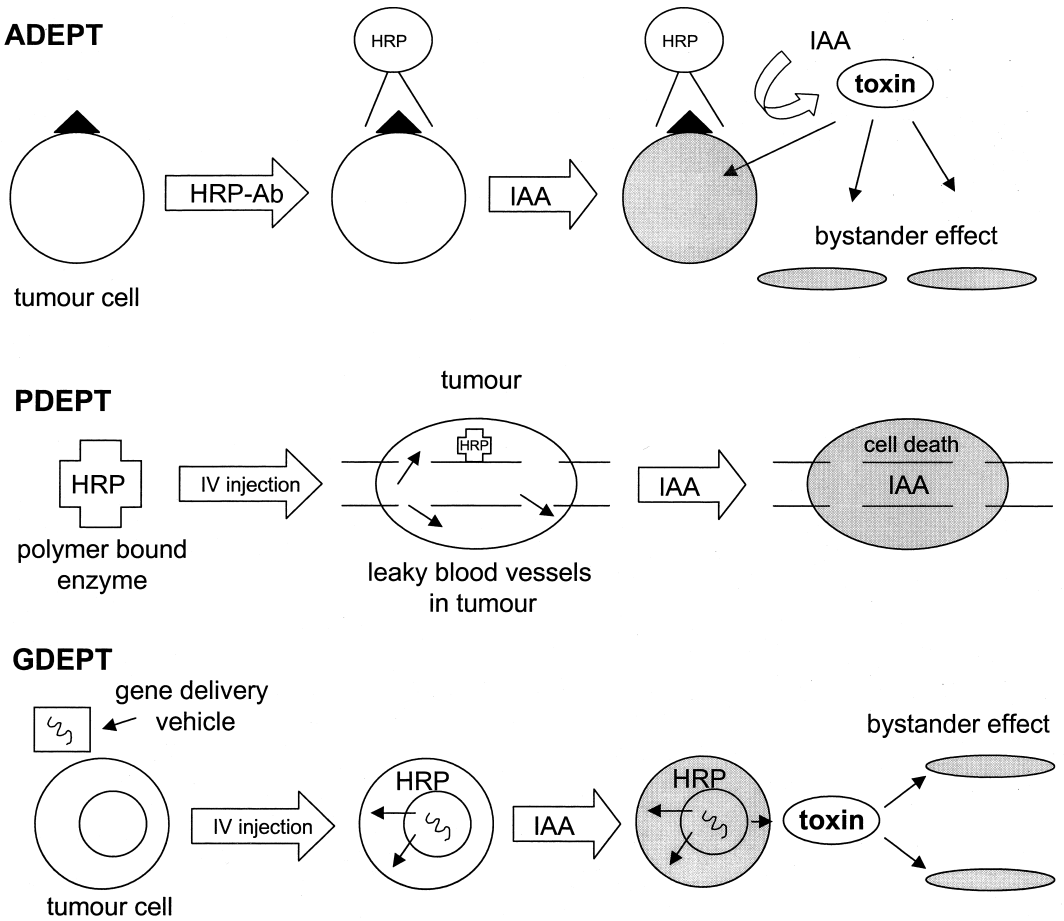

Fig. 4. Representation of main targeting strategies of potential value in cancer therapy.

bicin) to tumours [32] and has recently completed phase I trials [33]. Conjugation of drugs or proteins to polymers can increase their circulatory time and reduce their immunogenic effect. The polymer itself must be water soluble, biocompatible, non-immunogenic, and (in the case of protein carriers) not reduce their biological activity. In general, the larger the polymer (>40 kDa), the longer its circulatory time before clearance; however, long exposure times can have adverse effects. The concept of PDEPT relies upon the blood vessels in tumours being 'leaky', so that large, polymer-bound molecules enter the extracellular spaces in tumours but not very readily into normal tissues. This uptake requires the interstitial pressure of the tumour to be low to allow the net filtration from the blood into the tissue, which may be a potential problem. Clearance of the polymer from the tumour is slow because of the lack of lymphatic drainage. Once in the extracellular space, the polymer enters the cells by endocytosis, releasing the bound toxin by proteolytic action in the lysosomes, so causing cell death. The linker molecule between the polymer and prodrug can also be removed by the action of an enzyme bound to a different or the same polymer, so releasing the toxic drug. Physiological enzymes, e.g. esterases, can also be utilised to cleave the drug from its polymer.

We suggest that HRP, which has been successfully bound to various polymers [34], could be linked to a biologically compatible polymer and be allowed to accumulate in a tumour. Systemic administration of IAA would lead to the activation of the prodrug preferentially at the tumour, preventing normal tissue damage. Activation could occur in the extracellular space of a tumour or alternatively, after allowing greater time before administration of the prodrug, in the lysosomes. Lysosomes have a pH of around 5.5, close to the optimum pH of HRP, thereby increasing the turnover rate of IAA. Immunogenic effects are expected to be less severe or not to occur compared with ADEPT due to the masking of the foreign protein by the polymer. Repeat treatments might therefore be given, thus increasing the potential therapeutic benefit.

HRP has been successfully conjugated to PEG [34,35], increasing the molecular weight of the protein by approximately 25 kDa.[6] This conjugation retained 50% of the enzyme activity. Administration of HRP–PEG to tumour-bearing animals[7] indicates a problem of liver uptake,

[6] Folkes LK, unpublished results.
[7] Parkins CS and Folkes LK, unpublished results.

which can be reduced slightly by the pre-administration of mannan [36]. Modifications of HRP–PEG to try and reduce liver uptake are in progress, although targeting to liver cells may allow the treatment of primary liver cancer.

9. GDEPT

GDEPT [37] is also in clinical trials and is a two-step approach using the addition of a gene encoding an enzyme to a cell followed by the administration of a prodrug. The gene can be delivered to the target cell by viral or non-viral vehicles. Expression of the gene at the target allows production of an enzyme that is able to activate a prodrug to a toxic agent. Moreover, a bystander effect can take place, whereby not only the cells synthesising the enzyme are killed but also neighbouring ones. This gene therapy approach allows the enzyme to be expressed for longer times compared with ADEPT and in closer contact with intracellular targets, enabling a higher total metabolism of a prodrug in most cases.

One potential problem (or perhaps advantage) with the GDEPT IAA/HRP approach is that the enzyme is unlikely to be translocated across the cell membrane, unless DNA sequences have been added for specific cellular targeting; therefore, oxidation of the prodrug will only occur intracellularly. This is different from the ADEPT approach and using free HRP, where activation of the prodrug occurs extracellularly. If DNA damage is the reason for toxicity, one could assume that intracellular activation would increase the possibility of the active drug reaching the nucleus. However, if the key cellular target is the extracellular membrane, as in our original hypothesis, then intracellular activation would reduce the toxicity of the drug. Although HRP can be produced for longer periods by GDEPT compared with the ADEPT/PDEPT approach, steady-state enzyme levels may be too low to produce enough active drug to cause toxicity. Although HRP will not oxidise the majority of substrates without the presence of peroxide, steady-state peroxide levels in a cell may allow its reaction with some cellular components, e.g. tyrosine. Targeting of the gene to a tumour cell by selective gene delivery and expression would remove the possibility of normal tissue damage from over-expression of HRP.

The gene sequence of HRP has been elucidated and expressed in bacterial cells [38] and mammalian cells [39,40]. Transfection of human bladder carcinoma T24 cells with the HRP gene [40] has shown reduced colony formation in the presence of IAA compared with un-transfected cells, encouraging further development of the IAA/HRP GDEPT approach.

10. Conclusion

IAA is a potential prodrug with very low toxicity, transformed into a potent cytotoxin when activated by HRP. Endogenous mammalian peroxidases are very inefficient in achieving comparable oxidative activation, but non-enzymatic oxidations have proven useful mechanistic tools. The robust nature of the activating enzyme and the low toxicity of the prodrug together provide an attractive basis for the assessment of the combination in targeted tumour therapy. Although MOI may be responsible for some or all of the toxicity of IAA, studies with analogues where this product is unlikely to be formed suggest that other toxic pathways remain to be elucidated. Further structural modifications are being made to increase understanding of these mechanisms. The results from studies in mammalian and plant systems may be of mutual interest.

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References

[1] Folkes LK, Dennis MF, Stratford MRL, Candeias LP, Wardman P, Peroxidase-catalysed effects of indole-3-acetic acid and analogues on lipid membranes, DNA and mammalian cells *in vitro*. Biochem Pharmacol 1999;57:375–82.
[2] Mirsky A, Diengott D, Hypoglycemic action of indole-3-acetic acid by mouth in patients with diabetes mellitus. Proc Soc Exp Biol Med 1956;93:109–10.
[3] Engvild KC, The death hormone hypothesis. Physiol Plant 1989;77:282–5.
[4] Bandurski RS, Cohen JD, Slovin JP, Reinecke DM, Auxin biosynthesis and metabolism. In: Davies PJ, editor. Plant hormones—physiology, biochemistry and molecular biology. Dordrecht: Kluwer Academic Publishers, 1995. p. 39–65.
[5] Dunford HB, Heme peroxidases. New York: Wiley-VCH, 1999.
[6] Candeias LP, Folkes LK, Dennis MF, Patel KB, Everett SA, Stratford MRL, Wardman P, Free-radical intermediates and stable products in the oxidation of indole-3-acetic acid. J Phys Chem 1994;98:10131–7.
[7] Gazarian IG, Lagrimini M, Mellon FA, Naldrett MJ, Ashby GA, Thorneley RNF, Identification of skatolyl hydroperoxide and its role in the peroxidase-catalysed oxidation of indol-3-yl acetic acid. Biochem J 1998;333:223–32.
[8] Kanofsky JR, Singlet oxygen production from the peroxidase-catalysed oxidation of indole-3-acetic acid. J Biol Chem 1988;263:14171–5.
[9] Escobar JA, Vásquez-Vivar J, Cilento G, Free radicals and excited species in the metabolism of indole-3-acetic acid and its ethyl ester by horseradish peroxidase and by neutrophils. Photochem Photobiol 1992;55:895–902.
[10] Candeias LP, Folkes LK, Porssa M, Parrick J, Wardman P, Rates of reaction of indoleacetic acids with horseradish peroxidase compound I and their dependence on the redox potentials. Biochemistry 1996;35:102–8.

[11] Candeias LP, Folkes LK, Wardman P, Amplification of oxidative stress by decarboxylation: a new strategy in anti-tumour drug synthesis. Biochem Soc Trans 1995;23:262S.
[12] Brightwell J, Tseng MT, Peroxidase content in cell subpopulations of 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors in rats. Cancer Res 1982;42:4562–6.
[13] Candeias LP, Folkes LK, Porssa M, Parrick J, Wardman P, Enhancement of lipid peroxidation by indole-3-acetic acid and derivatives: substituent effects. Free Radic Res 1995;23:403–18.
[14] Candeias LP, Folkes LK, Wardman P, Enhancement of peroxidase-induced lipid peroxidation by indole-3-acetic acid: effect of antioxidants. Redox Rep 1996;2:141–7.
[15] Krylov SN, Dunford HB, Reaction of horseradish peroxidase with indole-3-acetic acid. In: Obinger C, Burner U, Erberman R, Penel C, Greppin H, editors. Plant peroxidases: biochemistry and physiology. Geneva: University of Geneva, 1996. p. 59–69.
[16] Folkes LK, Candeias LP, Wardman P, Toward targeted "oxidation therapy" of cancer: peroxidase-catalysed cytotoxicity of indole-3-acetic acids. Int J Radiat Oncol Biol Phys 1998;42:917–20.
[17] Pires de Melo M, Curi TCP, Curi R, Di Mascio P, Cilento G, Peroxidase activity may play a role in the cytotoxic effect of indole acetic acid. Photochem Photobiol 1997;65:338–41.
[18] Pires de Melo M, Curi TCP, Miyasaka CK, Palanch AC, Curi R, Effect of indole acetic acid on oxygen metabolism in cultured rat neutrophil. Gen Pharmacol 1998;31:573–8.
[19] Hinman RL, Bauman C, Lang J, The conversion of indole-3-acetic acid to 3-methyleneoxindole in the presence of peroxidase. Biochem Biophys Res Commun 1961;5:250–4.
[20] Fukuyama TT, Moyed HS, Inhibition of cell growth by photooxidation products of indole-3-acetic acid. J Biol Chem 1964;239:2392–7.
[21] Still CC, Fukuyama TT, Moyed HS, Inhibitory oxidation products of indole-3-acetic acid. J Biol Chem 1965;240:2612–8.
[22] Harman LS, Carver DK, Schreiber J, Mason RP, One- and two-electron oxidation of reduced glutathione by peroxidases. J Biol Chem 1986;261:1642–8.
[23] Demorest DM, Stahman MA, The binding of the peroxidase oxidation products of indole-3-acetic acid to histone. Biochem Biophys Res Commun 1972;47:227–33.
[24] Bednar TW, Linsmaier-Bednar EM, King CM, Peroxidase-H_2O_2 catalysed incorporation of auxin derivatives into sRNA. Biochem Biophys Res Commun 1976;72:761–7.
[25] Sabater F, Sánchez-Bravo J, Acosta M, Effects of enzyme/substrate ratio and of cofactors on the oxidation products of indole-3-acetic acid catalysed by peroxidase. Rev Esp Fisiol 1983;39:169–74.
[26] Griffiths JR, Are cancer cells acidic? Br J Cancer 1991;64:425–7.
[27] Kobayashi S, Sugioka K, Nakano M, Takyu C, Yamagishi A, Inaba H, Excitation of indole acetate in myeloperoxidase-H_2O_2 system: possible formation of indole acetate cation radical. Biochem Biophys Res Commun 1980;93:967–73.
[28] Bagshawe KD, Sharma SK, Springer CJ, Rogers GT, Antibody directed enzyme prodrug therapy. Ann Oncol 1994;5:879–91.
[29] Melton RG, Sherwood RF, Antibody-enzyme conjugates for cancer therapy. J Natl Cancer Inst 1996;88:153–65.
[30] Pedley RB, Boden JA, Begent RHJ, Turner A, Haines AMR, King DJ, The potential for enhanced tumour localisation by poly(ethylene glycol) modification of anti-CEA antibody. Br J Cancer 1994;70:1126–30.
[31] Duncan R, Dimitrijevic S, Evagorou EG, The role of polymer conjugates in the diagnosis and treatment of cancer. STP Pharma Sci 1996;6:237–63.
[32] Connors TA, Duncan R, Knox RJ, The chemotherapy of colon cancer. Eur J Cancer 1995;31A:1373–8.
[33] Vasey PA, Kaye SB, Morrison R, Twelves C, Wilson P, Duncan R, Thomson AH, Murray LS, Hilditch TE, Murray T, Burtles S, Fraier D, Frigerio E, Cassidy J on behalf of the Cancer Research Campaign Phase I/II Committee. Phase I clinical and pharmacokinetic study of PK1 [*N*-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents—drug-polymer conjugates. Clin Cancer Res 1999;5:83–94.
[34] Fortier G, Laliberté M, Surface modification of horseradish peroxidase with poly(ethylene glycol)s of various molecular masses. Biotechnol Appl Biochem 1993;17:115–30.
[35] Laliberté M, Gayet JC, Fortier G, Surface modification of horseradish peroxidase with poly(ethylene glycol)s of various relative molecular masses (M_r): relationship between the M_r of poly(ethylene glycol) and the stability of horseradish peroxidase-poly(ethylene glycol) adducts under various denaturing conditions. Biotechnol Appl Biochem 1994;20:397–413.
[36] Mumtaz S, Bachhawat BK, Enhanced intracellular stability of dextran-horse radish peroxidase conjugate: an approach to enzyme replacement therapy. Biochim Biophys Acta 1992;1117:174–8.
[37] Connors TA, The choice of prodrugs for gene directed enzyme prodrug therapy. Gene Ther 1995;2:702–9.
[38] Smith AT, Santama N, Dacey S, Edwards M, Bray RC, Thorneley RNF, Burke JF, Expression of a synthetic gene for horseradish peroxidase C in *Escherichia coli* and folding and activation of the recombinant enzyme with Ca^{2+} and heme. J Biol Chem 1990;265:13335–43.
[39] Connolly CN, Futter CE, Gibson A, Hopkins CR, Cutler DF, Transport into and out of the Golgi complex studied by transfected cells with cDNAs encoding horseradish peroxidase. J Cell Biol 1994;127:641–52.
[40] Greco O, Dachs GU, Wardman P, Folkes LK, Chaplin DJ, Development of an enzyme/prodrug combination for gene therapy of cancer. Proc Am Assoc Cancer Res 1999;40:478.