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Procarbazine in haematology: an old drug with a new life?

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Abstract

Procarbazine (PCB) was developed in the 1960s and was rapidly recognised as an active agent in lymphoid malignancies. PCB was one of the four drugs combined in mechlorethamine, vincristine, PCB, prednisolone (MOPP), one of the first combination chemotherapy regimens to show that advanced-stage disease could be cured in humans. During the last few decades, comprehensive studies have clarified cellular pathways involved in the modes of action of PCB and its drug resistance mechanisms. However, late toxicities, especially secondary leukaemias and sterility, led to its withdrawal from combination regimens used to treat Hodgkin's lymphomas (HLs). PCB was recently reintroduced in dose-intensified regimens and yielded impressive results. These new regimens (bleomycin, etoposide, doxorubicin, vincristine, PCB, and prednisone (BEACOPP) or escalated BEACOPP) are now being investigated versus the classic ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or ABVD-like combination chemotherapy regimens in the treatment of HLs.

Keywords: Procarbazine; Lymphoid malignancies; Chemotherapy; Drug resistance

1. Introduction

Procarbazine (PCB) was first synthesised as a monoamine-oxidase inhibitor, but was rapidly developed as an anticancer agent [1]. PCB belongs to an unconventional alkylating agent family including dacarbazine (DTIC), hexamethylamine (HMM), pentamethylmelamine (PMM) and temozolomide (TMZ). All these anticancer drugs contain an *N*-methyl group that is essential for their activity, but do not contain a chloroethyl group which is present in nitrogen mustard-type alkylating drugs. This explains why these agents lack bifunctionality and have been grouped as ''non-classical alkylating agents''. PCB is essentially a prodrug and undergoes a complex metabolic transformation into active intermediates. Although these metabolites have been characterised, their precise cellular mechanism of action and the clinical pharmacology of PCB is not fully understood. During the last few decades, PCB has been tested in the treatment of many human cancers and has shown activity in brain tumours and lymphomas, and especially in Hodgkin's lymphomas (HLs), where it has recently been reintroduced in combination with other anticancer agents. Nevertheless, the long-term toxicity and efficacy of these new regimens need to be carefully compared with the conventional standard doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) combination chemotherapy.

2. Mechanism of action and cellular pharmacology

PCB is a prodrug and its hepatic metabolism plays a major role in the generation of active metabolite species [2]. Potential activation mechanisms include chemical decomposition and microsomal oxidation [3]. It is metabolised to azoprocarbazine by microsomal cytochrome P-450 oxidoreductase or by mithochondrial monoamine oxidase enzymatic conversion [4,5]. Azo-PCB may be metabolised subsequently via different pathways that are not fully understood. Studies using alkaline elution techniques have confirmed that PCB and its metabolites can produce chromatid and single-strand DNA breaks in murine tumour cells *in vitro* [6]. The magnitude of the DNA breaks depends on the dose

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and time elapsed after treatment, suggesting that the breaks occur during or soon after DNA synthesis [7].

In addition to these effects on nuclear DNA, PCB can inhibit DNA, RNA and protein synthesis [6]. Inhibition of protein synthesis is delayed and can only be a late effect due to the inhibition of nucleic acid synthesis. Recent data suggest that PCB cytotoxicity is mainly mediated by its methylating properties. During PCB treatment, the generation of large amounts of ^{6}O-methylguanine (also known as a mutagenic and carcinogenic agent) could contribute to PCB cytotoxicity [8–10]. Delays in the growth of tumours lacking AGT (the enzyme mediating ^{6}O-methylguanine repair) were observed following the administration of PCB to thymic nude mice bearing xenografts derived from human malignant gliomas and medulloblastomas, compared with mice with normal AGT activity, emphasising the importance of this methylating pathway in PCB anticancer activity [11].

3. Drug resistance and clinical pharmacology

Recently, mechanisms of resistance to PCB have also suggested that the methylating activity of PCB plays a key role in its cellular toxicity. Resistance to PCB can develop rapidly in tumour cells. The above-mentioned relationship between PCB and AGT activities suggests that resistance to this agent could be secondary to AGT-mediated repair of ^{6}O-methylguanine activity and this is similar to resistance to nitrosourea which is also mediated by this enzyme [12]. Other drug-resistance mechanisms, such as hMSH2 mismatch-repair deficiency, have recently been identified in PCB drug-resistance [13–15]. The recent identification of these drug-resistance mechanisms could be instrumental in the development of new therapeutic strategies with PCB. Modulation of AGT activity has already been investigated.

The pharmacokinetics and metabolism of PCB have been studied mostly in laboratory animals, and such information in humans is incomplete. The rapid and extensive enzymatic metabolism of PCB plays a role in the complexity of its pharmacokinetic and excretion characteristics. PCB hydrochloride is available in 50 mg capsules. In HLs, it is usually given at a daily dose of 100 mg/m^2 for 7–14 days [16]. After oral administration, the drug is rapidly and completely absorbed from the gastrointestinal tract. The biodistribution of PCB is not well known, but there is rapid equilibration of PCB between plasma and cerebrospinal fluid in humans. PCB treatment is able to alter its own metabolism: the total and relative plasma concentrations of PCB metabolites were changed after the administration of 14 daily oral doses. There is a significant increase in the azoPCB concentration over time, suggesting that prior exposure to PCB induces the production of this metabolite or delays its clearance. The major urinary metabolite is the biologically inactive *N*-isopropylterephtamilic acid (70% of the drug during the first 24 h). There is minimal faecal and respiratory excretion of the drug (from 4% to 12% and 20% to 30%, respectively).

4. Toxicity and drug interactions

Long-term PCB toxicities remained a major problem and were responsible for the withdrawal of PCB from recent combination chemotherapy regimens, for example, ABVD in HLs. However, given the high activity of PCB in this disease, it has recently been reintroduced in new dose-intensified regimens, but at lower doses than in the classic mechlorethamine, vincristine, PCB, prednisolone (MOPP) regimen. PCB has profound azoospermic, teratogenic, mutagenic and carcinogenic effects that can limit its use in humans [17–20].

Rare side-effects, usually observed when large cohorts of patients are treated with a drug, have also been reported in recent decades: haemolysis in individuals with glucose 6-phosphate dehydrogenase deficiency; hypersensitivity reactions, including maculo-papular skin rash, eosinophilia, pulmonary infiltrates, or, rarely, transient hepatic dysfunction. PCB pulmonary toxicity necessitates discontinuation of the drug [21–23].

Many drug–drug or drug–food interactions are observed with PCB because of its extensive hepatic microsomal metabolism and its capacity to inhibit monoamine oxidase. Patients taking barbiturates, phenothiazines, narcotics, and hypnotics or sedatives may experience potentiated effects of these drugs during PCB therapy. Other drugs such as cimetidine or phenobarbital (pretreatment with phenobarbital results in increased PCB clearance) can affect PCB hepatic metabolism.

The inhibition of monoamine oxidase also predisposes patients to acute hypertensive reactions after concomitant therapy with tricyclic antidepressants and sympathomimetic drugs, as well as after ingestion of tyramine-rich beverages or foods, such as red wine, bananas, ripe cheese and yogurt. After ingestion of alcohol, disulfiram-like reactions (sweating, facial flushing and headache) may occur.

5. PCB in Hodgkin's lymphoma therapy

The development of cytotoxic drugs in the early 1960s dramatically improved the survival of patients with advanced HL [24]. Nitrogen mustard, PCB, vincristine and prednisolone were shown to be active in HL as single agents (see Table 1), but few patients achieved a complete response [25]. These responses were not long-lived and cure was not achieved. At the National Cancer Institute, DeVita and co-workers [16] developed a 4-drug chemotherapy regimen MOPP which was the first to

Table 1
Efficacy of single agent chemotherapy in Hodgkin's lymphoma

Drug	Response rate (%)	Complete response (%)
Mechlorethamine	63	16
Vincristine	64	36
Procarbazine	69	38

demonstrate that HL could be cured with a combination of anticancer agents. The complete remission (CR) rate attained was 84%, with relapse-free and overall survival reaching 66% and 48%, respectively, after more than 20 years. PCB certainly played a major role by producing a synergistic effect with other drugs. The Stanford group found that the dose and dose rate of nitrogen mustard, vincristine and procarbazine were crucial factors in obtaining a complete remission [26]. MOPP rapidly became the ''gold standard'' therapy for patients with advanced HL. Associated acute toxicities were common and manageable, but infertility and secondary acute leukaemia (sAML) were serious long-term toxicities.

The risk of developing sAML was mainly due to the association of PCB with mechlorethamine and to the cumulative dose of these alkylating agents [27,28]. sAML began 2 years following therapy, and declined by 11 years, with a peak-incidence at 6 years. These sAML often presented with total or partial deletion of chromosomes 5 and 7 at cytogenetic analysis, were poorly chemosensitive and the prognosis was extremely dismal with a median survival of only 0.4 of a year [29,30]. Infertility arose in more than 90% of males after treatment with a PCB-based regimen like MOPP: azoospermia and oligospermia were irreversible and often occurred after treatment with more than 3 cycles of MOPP. The remaining 10% of male patients recovered spermatogenesis within 1–7 years following the end of chemotherapy. 80–100% of women over the age of 25 years also became infertile [25,28,31].

Many MOPP-like combination regimens were developed to improve overall survival and reduce long-term toxicities. The only irreplaceable drug was PCB. MOPP-like regimens reduced some of the acute toxicities associated with MOPP, but failed to improve overall or disease-free survival.

ABVD was introduced in the 1970s by Bonadonna as a non-cross-resistant salvage regimen for patients failing on MOPP [32]. The regimen was shown to yield good activity in patients with MOPP-resistant disease, causing less acute myelotoxicity, sterility and particularly, less sAML.

Alternating MOPP with ABVD was studied based on the hypothesis put forward by Goldie–Coldman. Better failure-free survival (FFS) was achieved, but the complete response rate was not significantly improved and overall survival did not improve, as confirmed after long-term follow-up.

In 1992, the pivotal Cancer and Leukemia Group B (CALG B) trial in advanced HL, which compared MOPP with ABVD and with MOPP/ABVD, showed equivalent therapeutic results for ABVD and MOPP/ABVD, (CR rates were 81% and 82%, respectively, and the FFS rate was 64% for both regimen's). Both regimens were superior to MOPP (69% CR and 48% FFS at 3 years).

In 2003, an Intergroup trial compared ABVD versus a MOPP/ABV hybrid in a randomised study. The CR rates (76% vs. 80%, $P = 0.16$), FFS at 5 years (63% vs. 66%, $P = 0.42$) and overall survival at 5 years (82% vs. 81%, $P = 0.82$) were similar for the ABVD and MOPP/ABV hybrid regimens, respectively. MOPP/ABV was, as expected, significantly associated with a higher incidence of secondary myelodysplasia and sAML, but also with a higher risk of azoospermia. The ABVD regimen therefore became the standard chemotherapy regimen for advanced HL [33,34].

Dose-intensified regimens have been developed in an attempt to improve survival. The 12-week Stanford V regimen (doxorubicin, vinblastine, mechlorethamine, vincristine, bleomycin, etoposide, prednisone) combined with radiation yielded a 5 year survival rate of 96% and a freedom-from-disease-progression rate of 89%, which compare favourably with the ABVD results. No sAML was observed which could be related to the decision not to include PCB in this new regimen [35]. The German Hodgkin's study Group recently developed the BEACOPP regimen where PCB is administered at a dose of 100 mg/m^2 over 7 days, which represents half the classic dose delivered over 14 days in the MOPP regimen.

In a randomised study, COPP/ABVD, and standard-dose BEACOPP were compared with an escalated-dose BEACOPP. Freedom-from-treatment failure (69% vs. 76% vs. 87% $P \leqslant 0.001$) and overall survival at 5 years (83% vs. 88% vs. 91%, $P = 0.002$) favoured the escalated BEACOPP combination compared with the other regimens tested. Once again myelodysplasia and sAML were observed with an expected actuarial rate at 5 years

Table 2
Comparison of COPP–ABVD, BEACOPP, increased-dose BEACOPP [36]

	FFTF at 5 years (%)	OS at 5 years (%)	Acturial rate of sAML at 5 years (%)
COPP–ABVD	69	83	0.4
BEACOPP	76	88	0.6
Increased-dose BEACOPP	87 ($P < 0.001$ comparison with COPP–ABVD)	91 ($P = 0.002$ comparison with COPP–ABVD)	2.5 ($P = 0.03$ comparison with COPP–ABVD)

of 0.4%, 0.6% and 2.5% ($P = 0.03$) for the COPP/ABVD, standard-dose BEACOPP and escalated-dose BEACOPP, respectively (see Table 2) [36].

These data suggest that PCB could indeed remain a major anticancer drug in HL and could possibly be a part of the new "gold standard" combination chemotherapy regimen. However, many questions remain unanswered. Is the new escalated BEACOPP regimen really superior to the ABVD combination? Did all HL need to be treated with aggressive chemotherapy such as the escalated BEACOPP regimen and what precisely is the long-term toxicity observed with BEACOPP and especially the incidence of sAML. Ongoing clinical trials in Europe should answer all these important questions in the near future.

References

1. Kenis Y, DeSmedt J, Tagnon HJ. Action du natulan dans 94 cas de tumeurs solides. *Eur J Cancer* 1966, **2**, 51–57.
2. Alley MC, Powis G, Appel PL, *et al*. Activation and inactivation of cancer chemotherapeutic agents by rat hepatocytes co-cultured with human tumor cell lines. *Cancer Res* 1984, **44**, 549–556.
3. Sinha BK. Metabolic activation of PCB: evidence for carbon-centered free radical intermediates. *Biochem Pharmacol* 1984, **33**, 2777–2781.
4. Moloney SJ, Wiebkin P, Cummings SW, Prough RA. Metabolic activation of the terminal *N*-methyl group of *N*-isopropyl-alpha-(2 methylhydrazino)-*p*-toluamide hydrochloride (procarbbazine). *Carcinogenesis* 1985, **6**, 397–401.
5. Prough RA, Tweedie DJ. Procarcbazine. In Powis G, Prough RA, eds. *Metabolism and action of anticancer drugs*. London, Taylor and Francis, 1987, pp 2–9.
6. Therman E. Chromosome breakage by 1-methyl-2-benzyl-hydrazine in mouse cancer cells. *Cancer Res* 1972, **32**, 1133–1136.
7. Blijleven WG, Vogel E. The mutational spectrum of PCB on drosophila melanogaster. *Mutat Res* 1977, **45**, 47–59.
8. Meer L, Schold SC, Kleihues P. Inhibition of the hepatic O6-alkylguanine-DNA alkyltransferase *in vivo* by pretreatment with antineoplastic agents. *Biochem Pharmacol* 1989, **38**, 929–934.
9. Rossi SC, Conrad M, Voigt JM, *et al*. Excision repair of O6-methylguanine synthesised at the rat H-ras*N*-methyl-*N*-nitrosourea activation site and introduced in escherichia coli. *Carcinogenesis (London)* 1989, **10**, 373–377.
10. Hall J, Kataoka H, Sephenson C, *et al*. The contribution of O6-methylguanine and methylphosphotriesters to the cytotoxicity of alkylating agents in mammalian cells. *Carcinogenesis (London)* 1988, **9**, 1587–1593.
11. Schold Jr SC, Brent TP, von Holfe E, *et al*. O6-alkylguanine-DNA alkyltransferase and sensitivity to PCB in human brain tumor xenografts. *J Neurosurg* 1989, **70**, 573–577.
12. Pegg AE. Mammalian O6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* 1990, **50**, 6119–6129.
13. Friedman HS, Jonhson SP, Dong Q, *et al*. Methylator resistance mediated by mismatch repair deficiency in a glioblastoma multiforme xenograft. *Cancer Res* 1997, **57**, 2933–2936.
14. Kat A, Thilly WG, Fang WH, *et al*. An alkylation-tolerant mutator human cell line is deficient in strand specific mismatch repair. *Proc Natl Acad Sci USA* 1993, **90**, 6424–6428.
15. Koi M, Umar A, Chauhan DP, *et al*. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Cancer Res* 1994, **54**, 4308–4312.
16. DeVita VT, Serpick AA, Carbonne PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Intern Med* 1970, **73**, 881–895.
17. Schilsky RL, Sherins RJ, Hubbard SM, *et al*. Long-term follow up of ovarian function in women treated with MOPP chemotherapy for Hodgkin's disease. *Am J Med* 1981, **71**, 552–556.
18. Horning SJ, Hoppe RT, Kaplan HS, *et al*. Female reproduction potential after treatment for Hodgkin's disease. *N Engl J Med* 1981, **304**, 1377–1382.
19. Grunwald HW, Rosner F. Acute myeloid leukemia following treatment for Hodgkin's disease. *Cancer* 1982, **50**, 676–686.
20. Tucker MA, Coleman CN, Cox RS, *et al*. Risk of second cancers after treatment for Hodgkin's disease. *N Engl J Med* 1988, **318**, 76–81.
21. Sponzo RW, Arseneau J, Canellos GP. Procarbazine induced oxidative haemolysis: relationship to *in vivo* red cell survival. *Br J Haematol* 1974, **27**, 587–595.
22. Dunagin WG. Clinical toxicity of chemotherapeutic agents: dermatologic toxicity. *Semin Oncol* 1982, **9**, 14–22.
23. Garbes ID, Henderson ES, Gomez GA, *et al*. PCB-induced interstitial pneumonitis with a normal chest X-ray: a case report. *Med Pediatr Oncol* 1986, **14**, 238–241.
24. Mauch PM, Armitage JO, Diehl V, Hoppe RT, Weiss LM. *Hodgkin's Disease*. philadephia, Lippincott, Williams & Wilkins, 1999.
25. Coltman CA. Chemotherapy of advanced Hodgkin's disease. *Semin Oncol* 1980, **7**, 155–173.
26. Carde P, Mackintosh R, Rosenberg SA. A dose and time response analysis of the treatment of Hodgkin's disease with MOPP therapy. *J Clin Oncol* 1983, **1**, 146–153.
27. Travis LB, Curtis RE, Stovall M, *et al*. Risk of leukemia following treatment of non-Hodgkin's lymphoma. *J Nat Cancer Inst* 1994, **86**, 1450–1457.
28. Yung L, Linch D. Hodgkin's lymphoma. *Lancet* 2003, **361**(9361), 943–951.
29. Blayney DW, Longo DL, Young RC, *et al*. Decreasing risk of leukemia with prolonged follow-up after chemotherapy and radiotherapy for Hodgkin's disease. *N Engl J Med* 1987, **316**, 710–714.
30. Andrea KNg, Patricia Bernardo MV, Weller E, *et al*. second malignancy after Hodgkin disease treated with radiation therapy with or without chemotherapy: long-term risks and risk factors. *Blood* 2002, **100**(6), 1989–1996.
31. Pazdur R, Coia LR, Hoskins WJ, Wagman LD. *Cancer management: a multidisciplinary approach*. New York, The Oncology Group, 2003.
32. Santoro A, Bonfante V, Bonadonna G. Salvage chemotherapy with ABVD in MOPP-resistant Hodgkin's disease. *Ann Intern Med* 1982, **96**, 139–143.
33. Canellos GP, Anderson JR, Propert KJ, *et al*. Chemotherapy of advanced Hodgkin's disease with MOPP,ABVD, MOPP alternating with ABVD. *N Engl J Med* 1992, **327**, 1478–1484.
34. Duggan DB, Petroni GR, Jojnson JL, *et al*. Randomized comparison of ABVD and MOPP/ABV hybrid for the treatment of advanced Hodgkin's disease: report of an Intergroup trial. *J Clin Oncol* 2003, **21**, 607–614.
35. Stanford V and radiotherapy for locally extensive and advanced Hodgkin's disease: mature results of a prospective clinical trial. *J. Clin. Oncol* 2002, **20**, 630–37.
36. Diehl V, Franklin J, Pfreundschuh M, *et al*. Standard and increased-dose BEACOPP chemotherapy compared with COPP-ABVD for advanced Hodgkin's disease. *N Engl J Med* 2003, **348**, 2386–2395.