Plasma kinetics of procarbazine and azo-procarbazine in humans

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The plasma kinetics of procarbazine (PCB) and its major metabolite azo-procarbazine (azo-PCB) were systematically investigated in humans for the first time. Eight therapy-refractory tumor patients with normal liver and renal function were given a single oral dose of 300 mg PCB hydrochloride as a drinking solution under fasting conditions. With the exception of the single i.v. administration of 10 mg ondansetron hydrochloride immediately before the administration of PCB, the patients were free of any co-medication 4 weeks before and during the study. PCB and azo-PCB were determined by a specially developed HPLC-UV method. PCB was absorbed very rapidly. Mean maximum plasma concentration was 12.5 min. A high elimination rate of PCB from plasma was found. The mean apparent oral systemic clearance and the plasma elimination half-life were estimated at 35.8 l/min and 9.2 min, respectively. Considerable amounts of azo-PCB are found in the plasma of the eight tumor patients. The mean C_{max} and AUC ratios of azo-PCB/PCB were estimated at 5.5 and 45.2. Azo-PCB is formed very rapidly from PCB, but eliminated much more slowly from plasma than PCB. Considerable interindividual differences in the conversion rate of azo-PCB to its further metabolites were observed which should have consequences for the individual tumor therapeutic efficiency of PCB. No toxic side-effects or symptoms such as nausea or vomiting were observed during the entire study. *Anti-Cancer Drugs* 17:75–80 © 2006 Lippincott Williams & Wilkins.

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Introduction

Procarbazine [PCB; *N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide hydrochloride], first synthesized as a monoamine oxidase inhibitor, has been used for many years in the treatment of Hodgkin's disease and brain tumors [1]. Reintroduced in the dose-intensified BEACOPP (bleomycin, etoposide, doxorubicin, vincristine, PCB and prednisone) regimen, PCB yielded impressive therapeutic results in the treatment of advanced Hodgkin's disease [2,3].

Like dacarbazine and temozolomide, PCB must be assigned to a distinct class of *N*-methyl anti-tumor alkylating agents which are inactive prodrugs, require conversion to tumorstatic active metabolites, and develop their tumorstatic activity probably by forming the highly reactive, electrophilic methyldiazonium ion that alkylates DNA and proteins [4–10].

Extensive work has been done on the metabolic activation process of PCB; however, our knowledge of PCB metabolism is far from complete. A proposed metabolic pathway for PCB based on chemical decomposition and experiments on rat liver preparations and rat hepatocytes is given in Fig. 1.

PCB is oxidized by both mitochondrial monoamine oxidase and hepatic microsomal cytochrome P450 isoenzymes to azo-PCB, which tautomerizes to the hydrazone derivative that further undergoes hydrolysis to the aldehyde derivative and methyl hydrazine [11–14].

Azo-PCB is microsomally oxidized to azoxyderivatives [15,16], which are finally converted in a mixed reaction of chemical decomposition, microsomal oxidation and enzymatic cytosolic conversion (aldehyde dehydrogenase and xanthine dehydrogenase) to *N*-isopropyl-terephthalamic acid – the major urinary metabolite [4,12,17–21].

Methylazoxy-PCB seems to be the major cytotoxic intermediate involved in the mechanism of anti-cancer action of PCB. In murine and human L1210 leukemia tumor cells methylazoxy-PCB caused a several fold higher cytotoxicity than benzylazoxy-PCB, azo-PCB and PCB [4,20,22].

Our knowledge of metabolism and kinetics of PCB in humans is very incomplete. There are no data on metabolism of PCB and its further metabolic species in human liver preparations and human hepatocytes. The kinetics of PCB and its metabolites in human plasma have not yet been systematically investigated. He *et al.* [23] were the first to describe the plasma kinetics of PCB using a reliable detection method (HPLC-MS). Fast absorption (t_{max} = 34–37 min) and elimination of PCB

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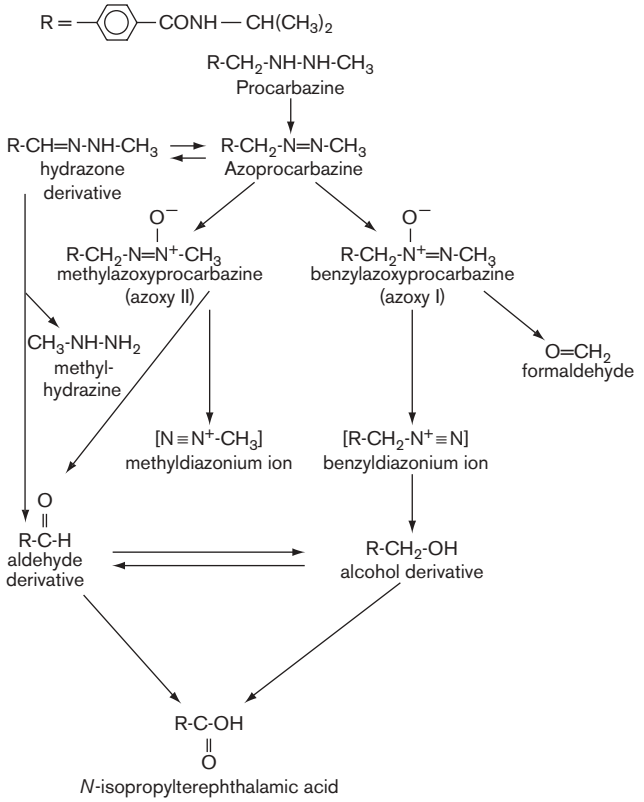
Fig. 1



Proposed pathway for PCB (based on [7,14]). Products in brackets have not been identified.

was observed after its oral application in two tumor patients. PCB metabolites were not investigated. In earlier investigations PCB showed an elimination half-life of 7 min after i.v. application in five tumor patients [24]. The 24-h urinary excretion rates were 67–70% of the administered PCB dose measured as radiolabeled fraction in three patients and 15% measured as a mixed metabolite fraction in two patients [18,25].

The aim of the present study was to investigate the plasma kinetics of PCB systematically in humans for the first time. The plasma levels of PCB and its first oxidative species azo-PCB were measured after a single oral dose of PCB in eight tumor patients.

Patients and methods

Patients

Eight therapy-refractory tumor patients (three females and five males, aged 60.9 ± 9.7 years, range 44–72 years) were included into the study after giving their written informed consent. The study was approved by the local Ethics Committee. All patients had a normal liver and renal function before the administration of PCB. The plasma levels of bilirubin and creatinine and the thrombocyte and leukocyte counts were within the normal range at the start of PCB administration. The Karnofsky index in all patients was above 50%.

None of the patients had received any chemotherapy, hormone therapy or radiotherapy within the 4 weeks prior to inclusion into the study. With the exception of the i.v. bolus injection of 10 mg ondansetron hydrochloride (Zofran; 8 mg) as an anti-emetic therapy immediately before the administration of PCB, no further drugs were given within 7 days before and after the single-dose administration of PCB.

Study design

A single dose of 300 mg crystalline PCB hydrochloride supplied by Hoffmann-La Roche (Basel, Switzerland) was dissolved in 100 ml water at room temperature. The solution of PCB was freshly prepared immediately before the administration in each case. The time between addition of PCB to water and oral administration was standardized on 2 min. The 100-ml PCB solution was administered between 8 and 9 a.m. Immediately after administration of the PCB solution the subjects were given 100 ml water from the same drinking glass which was used for preparation of the PCB solution. The subjects fasted for 12 h prior to dosing and until 4 h after dosing.

Drinking was not allowed for 1 h prior to dosing and until 1 h after dosing except for the two 100-ml water fractions administered with the study medication. Alcoholic and caffeinated drinks were not allowed.

Blood samples for the determination of PCB and azo-PCB were collected in water ice-cooled heparinized containers (9 ml NH_4-Monovette; Sarstedt, Nümbrecht, Germany) prior to dosing, and at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140 and 160 min, and 3, 3.5, 4, 5, 7, 9 and 12 h post-dose. Immediately after blood withdrawal, plasma was separated by centrifugation at 1900 *g* for 5 min at 4°C, transferred into water ice-cooled polypropylene tubes and immediately frozen at – 70°C until analysis.

Materials

Methanol (HPLC grade), acetonitrile (Ultra Gradient HPLC grade) and bidistilled water were obtained from Baker (Deventer, The Netherlands). Diethyl ether and 4-methylacetophenone were obtained from Merck (Darmstadt, Germany). PCB and azo-PC1B were kindly provided by Hoffmann-La Roche.

The analytic HPLC column (Nucleosil 100 C_{17} µm, 250 × 4.6 mm) was purchased from Knauer (Berlin, Germany).

Drug analysis

PCB

Plasma (1 ml) was extracted twice with 2 ml chloroform and the organic layer was evaporated by means of nitrogen. The residue was dissolved in 200 µl buffer (0.05 mmol/l KH_2PO_4, pH 2.3) and 20-µl aliquots of this solution were injected onto the HPLC column for analysis. The chromatographic system consisted of a Nucleosil C18 (250 × 4.6 mm, 7 µm) column, a ConstaMetric 4100 MS pump [Thermo Separation Products (TSP), Riviera Beach, California, USA] with a membrane degasser and a SpectroMonitor 3200 UV detector (TSP) operating at 254 nm. Chromatographic separation was achieved by an isocratic procedure. The mobile phase consisted of 7% acetonitrile and 93% 0.05 mmol/l KH_2PO_4 buffer (pH 2.3). A flow rate of 0.5 ml/min was used. The limit of quantification was 25 ng/ml (6 × noise). The within-run and between-run precision values for 752.5, 378.1 and 151.7 ng/ml PCB were 9.3, 8.7 and 10.3% as well as 12.9, 11.4 and 10.1%, respectively ($n = 6$). As within-run precisions were smaller than between-run precisions, a daily calibration curve was used for quantification. The recovery rate was 53% for 378.1 ng/ml. All samples were stored at −70°C. After 4 months of storage we determined the concentration to be about 90% (378.1 ng/ml). All samples were measured within this time. No interferences were observed in all blank values.

Azo-PCB

Plasma (1 ml) was extracted twice with 3 ml chilled diethyl ether and the organic layer was evaporated by means of nitrogen. The residue was dissolved in 200 µl methanol with 4-methylacetophenone (215 ng) as internal standard and 20-µl aliquots of this solution were injected onto the HPLC column for analysis. The chromatographic system consisted of a Nucleosil C18 (250 × 4.6 mm, 7 µm) column, a ConstaMetric 4100 MS pump (TSP) with membrane degasser and a SpectroMonitor 3200 UV detector (TSP) operating at 254 nm. The mobile phase consisted of 45% methanol (pH 7.0) and bidistilled water with a flow rate of 0.6 ml/min. The limit of quantification was 40 ng/ml (6 × noise). The within-run and between-run precision values for 8.762, 1.822 and 0.915 µg/ml were 7.3, 3.7 and 5.3% as well as 7.9, 6.7 and 5.7%, respectively ($n = 6$). The recovery rates for these concentrations were 90.4, 84.5 and 88.7%, respectively. The peak height ratio of drug to internal standard and a daily calibration curve were used for quantification (0.2–8.7 µg/ml). All samples were stored at −70°C. After 4 months of storage at −70°C we determined 89.6 and 95% for 8.762 and 1.822 µg/ml, respectively. All samples were measured within this time. No interferences were observed in all blank values.

Pharmacokinetic analysis

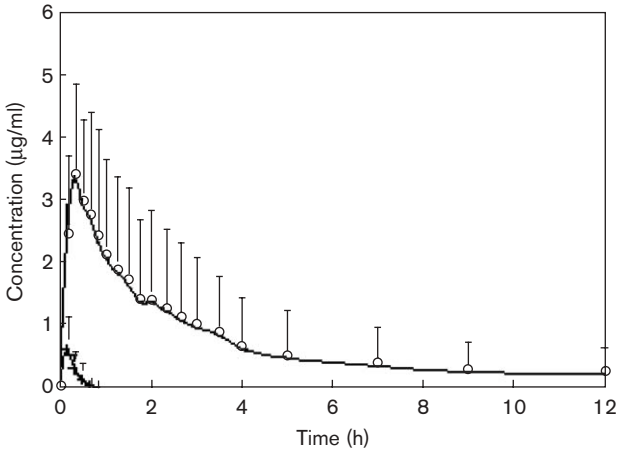
Non-compartmental pharmacokinetic evaluations were performed using the computer program Win Nonlin 4.0. Maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were taken directly from the plasma concentration–time profiles. Terminal elimination half-lives ($t_{1/2z}$) were obtained by linear regression analysis of data points during elimination phase after log-transformation of the data by means of Win Nonlin 4.0. Area under the plasma concentration time curve up to infinity ($AUC_{0-\infty}$) was calculated using trapezoidals from time 0 to the last quantifiable concentration (C_{last}) by adding C_{last}/λ_z. The apparent oral systemic clearance uncorrected for bioavailability (Cl/F) was calculated as oral dose/$AUC_{0-\infty}$. The apparent oral volume of distribution during the terminal phase (V_z/F) was calculated as Cl/F, divided by λ_z. Mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$, where AUMC represents the area under the first moment curve.

Results

No patient withdrew from the study and no serious adverse events were observed throughout the study. No patient showed symptoms of nausea or vomiting.

The mean plasma level versus time profiles of PCB and azo-PCB after oral administration of a single dose of 300 mg PCB hydrochloride in form of a 100-ml drinking solution are shown in Fig. 2. The corresponding individual and mean pharmacokinetic parameters of PCB and azo-PCB are presented in Tables 1 and 2. PCB was rapidly absorbed from the gastrointestinal tract, reaching mean maximum plasma concentrations at 0.208 ± 0.077 h (12.5 ± 4.6 min). Interindividual t_{max} values varied only 3-fold within 10–30 min post-dose. In contrast, the amount of PCB absorbed varied much more strongly.

Fig. 2



Mean plasma concentration of PCB (crosses) and azo-PCB (circles) after a single oral dose of 300 mg PCB hydrochloride in eight tumor patients.

Table 1 Pharmacokinetic parameters of PCB after a single oral dose of 300 mg PCB hydrochloride in eight tumor patients

Patient no.	t_{max} (h)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg·h/ml)	MRT (h)	Cl/F (l/min)	V_z/F (l)	$t_{1/2\ z}$ (h)
1	0.167	0.206	0.047	0.233	107.00	843	0.091
2	0.333	0.284	0.107	0.453	46.70	662	0.163
3	0.167	0.306	0.155	0.505	32.30	831	0.297
4	0.167	1.000	0.216	0.228	23.20	204	0.102
5	0.167	0.946	0.276	0.270	18.10	173	0.110
6	0.333	0.415	0.166	0.463	30.10	459	0.177
7	0.167	1.047	0.506	0.438	9.88	197	0.230
8	0.167	1.328	0.262	0.200	19.10	109	0.066
Mean	0.208	0.692	0.217	0.349	35.80	435	0.154
SD	0.077	0.434	0.140	0.127	30.84	307	0.079

Table 2 Pharmacokinetic parameters of azo-PCB after a single oral dose of 300 mg PCB hydrochloride in eight tumor patients

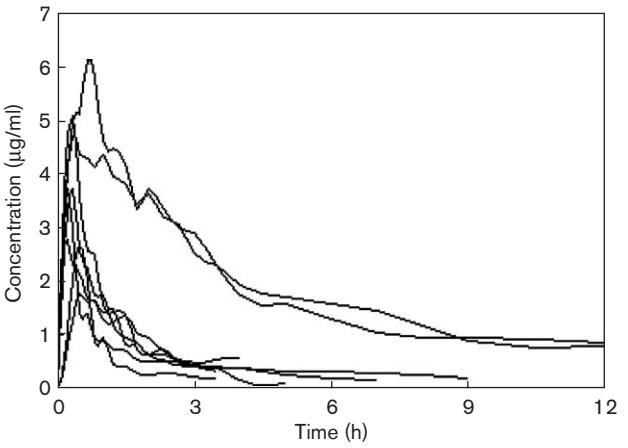
Patient no.	t_{max} (h)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg·h/ml)	$t_{1/2}$ (h)
1	0.333	3.71	4.28	0.91
2	0.500	1.76	4.54	2.17
3	0.333	4.71	26.68	4.19
4	0.167	2.66	4.14	1.09
5	0.333	5.08	5.22	0.85
6	0.500	2.66	2.01	0.50
7	0.667	6.12	26.52	4.00
8	0.167	3.52	5.00	2.59
Mean	0.375	3.78	9.80	2.04
SD	0.173	1.45	10.42	1.45

More than 6-fold differences in the C_{max} values of PCB were found in the eight tumor patients studied.

PCB also showed a very fast elimination from plasma which must be primarily interpreted as a high conversion rate to its metabolites in the first step to the azo-derivative. The mean apparent oral systemic clearance of PCB was estimated as 35.8 l/min, whereas a more than 10-fold interindividual variation was found in the eight tumor patients studied. A short plasma elimination half-life of the alkylating drug was found, corresponding to the high apparent oral clearance of PCB. Nearly 5-fold varying $t_{1/2z}$ values were estimated, with a mean value at 0.154 h (9.2 min). Consistent with the fast absorption and elimination of PCB, a short MRT was calculated in the eight tumor patients, with a mean of 0.349 h (20.9 min). The apparent oral volume of distribution during the terminal phase was calculated with a mean value of 435 l (109–843 l).

The plasma kinetics of azo-PCB, the first and fundamental metabolite of PCB, was quite different from that of PCB. In six of eight tumor patients higher t_{max} values for azo-PCB than for PCB were found (Tables 1 and 2). For azo-PCB, mean t_{max} values of 0.375 h (22.5 min) were obtained. Considerably higher plasma levels were found for azo-PCB than for PCB. On average, 5- or 50-fold higher values for C_{max} and AUC, respectively, were found for azo-PCB. Azo-PCB is eliminated much more slowly from plasma than PCB. In all eight studied tumor patients $t_{1/2z}$ was found to be larger for azo-PCB than for PCB, with a mean of 2.04 (122.4) versus 0.154 h (9.2 min). Two patients (nos 3 and 7) differed considerably in their azo-PCB plasma profile from that of the other six patients (Fig. 3). Whereas the azo-PCB C_{max} and t_{max} values in both patients did not differ clearly from those of the other six patients, the azo-PCB plasma elimination was strongly retarded. The corresponding $t_{1/2z}$ of azo-PCB was found to be larger in both patients than in the remainder.

Fig. 3



Plots of individual plasma concentration–time profiles of azo-PCB in eight tumor patients after a single oral dose of 300 mg PCB hydrochloride

Discussion

The results presented here systematically describe the plasma kinetics of PCB and its metabolite azo-PCB in humans for the first time.

Eight tumor patients with normal liver and renal function were included in the study. With the exception of the study medication and a single i.v. administration of 10 mg ondansetron hydrochloride (Zofran) immediately before PCB administration, all patients were without any co-medication during the entire study. PCB was given

as a freshly prepared drinking solution under fasting conditions. No patients showed symptoms of vomiting so that an undisturbed oral absorption of the study drug should be assumed.

PCB was given in a total dose of 300 mg. This is somewhat higher than the daily PCB dose usually administered. PCB is regularly given in a daily oral dose of 100 mg/m^2 in different combination regimes used to treat Hodgkin's lymphomas.

PCB undergoes oxidation in aqueous solution to give azo-PCB which tautomerizes to the hydrazone derivative that undergoes chemical hydrolysis to the aldehyde derivative and methyl hydrazine (see left part of Fig. 1) [14,25]. A bedside technique was used to eliminate possible influences due to a spontaneous degradation of PCB and azo-PCB. All sample collection steps were performed under ice-cooled conditions and sample preparation was carried out under acidified conditions. In contrast to aqueous solution, PCB is stable in plasma. In human plasma the drug degraded with a mean half-life of 47.6 h when incubated at 37.5°C [23].

We found a very fast oral absorption and plasma elimination of PCB in the eight tumor patients studied. The mean t_{max} and $t_{1/2}$ values were estimated at 12.5 and 9.2 min. These results are in a good agreement with the sparse human kinetic data described in the literature so far. After an oral administration of PCB in two tumor patients, He *et al.* [23] found t_{max} values of 34 and 37 min. The galenic form of the oral PCB medication used in this study was not described; however, the drug was probably administered in a tablet form because both malignant glioma patients received PCB orally once a day for 5 days 4-weekly during a phase I clinical trial. The different galenics of the oral PCB applications used in the study of He *et al.* [23] and the presented study may be responsible for the small difference in the estimated t_{max} values between both studies. He *et al.* [23] found C_{max} values of 546 and 669 ng/ml in both patients after an orally administered PCB dose of 294 mg/m^2. In our eight tumor patients, we found mean C_{max} values of PCB of 692 ng/ml after a total oral dose of 300 mg PCB hydrochloride (Table 1). The total orally administered PCB dose in the study of He *et al.* [23] was approximately 1.5- to 2.0-fold higher than in the present study, but the different oral PCB forms used (tablet versus drinking solution) should have contributed to the comparable C_{max} values in both studies.

We found a very high plasma clearance of PCB. In the eight patients studied, a mean apparent systemic clearance (Cl/*F*) of 35.8 l/min and a mean terminal elimination half-life ($t_{1/2z}$) of 0.15 h (9.2 min) were estimated (Table 1). In accordance with this, a mean plasma elimination half-life of 7 min was found in five tumor patients after i.v. administration of 250 mg PCB [24].

The high oxidation rate of PCB to its azo-derivative should be responsible for the fast disappearance of PCB from plasma.

There has been no reliable data on the pharmacokinetics of azo-PCB in humans in the literature up to now. The plasma kinetics of azo-PCB was only described in one tumor patient [26]; however, the orally administered PCB dose was very high (250 mg/kg), and the plasma levels of PCB and azo-PCB were very low (in each case below 1 ng/ml). In our study of eight patients with tumors, we found considerably higher plasma levels for azo-PCB than PCB (Fig. 2). The mean values for C_{max} were 5.5-fold and those for $AUC_{0-\infty}$ were 45-fold higher for azo-PCB than for PCB (Tables 1 and 2).

Formation of azo-PCB from PCB is rapid. Mean t_{max} was estimated at 0.375 h (22.5 min) for azo-PCB and 0.208 (12.5 min) for PCB. It can be assumed that the main part of azo-PCB detected in plasma is formed by *in vivo* oxidation of PCB. However, it cannot be excluded that azo-PCB is also absorbed in the upper intestinal (duodenal, jejunal) tract as an intact compound after its chemical formation from PCB in this intestinal compartment. A rapid degradation of PCB to azo-PCB in a weakly alkaline medium has been reported by Gorsen *et al.* [27]. The oral administration of PCB as a drinking solution carried out in the present investigation enables this process to be performed kinetically. However, an oral administration of azo-PCB can be excluded. No azo-PCB could be detected in the drinking solution.

We have further found that azo-PCB disappears from plasma much more slowly than PCB. Mean $t_{1/2z}$ was estimated at 122.4 and 9.2 min for azo-PCB and PCB, respectively. In two tumor patients (nos 3 and 7), the elimination of azo-PCB from plasma was exceedingly slow (Fig. 3). In both patients the plasma elimination half-life was estimated at 251 and 240 min, respectively, whereas in the other six patients the mean $t_{1/2z}$ of azo-PBC was estimated at 81 min.

The reason for this divergent azo-PCB plasma elimination profile in two patients is unclear. In rat experiments it was shown that the metabolism of azo-PCB to its two azoxy derivatives is mediated by the CYP isoenzymes 1A1, 2C6 and one or more members of the gene subfamily 3A [15,16]. Genetic deficiencies of human CYP 1A2 and 2C9 enzyme activities in the Caucasian population are known, but their frequencies are very low. Therefore, it seems unlikely that the delayed azo-PCB elimination found in the two patients is caused by genetic

deficiencies. With the exception of a single administration of ondansetron, all eight tumor patients investigated were free of co-medication, so that drug-induced inhibition of CYP isoenzymes that would have mediated azo-PCB metabolism can be excluded. A reduced liver function should be most likely discussed as the cause for the delayed azo-PCB elimination in the two tumor patients. All eight patients had normal serum bilirubin levels (below 1.1 mg/dl) during the entire study; however, the serum bilirubin level must be considered as a raw parameter in the prediction of hepatic drug-metabolizing capacity. CYP isoenzymes marker substrates were not investigated to check the microsomal oxidase activities in the present study.

A reduced conversion of azo-PCB to its further metabolites should be accompanied by a reduction in tumorstatic activity of PCB. Further studies are required to clarify the relation between a restriction of liver function and metabolic activation as well as tumorstatic activity of PCB.

In summary, the present study systematically describes the plasma kinetics of PCB and its first metabolite azo-PCB in humans for the first time. After an oral administration PCB is very rapidly absorbed, and subsequently transformed to its first and fundamental metabolite azo-PCB. The area under the plasma concentration time curve is more than 40-fold higher for azo-PCB than for PCB. Considerable interindividual differences in the conversion of azo-PCB to its further metabolites were also observed in tumor patients with normal renal function and inconspicuous liver function, which should have consequences in the individual tumor therapeutic efficiency of PCB.

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