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Mechanisms of the Inhibition of Human Erythrocyte Pyridoxal Kinase by Drugs

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ABSTRACT. The aim of this study was to investigate the interaction between drugs chosen for their clinical neurotoxicity or chemical structure and vitamin B_6 metabolism. After a preliminary screening of drugs to determine their potential inhibitory effect on erythrocyte nonpurified pyridoxal kinase (PLK) (EC 2.7.1.35), additional investigations, including kinetic studies and detection of chemical reactivity between the inhibiting drugs and pyridoxal (PL) or pyridoxal-5′-phosphate (PLP), using UV-visible spectrophotometry and mass analysis, were carried out to specify the mechanism of PLK inhibition. Depending on the results, the inhibiting drugs were divided into three groups. The first group included theophylline and progabide and inhibited PLK using either PL or pyridoxamine (PM) as substrate and thereby were true inhibitors. Moreover, they did not form covalent complexes with PL or PLP. The second group, which included cycloserine, dopamine, isoniazid, and thiamphenicol glycinate, inhibited PLK using PL, but not PM, as substrate. They were able to react with PL or PLP to form covalent complexes, and kinetic studies suggested that the observed PLK inhibition was due to these formed complexes. A third group, which consisted of levodopa, D-penicillamine, and muzolimine, inhibited PLK using PL, but not PM, as substrate. They formed, with PL or PLP, chemical derivatives that probably had no inhibitory effect on PLK. These results and the clinical consequences of such interactions are discussed and compared with results of previous studies. BIOCHEM PHARMACOL **54**;8:863–870, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. pyridoxal kinase; enzymatic inhibition; pyridoxal; pyridoxal-5′-phosphate; drug interactions; vitamin B_6

Many interactions between drugs and vitamins have been described. They explain certain adverse effects of drugs which can be prevented by the administration of the appropriate vitamin. Among these interactions, we particularly focused our attention on the effects of drugs on vitamin B_6 status and function in order to explain their neurological side-effects. Such interactions have already been described in vitamin B_6 status and metabolism studies in patients [1–5] or in various animal species [6–13] treated with the interacting drugs administered at therapeutic or toxic levels. Other studies have shown the partial or total protective effect of vitamin B_6 against drug toxicity [14–17]. Two main mechanisms account for the functional hypovitaminosis B_6 induced by drugs. The first is the ability of some drugs containing an amine or hydrazine function, such as penicillamine, cycloserine, dopamine, levodopa, and gentamicin, to bind to pyridoxal (PL)† and/or to pyridoxal 5′-phosphate (PLP) via a Schiff's base formation [11, 16, 18, 19]; the second is an inhibition of pyridoxal kinase (PLK, EC 2.7.1.35) by methylxanthines [3, 20]. PLK is a cytoplasmic enzyme widely present in the organism that catalyzes phosphorylation of pyridoxine, pyridoxamine (PM), and PL with ATP as phosphate donor. To become active, the enzyme requires the presence of K^+ [21–23] and bivalent cations such as Mg^{2+}, Zn^{2+}, and Co^{2+}, which form a complex with ATP [24].

To investigate new drug-B_6 interactions and to determine the mechanisms of well recognized interactions that have not been fully elucidated, we undertook an *in vitro* preliminary screening of drugs to determine their potential inhibitory effect on human erythrocyte PLK. The tested drugs were chosen on the basis of three criteria: their well established interaction with B_6 as reference drugs; their neurological adverse effects such as peripheral neuropathy or convulsions, which are frequently associated with vitamin B_6 deficiency [25]; and a chemical structure suggesting that they could chemically react with PLP and/or PL. Additional investigations, including kinetic studies of inhibition types and detection of chemical reactivity between drugs and aldehyde derivatives of vitamin B_6, were carried out to determine the mechanism of the inhibition.

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† *Abbreviations:* PL, pyridoxal; PLK, pyridoxal kinase; PLP, pyridoxal-5′-phosphate; PM, pyridoxamine; PLSC, pyridoxal semicarbazone; PLPSC, pyridoxal-5′-phosphate semicarbazone; PMP, pyridoxamine-5′-phosphate.

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MATERIALS AND METHODS

Enzymatic Investigations

REAGENTS. PLP, PL, PM, pyridoxamine-5′-phosphate (PMP), trichloroacetic acid, semicarbazide chloride, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, triethanolamine, and magnesium chloride were purchased from Merck (Darmstadt, Germany); Triton X-100, mercaptoethanol, and ATP magnesium salt (ATP-Mg) were from Sigma (St Louis, MO, USA); HPLC grade acetonitrile was from Carlo Erba (Milan, Italy); PIC reagent A was from Waters-Millipore Corp. (Milford, MA, USA); and the other reagents were from Prolabo (Paris, France). The tested drugs were obtained from their manufacturer.

APPARATUS. The apparatus consisted of a pump (307 Gilson model, Villiers le bel, France), an integrator (HP 3395 Hewlett Packard, Les Ulis, France), an autosampler (Wisp 710 B model, Waters, Marlborough, MA, USA), a fluorimetric detector (SFM 25 model, Kontron Analytical, Zürich, Switzerland), an S5 ODS 1 15-cm × 4.6-mm ID spherisorb column (Phase Sep, Saint Quentin en Yvelines, France), and a pump (constametric I model, LDC Milton-Roy, Riviera Beach, FL, USA) for the postcolumn derivatization.

PREPARATION OF ERYTHROCYTE EXTRACTS. Hemolysate was prepared from one O Rh+ donor with no drug therapy, according to the method proposed by Ubbink and Schnell [26]. It was then dialyzed for 24 hr at 4° in a dark room against 5 mM tris-buffer containing 50 mM mercaptoethanol, pH adjusted to 7.4 with orthophosphoric acid (approximately 4 × 1 litre of dialysis for 40 mL of hemolysate). Immediately after dialysis, the hemoglobin content was measured by a spectrophotometric method (Reagent JT Baker, Deventer, the Netherlands), and the hemolysate was stored at −80° in 500-μL aliquots.

DETERMINATION OF PLK ACTIVITY. PLK activity, using PL and ATP-Mg as substrates, was measured by the method of Ubbink *et al.* [3, 26], which is based on HPLC separation and fluorimetric detection of PL and PLP, the substrate and the product of the reaction, respectively, as semicarbazone derivatives obtained by the reaction with semicarbazide (pyridoxal semicarbazone (PLSC) and pyridoxal-5′-phosphate semicarbazone (PLPSC)) [27]. Enzymatic activity was also determined by measuring the production of PMP from PM as substrate under the same experimental and instrumental conditions, with the following modifications: semicarbazide derivatization was not used; the mobile phase was a 50 mM KH_2PO_4 buffer containing 4 mL of PIC reagent A per liter, pH adjusted to 3 with orthophosphoric acid; and the excitation wavelength was 320 nm, and the emission wavelength was 420 nm.

STUDIES OF THE INHIBITORY EFFECTS OF SOME DRUGS ON PLK ACTIVITY. As a first step, the initial rate (v, expressed as nanomole of PLP formed per gram of hemoglobin per hour) was measured with each tested drug at a final concentration of 100 μM under two experimental conditions: at a saturating ATP-Mg concentration of 2 mM and a single fixed PL concentration of 3 μM (approximately three times the k_m value), and reciprocally at a saturating PL concentration of 98 μM and a single fixed ATP-Mg concentration of 100 μM (approximately five times the k_m value). When a drug showed an inhibitory effect toward PL higher than 15%, its effect as inhibitor was also tested at a saturating ATP-Mg concentration of 2 mM and a single fixed PM concentration of 2.4 μM (approximately three times the k_m value).

Kinetic studies of the inhibition type were carried out by monitoring PLP production in hemolysate from pyridoxal (0.375 to 6 μM) at a saturating ATP-Mg concentration (2 mM) and reciprocally from ATP-Mg (3.125 to 50 μM) at a saturating PL concentration (98 μM) in the absence and the presence of three fixed levels of drugs. The kinetic data were analyzed using the GraFit program (Erithacus Software Ltd, Staines UK). All the initial velocity values used for individual kinetic analyses resulted from a single determination carried out the same day in the same batch. These values were fitted by nonlinear regression analysis, using simple weighting, to the Michaelis-Menten equation and to the equations of the enzymatic inhibition. The GraFit program performs nonlinear regression by the Marquart's method, using a numerical second order method to calculate partial differentials. Regression analysis provides the best-fit values for kinetic parameters, and their standard error is calculated by the matrix inversion method.

Investigation of the Binding of Inhibiting Drugs to Either PLP or PL

To seek the *in vitro* formation of PLP- and/or PL-drug covalent complexes, UV-visible absorption spectra were obtained under the following conditions: all reactions were performed in a 90 mM K_2HPO_4 buffer, pH adjusted to 7.4 with orthophosphoric acid; the PLP or PL concentration was always 0.1 mM; the drug concentration was always 0.5 mM; and spectra were recorded after a 15-min incubation of both components at 37° and scanned from 250 to 500 nm on a DU-7 spectrophotometer from Beckman (Fullerton, CA, USA). Absorption spectra of each drug in the absence of B_6 vitaminers were also recorded.

The binding of drugs to PLP or PL was also investigated chromatographically by measuring remaining free PLP or PL (not bound to drug) as semicarbazone derivatives after incubation of PLP or PL (0.16 mM) and drug (0.5 mM) for 1 hr at 37° in a 90 mM K_2HPO_4 buffer, pH 7.4. It was also possible to detect the reversibility of binding by semicarbazide by this method.

MS analysis completed the spectrophotometric study and gave the molecular weight of the *in vitro*-formed complexes,

TABLE 1. Screening study of the inhibitory effect of certain drugs on PK activity

Drug	Inhibition (%) S = PL	S = PM	Drug	Inhibition (%) S = PL	S = PM	Drugs	Inhibition (%) S = PL	S = PM
Allopurinol	0	—	Didanosine	0	—	Nitrofurantoin	0	—
Almitrine	0	—	Dopamine	52	0	Pefloxacin	0	—
Amantadine	0	—	Ethambutol	0	—	D-Penicillamine	20	0
Amineptine	0	—	Ethionamide	0	—	Phenytoin	0	—
Captopril	0	—	Flecainide	0	—	Procarbazine	0	—
Carbamazepine	0	—	Flucytosine	0	—	Progabide	50	26
Cefotaxime	0	—	5-Fluorouracile	0	—	Progabide acid	0	—
Chloramphenicol	0	—	Gabapentin	0	—	Progesterone	0	—
Chloroquine	0	—	Gentamicin	0	—	Pyrazinamide	0	—
Cisplatin	0	—	Imipramine	0	—	Pyrimethamine	0	—
Colchicine	0	—	Isoniazid	81	0	Theophylline	86	88
Cycloserine	42	0	Levodopa	16	0	Thiamphenicol (*glycinate*)	31	0
Dacarbazine	0	—	Metronidazole	0	—			
Dantrolene	0	—	Muzolimine	27	0	Thiamphenicol	0	—
Desipramine	0	—	Nalidixic acid	0	—	Vigabatrin	0	—

S, substrate. Results obtained at [PL] = 3 μM, [PM] = 2.4 μM, [ATP] = 2 mM, and [drug] = 100 μM.

in 5 mM ammonium acetate, pH 7.4, from PLP or PL (0.1 mM) and drug (0.5 mM) after a 15-min incubation at 37°. A Perkin-Elmer PE SCIEX API 300 LC/MS/MS system (Forster City, CA, USA) was used for MS detection. Nitrogen (l'Air Liquide, Paris, France) was employed as the nebulizing gas. The instrument was operated in the positive ionization mode. Samples were introduced by continuous infusion at 5 μL/min, and MS data for PLP, PL, drugs, and their possible complexes were collected using total ion current with a range of m/z 50–650.

RESULTS

Enzymatic Investigations

The rapid screening carried out at 3 μM PL and 2 mM ATP-Mg allowed us to retain nine drugs with inhibiting properties (Table 1) in the following order of decreasing potency: theophylline, isoniazid, dopamine, progabide, cycloserine, thiamphenicol glycinate, muzolimine, D-penicillamine, and levodopa (Fig. 1). Screening at 98 μM PL and 100 μM ATP failed to find any other inhibiting drug. Among these nine drugs, only two, theophylline and progabide, were also able to significantly inhibit PLK activity when PM, instead of PL, was used as substrate.

The results of the kinetic studies are summarized in Table 2. No kinetic parameters were calculated with cycloserine, dopamine, isoniazid, and thiamphenicol glycinate (Fig. 2) because the Hanes plots versus [PL] intersected to the right of the vertical axis, showing inhibition only for the highest concentrations of PL. With the other drugs, the Hanes plots versus [PL] or [ATP] were linear, and initial velocity data were fitted to the various equations of the enzymatic inhibition to specify the type and kinetic parameters of the inhibition. Figure 3 illustrates the inhibition of PLK by progabide, theophylline, levodopa, and muzolimine. The k_i and k_{si} values reported in Table 2 show that theophylline was the most potent inhibitor preferentially binding to PLK on the PL site with a k_i value of 3 ± 0.4 μM. The interaction between PLK and progabide took place on the ATP rather than the PL site because the inhibition constants had their lowest values in the experiment performed at a saturating PL concentration and various ATP concentrations. The types of inhibition by drugs that did not inhibit the enzyme in the presence of PM were only apparent because these drugs were not true inhibitors.

Investigation of the Binding of Inhibiting Drugs to PLP and PL

At neutral pH, PLP absorbed with maxima at 330 nm and 388 nm, whereas PL absorbed with maxima at 252 nm and 316 nm. The drugs that caused a shift in the absorption spectrum of PLP or PL consistent with the formation of a covalent complex are shown in Table 3. Data noted as inconclusive resulted from drugs with maximum absorption in the same range of wavelength as PLP or PL. Seven drugs showed evidence of a PLP-drug complex formation, illustrated for levodopa, muzolimine, and D-penicillamine in Fig. 4. It was not possible to spectrophotometrically detect any PL-drug complex. Binding to PLP was demonstrated indirectly for six drugs by the chromatographic study (Table 4). With dopamine and levodopa, no PLPSC peak was detected on the chromatogram, suggesting that they were tightly bound to PLP without reversibility by semicarbazide, which was added for fluorimetric detection. In the mixture containing thiamphenicol glycinate and PLP, 100% of free PLP was recovered as a semicarbazone derivative, suggesting that the formed complex (spectrophotometrically detected) was reversed by semicarbazide. The results obtained with PL under the same conditions showed a 46% decrease in free PL after incubation with muzolimine and a moderate decrease in free PL (10%–20%) after incubation with isoniazid, dopamine, levodopa, and penicillamine. Mass detection confirmed the binding of some drugs to B_6 (Table

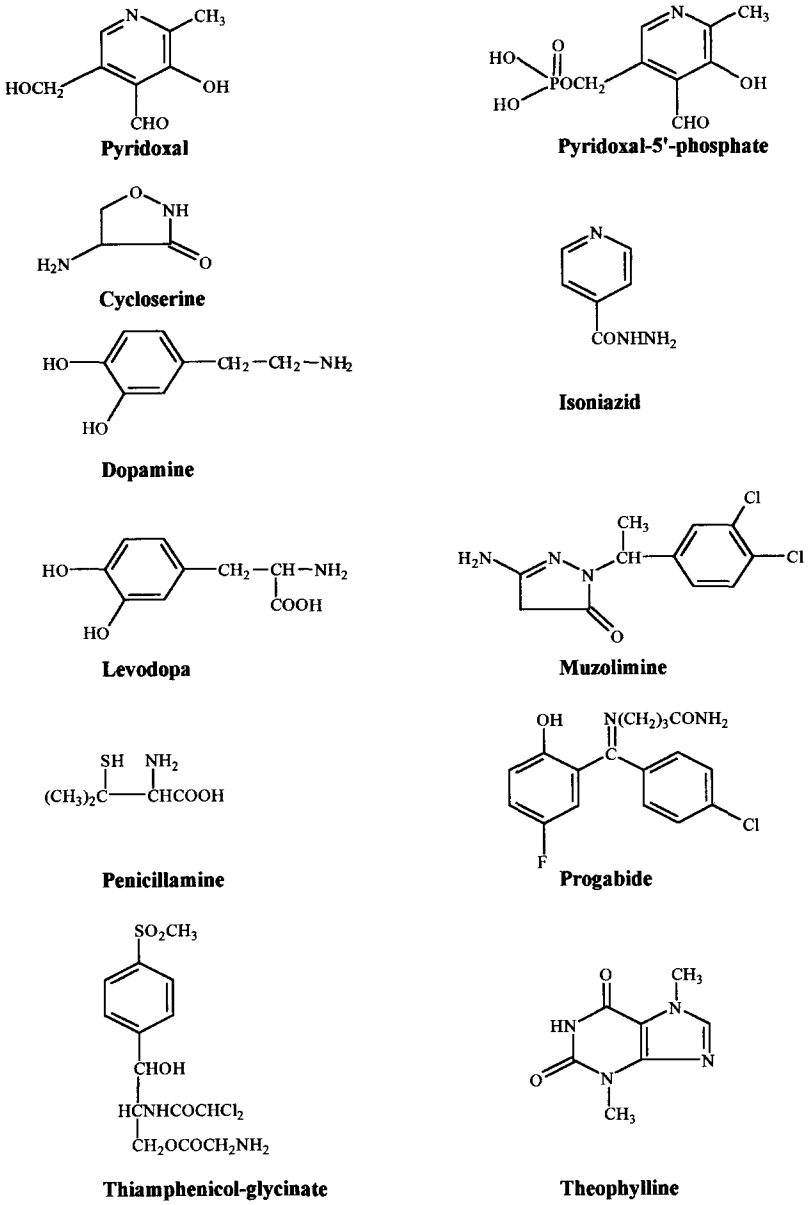

FIG. 1. Chemical structure of the aldehyde derivatives of vitamin B_6 and of drugs that inhibit PK activity.

TABLE 2. Type and kinetic parameters (calculated value ± standard error) of PK inhibition of drugs

	Variable S : PL; saturating S : ATP			Variable S : ATP; saturating S : PL		
Drug	Inhibition mechanism	k_i (μM)	k_{si} (μM)	Inhibition mechanism	k_i (μM)	k_{si} (μM)
Cycloserine	Inconclusive	—	—	*	—	—
Dopamine	Inconclusive	—	—	*	—	—
Isoniazid	Inconclusive	—	—	*	—	—
Levodopa	m NC	271 ± 67	388 ± 61	m NC	264 ± 8	392 ± 29
Muzolimine	C	198 ± 17	—	m NC	107 ± 16	312 ± 53
D-Penicillamine	m NC	375 ± 75	524 ± 69	NC	1050 ± 49	—
Progabide	NC	173 ± 8	—	m NC	22 ± 2	121 ± 18
Theophylline	m NC	3 ± 0.4	131 ± 61	m NC	77 ± 8	309 ± 27
Thiamphenicol (*glycinate*)	Inconclusive	—	—	*	—	—

*, not performed; S, substrate; k_i, apparent dissociation constant of the enzyme-inhibitor complex (enzyme being saturated by one of the substrates); k_{si}, apparent dissociation constant of the enzyme-substrate inhibitor complex (enzyme being saturated by one the substrates); C, competitive; NC, noncompetitive; m NC, mixed noncompetitive.

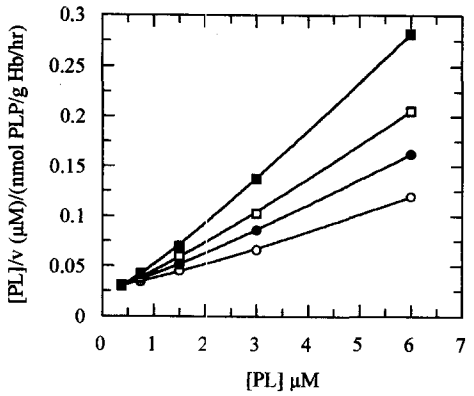

FIG. 2. Hanes plots of [PL]/v versus [PL] in absence (○) and in presence of thiamphenicol glycinate at 100 μM (●), 200 μM (□), and 400 μM (■).

TABLE 3. UV-visible spectrophotometric study of the binding of drugs to PLP and PL

Drug (0.5 mM)	PLP (0.1 mM)	PL (0.1 mM)
Cycloserine	+	–
Dopamine	+	Inconclusive
Isoniazid	+	Inconclusive
Levodopa	+	Inconclusive
Muzolimine	+	Inconclusive
D-Penicillamine	+	–
Progabide	Inconclusive	Inconclusive
Theophylline	–	Inconclusive
Thiamphenicol (*glycinate*)	+	–

(+) or (–), presence or absence of a shift in the absorption spectrum of PLP or PL consistent with formation of a covalent complex. Mixtures contained 100 μM of PLP or PL and 500 μM of each drug in 90 mM K_2HPO_4, pH 7.4, and were incubated for 15 min at 37°.

5). The m/z values of the protonated molecular ion of the complexes were consistent with the formation of a Schiff's base between a drug amino group and the aldehyde function of PLP or PL. However, mass detection failed to detect cycloserine-PLP or isoniazid-, dopamine-, levodopa-, and penicillamine-PL complexes, probably owing to their instability under our ionization conditions.

DISCUSSION

After an analysis of results, drugs that showed an apparent inhibitory effect on PLK can be divided into three groups. The first group includes theophylline and progabide, which are true inhibitors of PLK. Under our experimental conditions, they did not form covalent complexes with B_6 vitaminers. Ubbink *et al.* [3] were the first to demonstrate that theophylline-induced vitamin B_6 deficiency could result from a noncompetitive inhibition of nonpurified erythrocyte PLK, with an apparent k_i of 12.8 μM. Using the same methodology, except that our hemolysate was dialyzed, we obtained mixed noncompetitive (but almost competitive) inhibition with respect to PL (see Fig. 3). Later, Ubbink *et al.* [20], studying inhibition of purified PLK from sheep brain by methylxanthines, showed that theophylline is the most efficient competitive inhibitor with respect to PL (k_i of 8.7 μM). They concluded that the degree of enzyme purification may influence the pattern of the observed inhibition. An apparent k_i value lower than the therapeutic plasma level of theophylline [2] could explain an *in vivo* inhibition of PLK and the decreased

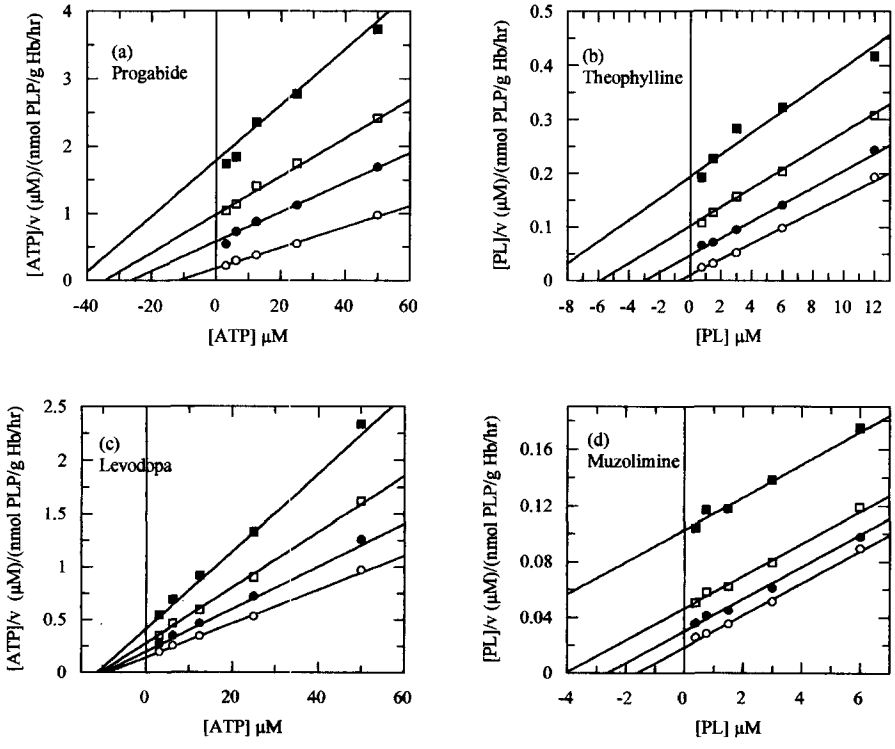

FIG. 3. Hanes plots of [S]/v versus [S]: a, in absence (○) and in presence of progabide at 50 μM (●), 100 μM (□), and 200 μM (■); b, in absence (○) and in presence of theophylline at 10 μM (●), 25 μM (□), and 50 μM (■); c, in absence (○) and in presence of levodopa at 125 μM (●), 250 μM (□), and 500 μM (■); and d, in absence (○) and in presence of muzolimine at 125 μM (●), 250 μM (□), and 1000 μM (■).

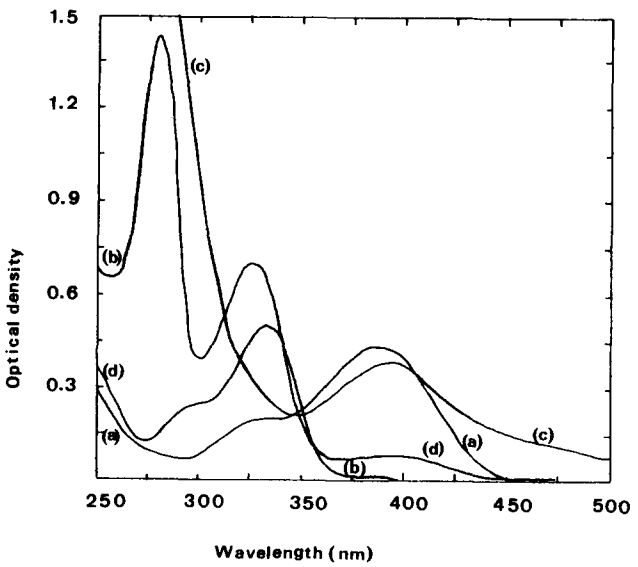

FIG. 4. UV-visible absorption spectra of PLP (a) and the following covalent complexes: levodopa-PLP (b), muzolimine-PLP (c), and D-penicillamine-PLP (d) in a 90 mM K_2HPO_4 buffer at pH 7.4.

plasmatic PLP levels observed in rabbits [13], in treated asthmatic patients, or in healthy volunteers [3, 28, 29].

Progabide and its main metabolite, progabide acid, are central γ aminobutyric acid ($GABA_A$) receptor agonist drugs [30, 31]. Although progabide is devoid of any neurotoxic effect, it was tested because its chemical structure contains a Schiff's base. Progabide is a less potent inhibitor than theophylline. Comparison of the values of apparent inhibition constants with plasmatic progabide levels in treated patients (which vary from 1.5 to 3 μM [32]) explains why PLK inhibition has no clinical significance in humans, especially as progabide acid does not itself inhibit PLK. The amide function of progabide seems to be an essential chemical structure in favor of its inhibitory effect.

A second group of drugs can be individualized, including cycloserine, dopamine, isoniazid, and thiamphenicol glycinate. By themselves, these drugs did not inhibit PLK with respect to PM. They were able to bind PLP, and two of them were also able to bind PL, as shown by at least one of the methods used. The observed inhibition may be related to two different mechanisms: first, an apparent inhibition due to PLP-drug covalent complex formation that prevents PLPSC formation in the reaction mixture and thereafter fluorimetric detection of PLP (except for thiamphenicol glycinate, whose complex with PLP can be reversed by SC); second, a true inhibition of PLK by the formed complexes themselves, which could account for the greater inhibition observed at the highest PL concentrations.

TABLE 4. Chromatographic study of the binding of drugs to PLP and PL.

Drug	Remaining free PLP (%)	Remaining free PL (%)
Cycloserine	55	100
Dopamine	0	80
Isoniazid	25	88
Levodopa	0	88
Muzolimine	47	54
D-Penicillamine	48	88
Progabide	100	100
Theophylline	100	100
Thiamphenicol (*glycinate*)	100	100

Mixtures contained 160 μM PLP or PL and 500 μ of each drug in 90 mM K_2HPO_4, pH 7.4, and were incubated for 1hr at 37°.

TABLE 5. Mass spectrometric study of the binding of drugs to PLP and PL

Drugs	m/z of the protonated molecular ion $[drug\text{-}PLP+H]^+$	m/z of the protonated molecular ion $[drug\text{-}PL+H]^+$
Cycloserine	—	—
Dopamine	383	—
Isoniazid	367	—
Levodopa	427	—
Muzolimine	501	421
D-Penicillamine	379	—
Progabide	—	—
Theophylline	—	—
Thiamphenicol (*glycinate*)	642	—

—, no detected complex under our experimental conditions. Mixtures contained 0.1 mM PLP or PL and 0.5 mM of each drug in 5 mM ammonium acetate, pH 7.4, and were incubated for 15 min at 37°.

For 40 years, isoniazid therapy has been recognized as responsible for the symptomatology of vitamin B_6 deficiency, particularly symmetrical, distal, and sensorimotor neuropathy [33]. In 1960, Braunstein [19] described a reduction of PLP availability by formation of hydrazone derivatives with isoniazid. McCormick and Snell [34], who studied the effects of inhibitors on PLK from various origins, found that the hydrazone derivative from isoniazid and PL was a potent inhibitor of the kinase, beef brain kinase being more sensitive than that of rat liver; on the contrary, the derivative from isoniazid and PLP (Fig. 5) was not an inhibitor. Kark *et al.* [1] demonstrated a lower PLP level than that of controls and significantly reduced erythrocyte PLK activity in humans treated by isoniazid, while other enzymatic activities of B_6 metabolism remained identical to controls.

Cycloserine has been extensively used in combination with isoniazid. Its high neurological toxicity currently limits its use to cases where microorganisms are resistant to other antituberculous drugs. Pyridoxine was effectively used to prevent and treat convulsions due to cycloserine [17]. The nonenzymatic reaction between cycloserine and PLP was studied by Roze and Strominger [35], who detected three major compounds: PMP and the mono- and di-PLP derivatives of β-aminoxyalanine. None of these products was revealed by mass spectrometry. However, spectrophotometric and chromatographic data led us to suppose that

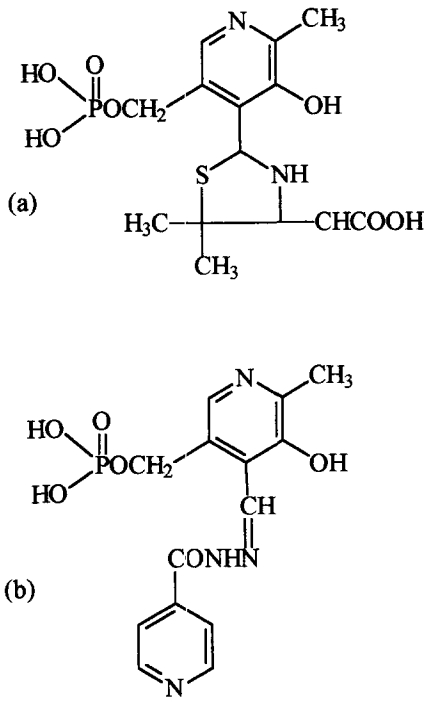

FIG. 5. Chemical structure of the supposed penicillamine-PLP complex (a) and the PLP isonicotinyl hydrazone (b).

covalent complexes having inhibitory property on PLK are produced in a mixture containing both components.

No neurological manifestation of B_6 deficiency has been linked to dopamine therapy in humans, but this drug is unable to cross the blood-brain barrier. However, its ability to react with PLP by forming Schiff's base, which can rapidly deplete PLP in animals intoxicated by dopamine, has been demonstrated [12, 16]. Asakura *et al.* [11] demonstrated PLK inhibition by dopamine when PL was used as substrate. They related this inhibitory effect to the formation of the PLP-dopamine derivative and, contrary to our own hypothesis, did not find any PLK inhibition brought about by the complex itself.

Long-term therapy with thiamphenicol or chloramphenicol is believed to be a possible cause of optic neuritis and sensitive peripheral neuropathy [36]. At 100 μM, thiamphenicol glycinate acts as a weak inhibitor of PLK, while chloramphenicol and thiamphenicol do not. After esterification by glycine, thiamphenicol presents an amino group that can react with the aldehyde function of PLP. The formed derivative seems less stable than those previously described because it can be reversed by semicarbazide. Further studies are needed to confirm this observation and the possibility of PLP deficiency in patients receiving thiamphenicol glycinate by parenteral route.

Levodopa, penicillamine, and muzolimine form a third group. The linearity of the Hanes plots and the ability of these drugs to react nonenzymatically with PLP and PL suggest that their apparent inhibitory effect only results from their chemical reactivity without PLK inhibition by the formed derivatives. Levodopa is known to induce B_6 deficiency [37]. Moreover, in patients with Parkinson's disease, its therapeutic effect is abolished together with its dyskinetic side effects when large doses of pyridoxine are simultaneously administered. The formation of a Schiff's base between levodopa and PLP is a well established explanation of this double antagonism. The chromatographic results show that levodopa, like dopamine, reacts with PLP more tightly than other drugs.

B_6 antagonism by penicillamine has been reported in animal species [8, 10]. Long-term therapy with L- or D-penicillamine increased xanthurenic acid and kynurenine excretion after tryptophan load indicating a B_6 deficient state [4], and Rothschild [38] proposed giving supplemental pyridoxine to patients receiving penicillamine. The amino and sulphydryl groups of penicillamine can react with the aldehyde function of PLP to form a thiazolidine compound (Fig. 5) [37]. The m/z value of the protonated ion of the PLP-penicillamine complex we obtained by MS detection is consistent with the molecular weight of a thiazolidine derivative.

Although chemically unrelated, muzolimine is a diuretic that reportedly has effects and uses similar to those of the loop diuretics. Cases of neuromyeloencephalopathy occurring in hemodialyzed patients after administration of high doses of muzolimine led to its withdrawal worldwide. This neurotoxicity was not related to vitamin B_{12} or folic acid deficiency. Administration of supplemental group B vitamins and cessation of muzolimine treatment were ineffective in improving symptomatology after 10 months [39]. Our data provide evidence of the ability of muzolimine to react with PLP and PL, but the question of whether this reactivity can lead to vitamin B_6 deficiency remains unanswered. However, eventual B_6 deficiency cannot entirely explain the symptomatology and its irreversibility.

In conclusion, our data confirm some known drug-B_6 interactions and report some new interactions between B_6 and progabide, thiamphenicol glycinate, and muzolimine, probably not of great clinical significance but worthy of further investigation.

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