Pergamon

Biochemical Pharmacology, Vol. 48, No. 10, pp. 1976–1978, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00369-6

Short Communication

THE INFLUENCE OF PICOLINES ON GLUTATHIONE TRANSFERASE ACTIVITY AND SUBUNIT COMPOSITION IN HUMAN LIVER DERIVED Hep G2 CELLS

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(*Received* 7 *April* 1994; *accepted* 15 *August* 1994)

Abstract—Hep G2 cells, an established cell line derived from a human hepatoma, have retained a number of hepatocytic phase I and II reactions. The influence of picolines (2-, 3- and 4-methylpyridine), related compounds and some classical enzyme inducers on specific glutathione transferase (GST) activity and its subunit composition in Hep G2 cells was investigated. Increased GST activity was observed for rifamycin, phenobarbital, pyrazine and the picolines, of which the 4-isomer was the strongest inducer. The GST subunits were analysed by HPLC. GSTP1, GSTM1a, GSTA1 and GSTA2 were present in control Hep G2 cells. GSTM1a disappeared or was strongly reduced under the influence of the test chemicals. All GST increases were due to augmented GSTA1 expression. Thus, picolines stimulate GST activity in Hep G2 cells by influencing the class alpha GSTA1.

Key words: glutathione transferase; subunits; Hep G2 cells; picolines; induction; class alpha

Nitrogen heterocycles are widely employed in the pharmaceutical and chemical industries. Pyridine is used as a solvent in drug manufacture, as reagent in industrial processes, in phosgenation processes for polycarbonate resins, and as intermediate in the preparation of antihistamines. Picolines (methylated pyridines) also have many applications. 2-Picoline is used mainly as an intermediate in the production of 2-vinylpyridine. It is also employed as an intermediate in the production of herbicides, fungicides and pharmaceuticals such as amprolium, methyridine and 2-pyridine aldoxime methiodide. 3-Picoline is primarily used in the synthesis of the vitamins niacin and niacinamide, and further in the synthesis of resins, dyestuffs, rubber accelerators and insecticides. 4-Picoline is mainly used in the synthesis of pharmaceuticals, of which isoniazid is the most important, and also in the production of pesticides, waterproofing agents, resins, and 4-vinylpyridine [1].

Many nitrogen heterocycles induce cytochrome P450, but little is known about their effect on GST† (EC 2.5.1.18). GSTs are dimeric multifunctional phase II enzymes, playing an important role in the biotransformation and detoxification of xenobiotics [2]. Their induction has been recently reviewed [3]. Primiano *et al.* [4] reported that 4-picoline induces a novel class alpha GST in rabbit liver cytosol. Important differences between rats and rabbits were observed for the induction of GST by nitrogen heterocycles and other inducers [5]. The influence of picolines on GST activity and its subunit composition in Hep G2 cells are reported here. Some related compounds and classical enzyme inducers were included for comparison purposes. Hep G2 cells are a highly differentiated human hepatoma cell line which has retained many specialized functions usually lost upon culturing [6]. The GST activity remains constant in function of the time in culture [7], but increases by culture in modified Earle's medium, although the effect of the medium composition on the GST activity is much less marked as the effect on mixed function oxidase activities [8].

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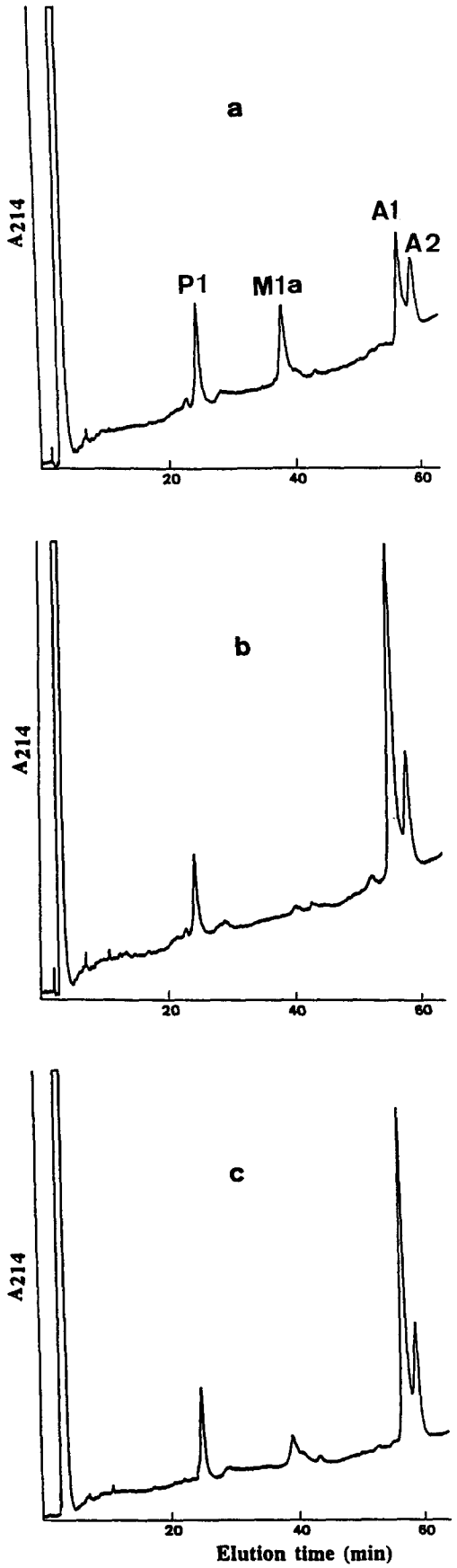
† Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GSH, reduced glutathione; GST, glutathione transferase.

Materials and Methods

Hep G2 cells (ATCC No. 8065-HB) were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin (complete medium). They were incubated at 37° in a 5% (v/v) CO_2 atmosphere.

For GST induction the cells were treated for 3 days with 5 mM nitrogen heterocyclic compounds, 50 μM rifamycin or 2 mM phenobarbital in complete medium supplemented with 5 μM hydrocortisone, or for 1 day with 20 μM benz[*a*]anthracene.

The cells were collected by trypsinization, washed with phosphate-buffered saline and homogenized in 22 mM sodium phosphate buffer, pH 7.0, in a motor driven Potter–Elvehjem homogenizer (1500 rpm), equipped with a teflon pestle. The clear cytosol, obtained by ultracentrifugation (1 hr, 100,000 *g*), was applied to a GSH affinity column (1.0 × 4.3 cm) [9]. Purified GST was eluted with 15 mM GSH in 50 mM Tris–HCl buffer, pH 9.6. The fractions with GST activity were pooled, concentrated by ultrafiltration on an Ultrafree-PF unit (Millipore) and further analysed by HPLC for the GST subunit composition as described in the legend to Fig. 1. The GST activity was determined according to Ref. 10 with 1 mM CDNB and 1 mM GSH as substrates. Specific activity is expressed in mU/mg protein as measured by the Lowry method [11], with bovine serum albumin as standard. The results are expressed as the means ± standard deviation (N = 3). They are statistically analysed by the Student's *t*-test.



Results and Discussion

Hydrocortisone and benz[*a*]anthracene had no effect on specific GST activity in Hep G2 cells (Table 1), confirming previous observations [12]. Pyridine reduced, while all other test chemicals increased specific GST activity, including the previously untested 2- and 3-picoline. 4-Picoline was the strongest GST inducer in Hep G2 cells (Table 1). Consequently, the well-known *in vivo* inducers phenobarbital and rifamycin [13] are also effective in cultured Hep G2 cells. Pyrazine, pyridine and phenobarbital (but not 4-picoline) stimulated GST expression in hepatic rat liver cytosol; neither of these agents, however, stimulated GST expression in rabbit liver [5].

In order to investigate whether the picolines induce specific GST(s) in Hep G2, GST subunit composition was further analysed by HPLC. Typical chromatograms are shown in Fig. 1 for control cells, 4-picoline and rifamycin treated cells. GST subunits are designated according to Ref. 14. GSTP1 (class pi), GSTM1a (class mu), GSTA1 and GSTA2 (class alpha) were eluted at 25.1, 38.9, 56.8 and 59.1 min, respectively. The occurrence of these GST subunits in Hep G2 was previously shown using other techniques [16–18]. The class mu GST subunit was designated GSTM1a (previously named GST μ) because it eluted closer to the GSTP1 peak than does GSTM1b (previously named GST ψ) [19].

GST subunit composition was the same in control and hydrocortisone treated cells (Table 1). Although benz[*a*]-anthracene did not alter specific GST activity, the relative subunit content was drastically changed. GSTA2 was the dominating remaining GST after pyridine treatment. Class mu GSTM1a disappeared or was strongly reduced under the influence of any test chemical (Table 1), which was observed by Primiano *et al.* [5] only for pyridine in rabbit liver, but not for other nitrogen heterocycles, neither in rats nor in rabbits. Class pi GSTP1 was reduced by phenobarbital, rifamycin and the picolines, especially 2-picoline. All total GST increases were due to an augmented class alpha GSTA1. These results are very similar to the increased GSTA1 expression in freshly isolated human hepatocytes cultured with various inducers [20]. 4-Picoline induced a novel class alpha GST in rabbit liver [4], while none of the picolines induced any new GST in Hep G2 cells (Table 1).

Induction of GST and other phase II drug-metabolizing enzymes is considered a major protection mechanism against chemical stress and carcinogenesis. Indeed, a correlation exists between GST induction and lower incidence of experimental cancer [3]. The differences in GST expression between rats and rabbits in response to nitrogen heterocycles [5] were extended in this study to the human hepatoma-derived established Hep G2 cell line. The picolines stimulate GST expression in Hep G2 cells mainly by influencing the class alpha GSTA1. The human origin of this cell line probably allows a safer extrapolation of these results to humans than do the results on rodents.

Fig. 1. HPLC separation of cytosolic Hep G2 GST subunits. Affinity-purified GST was subjected to HPLC analysis [15] on a Vydac 30 nm C18 wide-pore reverse-phase column (250 × 4.6 mm), eluted at a flow rate of 1 mL/min. Solvent A was 0.1% (v/v) trifluoroacetic acid in water and solvent B was 0.1% (v/v) trifluoroacetic acid in acetonitrile. The gradient consisted of a linear gradient from 35 to 40% (v/v) solvent B in 5 min, followed by a linear gradient from 40 to 54% (v/v) solvent B in 75 min. The absorbance was monitored at 214 nm. (a) Control cells, (b) cells treated with 4-picoline, (c) cells treated with rifamycin.

Table 1. GST activity and GST subunit composition in Hep G2 cytosol of cells treated with different test chemicals

Test compound	GST activity		% GST subunits			
	Sp. act.*	%	GSTP1	GSTM1a	GSTA1	GSTA2
Control	45.0 ± 2.8	100	21.9	21.0	32.9	24.2
Hydrocortisone	45.9 ± 3.1	102	26.8	15.3	34.3	23.6
Benz[*a*]anthracene	45.1 ± 2.5	100	15.8	tr.†	48.6	35.6
Rifamycin	53.4 ± 3.4‡	119	13.7	3.8	60.0	22.5
Phenobarbital	68.7 ± 5.6§	153	12.7	tr.†	64.0	23.3
2-Picoline	57.7 ± 3.1§	128	6.9	3.9	76.1	13.1
3-Picoline	54.4 ± 3.8‡	121	13.6	tr.†	56.4	30.0
4-Picoline	108.6 ± 7.1‖	241	10.6	tr.†	68.3	21.1
Pyridine	34.4 ± 2.0§	77	26.0	tr.†	33.8	40.3
Pyrazine	59.8 ± 3.9§	133	18.6	tr.†	55.7	25.7

* Specific activity in nmol/min/mg protein, mean ± standard deviation
† Trace
‡ $P < 0.05$
§ $P < 0.01$
‖ $P < 0.001$

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