The Metabolic Fate of Chlordimeform [*N*-(4-Chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine] in Human Embryonic Lung Cell Cultures

Tsu Hui Lin, Hanson H. North, and Robert E. Menzer*

The metabolism of [^{14}C]chlordimeform (*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine) by primary human embryonic lung cells in culture was studied. Chlordimeform was quite susceptible to degradation by the cell cultures. Over 97% of the applied chlordimeform was changed by the cells after 72 hr of incubation, with the major metabolites appearing as *N*-formyl-4-chloro-*o*-toluidine (81.9%) and 4-chloro-*o*-toluidine (2.3%). Other minor metabolites of chlordimeform included *N*-demethylchlordimeform (*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine) and two unknown metabolites. When [^{14}C]chlordimeform was incubated in medium without cells for 72 hr, the compound was decomposed to *N*-formyl-4-chloro-*o*-toluidine (10%) with the remainder being unchanged. Water-soluble metabolites of chlordimeform were formed in insignificant quantities in both controls and test flasks.

Chlordimeform (Galecron) (*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine) is a recently introduced acaricide, ovicide, and larvicide (Dittrich, 1967). The metabolism of chlordimeform has been studied in plants, rats, dogs, goats, rat liver microsomes, and microorganisms. Apple seedlings degraded chlordimeform to form *N*-demethylchlordimeform (*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine), *N*-formyl-4-chloro-*o*-toluidine, 4-chloro-*o*-toluidine, and a glucoside identified as *N*-(2-methyl-4-chlorophenyl)-D-glucosylamine (Sen Gupta and Knowles, 1970).

In mammals (rats, dogs, goats), chlordimeform was first demethylated to *N*-demethylchlordimeform. Both chlordimeform and its *N*-demethyl metabolite were cleaved at the carbon–nitrogen double bond to form *N*-formyl-4-chloro-*o*-toluidine which was either oxidized at the ring methyl group to *N*-formyl-5-chloroanthranilic acid or deformylated to 4-chloro-*o*-toluidine which was then oxidized to 5-chloroanthranilic acid. The anthranilic acids could form glucuronic acid or sulfate conjugates (Knowles and Sen Gupta, 1970; Sen Gupta and Knowles, 1970). Rat liver microsomes transformed chlordimeform to *N*-demethylchlordimeform, 4-chloro-*o*-toluidine, and aqueous metabolites (Ahmad and Knowles, 1971).

In bacteria (*Aerobacter aerogenes* and *Serratia marcesens*), fungi (*Fusarium moniliforme* and *Rhizopus nigricans*), and actinomycete (*Streptomyces griseus*), *N*-formyl-4-chloro-*o*-toluidine was the major degradation product (Johnson and Knowles, 1970).

This investigation of the metabolism of chlordimeform in human embryonic lung cell cultures was conducted to study the mechanism of demethylation. An earlier study (North and Menzer, 1972) using this system clarified the relationships between oxidative demethylation and desulfuration in dimethoate.

MATERIALS AND METHODS

Materials. [^{14}C]Chlordimeform with a specific activity of 4.72 mCi/mmol was labeled at the phenylmethyl group. The radioactive compound and its unlabeled derivatives used in this study were furnished by CIBA-Geigy, Ltd., Basel, Switzerland.

Cell Cultures. Human embryonic lung (HEL) cells were supplied by Flow Laboratories, Rockville, Md. These cells were freshly explanted from in vivo sources and seeded in tissue culture flasks with nutrient medium (Lin et al., 1975; North and Menzer, 1972).

Department of Entomology, University of Maryland, College Park, Maryland 20742.

Incubation Conditions. Radioactive chlordimeform (300,000 dpm, 5.6 μg) in chloroform was added to culture flasks by dispersing the material carefully over one surface of each flask upon which the cells would be seeded. A gentle stream of sterile air was applied to evaporate the solvent. Other incubation and extraction conditions were as previously described (Lin et al., 1975).

Commercially prepared TLC plates (silica gel F-254, 0.25 mm thickness, E. Merck) were used to resolve ether-extractable metabolites of chlordimeform by methods previously described (Lin et al., 1975). No attempt was made to separate water-soluble metabolites because of low radioactivity. TLC plates were developed with solvents as indicated in Figure 1. Radioassay procedures have been previously described (North and Menzer, 1972).

RESULTS

The extraction procedures proved to be adequate in view of the average 99% recovery of originally inoculated radioactivity in both test and control flasks. The combined ether extracts from the test flasks contained over 98% of recovered radioactivity, and the radioactivity in the aqueous fraction was less than 2% after incubation periods ranging from 6 to 72 hr (Table I).

Thin-layer chromatography separated the ether-extractable metabolites into seven spots (Table I). Results from control cultures indicated very little degradation resulting from the medium, with only about 10% conversion to *N*-formyl-4-chloro-*o*-toluidine. Identification by cochromatography of the metabolites with standard reference compounds indicated that four of the spots were identical with chlordimeform, 4-chloro-*o*-toluidine, *N*-demethylchlordimeform, and *N*-formyl-4-chloro-*o*-toluidine. One of the unknowns which remained at the origin on TLC plates could be an anthranilic acid derivative of chlordimeform. Over 97% of the applied chlordimeform was transformed to metabolites by the cells in 72 hr. The metabolites present in the greatest quantities were *N*-formyl-4-chloro-*o*-toluidine and 4-chloro-*o*-toluidine although a portion of the former probably resulted from hydrolysis in the medium. The radioactivity in the ether extract of the aqueous phase after glucuronidase hydrolysis was insufficient for resolving metabolites.

DISCUSSION

Chlordimeform was easily transformed by the cell cultures since only about 4% of the applied dose remained intact after 72 hr of incubation. It appears that chlordimeform was first demethylated and then cleaved at the carbon–nitrogen double bond to form *N*-formyl-4-chloro-*o*-toluidine in view of the increasing formation with incu-

Table I. [^{14}C]Chlordimeform Metabolism by HEL Cells: Percent of Metabolites Present in Recovered Radioactivity at Corresponding Period of Incubation

Metabolites	6 hr		12 hr		24 hr		48 hr		72 hr	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Chlordimeform	81.7	16.9	79.6	14.8	81.7	12.8	79.5	4.9	78.2	3.9
4-Chloro-*o*-toluidine	0	1.6	0	2.4	0	2.4	0	2.0	0	2.3
N-(4-Chloro-*o*-tolyl)-*N'*-methylformamidine	0	0.1	0	0.2	0	0.3	0	0.6	0	0.7
N-Formyl-4-chloro-*o*-toluidine	11.0	74.6	11.9	73.0	11.0	77.7	12.9	84.6	13.9	81.9
Unknown 1	0	0.2	0	0.1	0	0.1	0	0.2	0	0.3
Unknown 2	0	0.1	0	0.2	0	0.2	0	0.2	0	0.4
Unknown 3 (origin)	7.0	6.0	8.0	7.9	7.0	4.9	7.0	5.9	6.9	8.9
Total organosoluble	99.6	99.5	99.5	98.7	99.6	98.4	99.4	98.4	99.0	98.7
Total water soluble	0.4	0.5	0.5	1.3	0.4	1.6	0.6	1.6	1.0	1.3

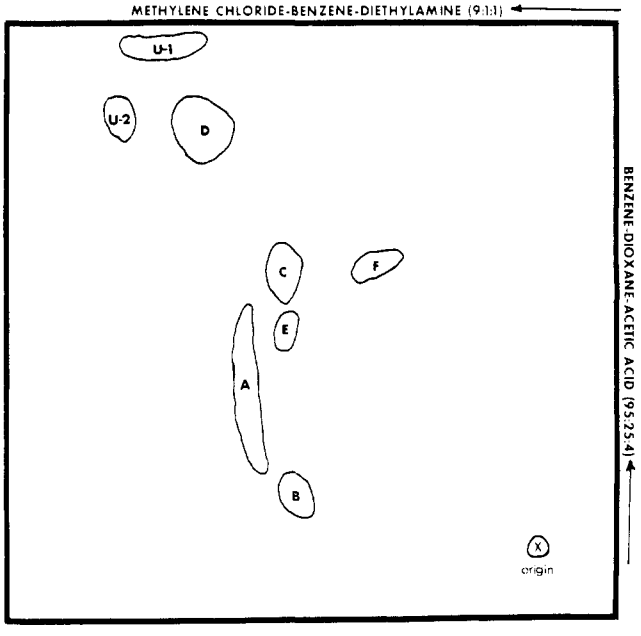

Figure 1. Two-dimensional thin-layer chromatogram of chlordimeform and its derivatives developed in solvent systems as shown: (A) chlordimeform; (B) *N*-dimethylchlordimeform (*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine); (C) *N*-formyl-4-chloro-*o*-toluidine; (D) 4-chloro-*o*-toluidine; (E) 5-chloroanthranilic acid; (F) *N*-formyl-5-chloroanthranilic acid.

bation time of *N*-demethylchlordimeform and *N*-formyl-4-chloro-*o*-toluidine. This is in agreement with Knowles and Sen Gupta (1970). Further metabolism of *N*-formyl-4-chloro-*o*-toluidine formed 4-chloro-*o*-toluidine and possibly anthranilic acid conjugates. The metabolic pattern of chlordimeform in HEL cell cultures was different from that in mammals. HEL cells are more efficient in forming oxidative metabolites and seem to be almost incapable of forming water-soluble metabolites (North and Menzer, 1972; Lin et al., 1975), whereas in mammals the opposite is true. In microorganisms, *N*-formyl-4-chloro-*o*-toluidine was reported to be the major degradation product of chlordimeform (Johnson and Knowles, 1970). This was similar to the case for the HEL cell culture system in which *N*-formyl-4-chloro-*o*-toluidine was also the major metabolite of chlordimeform. The formation of *N*-demethylchlordimeform was minute as compared to that of the *N*-formyl derivative. Perhaps HEL cells, being in the very early stage of development of metabolic enzyme systems, handle chlordimeform or other foreign compounds in a manner closer to microorganisms than to mammals, which have complex enzyme systems to deal with xenobiotics.

The unstable carbon–nitrogen double bond of chlordimeform may be responsible for the increased susceptibility of the compound to degradation by the cells. The absence of hydroxylated or hydrolytic products among metabolites of chlordimeform may also account for the low concentrations of water-soluble metabolites in cell cultures. It is generally accepted that hydroxylation or hydrolysis of foreign compounds results in formation of metabolites which are more water soluble than the parent.

The fact that oxidative metabolism of chlordimeform predominated over hydrolytic mechanisms is a further indication that this cell culture system is a very useful tool for studying oxidative degradative mechanisms.

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