Bull. Environm. Contam. Toxicol. 28, 500–503 (1982)

Carbaryl Binds to Proteins of Human Cells in Culture but Chlorinated Organic Chemicals Do Not

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We previously reported that upon incubation of cultured cells with various radioactive pesticides and environmental chemicals, persistent chlorinated organic compounds were taken up to a greater extent than non-persistent compounds such as organophosphorus pesticides (MURAKAMI & FUKAMI 1978). It was also found that carbaryl or Sevin, a broad spectrum insecticide with anticholinesterase activity, was taken up considerably by the cells. In the subsequent work, we demonstrated that carbaryl more remained in an insoluble cell fraction after treatments with perchloric acid and ethanol than benzo[a]pyrene, DDT and parathion (MURAKAMI & FUKAMI 1979). Recently we described that carbaryl was incorporated markedly into the crude nuclear fraction (MURAKAMI & FUKAMI 1980). These findings suggest that carbaryl may have a high binding potential to cellular macromolecules such as DNA or protein. Therefore, we have attempted to evaluate the interaction between carbaryl and the DNA or proteins of cultured human cells.

MATERIALS and METHODS

Human embryonic lung diploid cells (HEL 299) from the American Type Culture Collection (CCL 137) (Rockville, Maryland) were used. The general procedures of MURAKAMI & FUKAMI (1976 and 1979) for cell culture studies were used in the present investigation. A carbamate insecticide (carbaryl), four organochlorine chemicals (2,4-D, DDT, HCB and PCB) and a carcinogen (benzo[a]pyrene) were selected as test compounds (TABLE 1).

Cells were grown in 75 cm^2 tissue culture flasks containing 10 mL of medium to near-confluence as indicated by microscopic examination. The labeled chemicals were dissolved in ethanol and were added to the medium to give a final concentration of 1 % ethanol. The concentration of the chemicals in the medium was 4×10^{-6} M. This dose caused no visible cytotoxicity to the cells.

After incubation for 18 h at 37° C with the chemicals, DNA and protein fractions were prepared according to the methods of DIAMOND et al. (1967) and KUROKI & HEIDELBERGER (1971). The radioactive medium was re-

0007-4861/82/0028-0500 $01.00

TABLE 1
Radioactive Compounds Used in This Work

Common name	Chemical name	Specific activity mCi/mmole	Source[1]
Benzo[a]pyrene[2]		21.7	RCC
Carbaryl	1-Naphthyl N-methyl-^{14}C-carbamate	59.5	RCC
2,4-D	2,4-Dichlorophenoxy-2-^{14}C-acetic acid	28	RCC
DDT	Dichlorodi-U-^{14}C-phenyltrichloroethane	29.7	RCC
HCB	Hexachlorobenzene-^{14}C(U)	35.32	NEN
PCB[3]	Polychlorinated biphenyl-^{14}C(U)	31.3	NEN

1 Abbreviations : NEN, New England Nuclear, Boston, Massachusetts; RCC, Radiochemical Centre, Amersham, England.

2 Carbon 7 and 10 positions are labeled with ^{14}C.

3 Isomeric mixture containing approximately 54 % chlorine by weight.

moved, and the cell monolayers were washed twice with PBS:A. The cells were then scraped off by a rubber policeman and pelleted by centrifugation (1,000 rpm, 5 min). The cell pellet was washed twice with PBS·A by centrifugation and was frozen until use.

The cells were suspended in 4 mL of 5 % sodium p-aminosalicylate and lysed by the addition of 0.4 mL of 10 % sodium laurylsulfate. The resulting highly viscous solution was extracted with an equal volume (4.4 mL) of a phenol : m-cresol : 8-hydroxyquinoline : water mixture (500 : 70 : 0.5 : 55 by weight) for 30 min with stirring at room temperature.

After centrifugation at 12,000 x g for 30 min at 4° C, the DNA was prepared from the upper aqueous phase and counted according to the method of KUROKI & HEIDELBERGER (1971). The protein was precipitated from the intermediate and lower phenol layers by addition of 3 volumes of methanol. The protein was washed 5 times with methanol and once with ether and was dried. The protein was dissolved in 2 mL of 0.5 N NaOH. Radioactive chemicals bound to protein were determined by counting a 0.5 mL aliquot of the NaOH solution in 15 mL of a mixture of 5 volumes of toluene with dimethyl POPOP (0.01 g/L) and PPO (6 g/L) and 1 volume of Triton X-100 (MENEGHINI 1974). Protein was determined by assaying 0.2 mL of the NaOH solution according to the method of LOWRY et al. (1951). About 15×10^7 and 3×10^7 cells were used for isolation of DNA and protein, respectively.

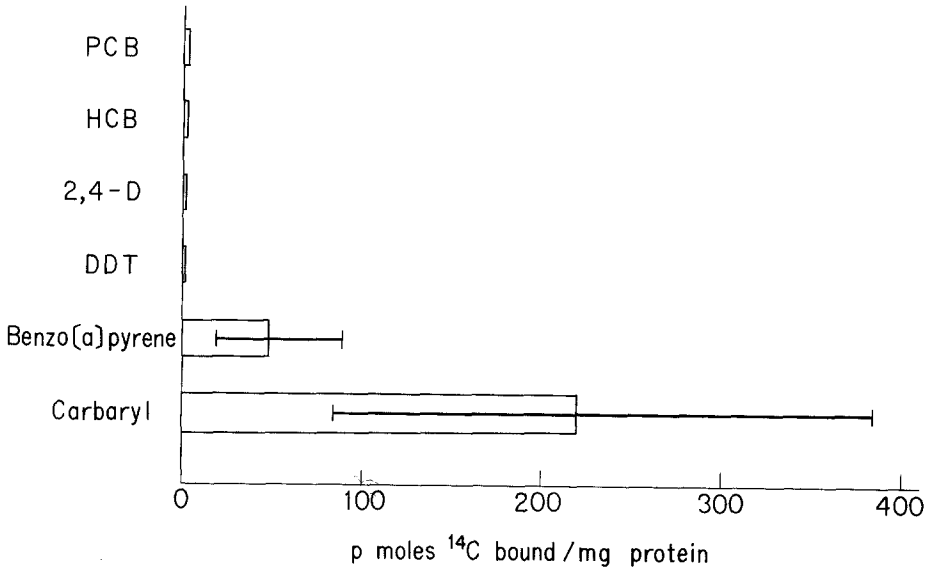

Fig. 1. Binding of ^{14}C-labeled chemicals to proteins from cultured human embryonic lung cells. Values are means of three determinations.

RESULTS and DISCUSSION

The radioactivity measurements on the protein isolated from the cultured cells show that a binding of carbaryl is greatest among chemicals tested (Fig. 1). Benzo[a]pyrene binds also to proteins, but the amount is much lower than that of carbaryl. No binding of chlorinated chemicals, 2,4-D, DDT, HCB and PCB, to cellular proteins can be detected. A binding of carbaryl to cellular DNA is extremely unlikely in the present cell culture system. MILLER et al. (1979) have reported that carbaryl binds covalently to amino acid residues of rat liver microsomal protein in vitro.

It has been generally accepted that the first step in the exertion of toxic action by a chemical involves binding of the chemical or one of its metabolites to a biological macromolecule. There is increasing evidence that DNA is the critical cellular target for hazardous chemicals, especially for carcinogens, but it is thought that binding of a chemical to proteins can induce some cellular damage through so-called "epigenetic" mode of toxicological reaction. Perhaps the high binding ability of carbaryl to proteins may serve as an index of toxicity of the insecticide. Recently NARBONNE & DAUBEZE (1980) have reported that hexachlorobiphenyl binds to rat liver

microsomal proteins in vitro, while no binding of polychlorinated biphenyl to the cellular proteins can be detected in the present study (Fig. 1). These results suggest that only chemicals with very high protein binding ability bind to proteins of cells in culture. We are now studying the detailed mechanism for binding of carbaryl to proteins of cultured cells and its consequences.

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