ヒト培養細胞における核酸およびタンパク質生合成に対するcarbarylの影響

誌名	日本農薬学会誌
ISSN	03851559
著者	村上, 誠 深見, 順一
巻/号	8巻3号
掲載ページ	p. 353-355
発行年月	1983年8月

J. Pesticide Sci. **8**, 353–355 (1983)

Note

Effects of Carbaryl on Nucleic Acid and Protein Biosyntheses in Cultured Human Cells

Makoto MURAKAMI and Jun-ichi FUKAMI

The Institute of Physical and Chemical Research, Hirosawa, Wako, Saitama 351, Japan

(Received December 3, 1982)

Monolayer cultures of human embryonic lung diploid cells (HEL 299) were exposed to an insecticidal chemical, carbaryl (1-naphthyl *N*-methylcarbamate), and its effect on nucleic acid and protein syntheses was measured. No significant differences among the effects on DNA, RNA and protein syntheses were observed. The rates of the syntheses of the macromolecules were inhibited severely at a concentration of 10^{-3} M. At lower dose levels between 10^{-7} M and 10^{-5} M, the syntheses of the macromolecules were virtually unaffected.

INTRODUCTION

Carbaryl (Sevin: 1-naphthyl *N*-methylcarbamate) is a broad spectrum insecticide with anticholinesterase activity. The cholinesterase inhibition by carbaryl is greater in insects than in mammals. In mammals its toxicity is low. Possible effects of carbaryl on reproduction and fetal development have been investigated and its fetal accumulation has been revealed by whole-body autoradiography.[1]

Recently we have investigated the binding of pesticides and environmental chemicals to proteins of cultured human embryonic cells and found that carbaryl binds to cell proteins while chlorinated organic chemicals, 2,4-D, DDT, hexachlorobenzene and PCB, do not. A carcinogen, benzo[a]pyrene, also binds to cell proteins, but the amount is much lower than that of carbaryl.[2] As a part of the extensive studies of carbaryl toxicity at the cellular level, we have examined the effects of the insecticide on the syntheses of DNA, RNA and protein in cultured human embryonic cells, and the results are described here. The toxic effects of carbaryl on the cells grown under *in vitro* conditions have been studied by several workers.[3–6] The results of these studies indicated that carbaryl was as toxic to cultured cells as common organochlorine and organophosphorus pesticides.

MATERIALS AND METHODS

Monolayer cultures of human embryonic lung diploid cells (HEL 299) from the American Type Culture Collection (CCL 137) (Rockvill, Maryland, U.S.A.) were used. The general procedures of Murakami and Fukami[7,8] for cell culture studies were used in the present investigation. Carbaryl was obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. It is the standard sample obtainable in Japan.

Effects of carbaryl on syntheses of nucleic acids and proteins were measured according to the method of Litterst *et al.*[4] Cells in 5 ml of growth medium were planted in 25 cm^2 tissue culture flasks. After 24 hr of incubation, this starter medium was replaced with 5 ml of medium, and cells in the fresh medium were incubated for an additional 24 hr. Various concentrations of the test compound in 0.05 ml of ethanol were added to the cultures. Controls were the cultures that had been treated only with ethanol. After incubation for 2 hr, one of the following radioactive precursors was added: L-[*U*-^{14}C]Leucine (1 μCi/5 ml of medium; 342 mCi/mmol), [2-^{14}C]uridine (0.5 μCi/5

ml of medium; 53 mCi/mmol), or [2-^{14}C]thymidine (0.5 μCi/5 ml of medium; 58 mCi/mmol). All labeled compounds were purchased from Amersham International Ltd., Amersham, England. After 1 hr incubation, the cells were rinsed twice with 5 ml of cold 0.9% saline. The cell layer was then immersed in cold 4% perchloric acid for 40 min to remove acid-soluble materials, rinsed successively with 80 and 100% ethanol, and anhydrous diethyl ether. After drying, the fixed monolayers were dissolved in 2 ml of 98% formic acid for 20 min. A 0.5 ml aliquot of the formic acid solution was counted in 10 ml of Aquasol-2 (New England Nuclear, Boston, Massachusetts, U.S.A.).

RESULTS AND DISCUSSION

The effect of varying concentrations of carbaryl on nucleic acid and protein syntheses in cultured human cells is illustrated in Fig. 1. None of the macromolecules appeared particularly sensitive to the action of the insecticide. The rates of the syntheses of the macromolecules were inhibited severely at a carbaryl concentration of 10^{-3} M. No significant inhibition of nucleic acid and protein syntheses by the pesticide was observed at concentrations between 10^{-7} M and 10^{-5} M.

As illustrated schematically in Fig. 2B, an antibiotic, anisomycin,[10] and alkaloids, tubulosine[11] and emetine,[12] which act by inhibiting the biosynthesis of protein, exhibit considerable inhibition of protein synthesis in intact HeLa cells at a concentration of 10^{-7} M and almost complete inhibition at 10^{-6} M. The compounds partially inhibit DNA synthesis, while RNA synthesis is virtually unaffected.

In contrast to these compounds, the effects of insecticides, chlordimeform and carbaryl, on nucleic acid and protein syntheses in cultured cells show quite different patterns as described in the previous paper[9] and in this note, respectively (Fig. 2A). No significant differences among the effects on DNA, RNA and protein syntheses were observed. The syntheses of the macromolecules were inhibited considerably at a concentration of 10^{-3} M. At lower dose levels, the syntheses of the macromolecules were virtually unaffected.

We have reported that carbaryl binds markedly to cell proteins.[2] Virtual ineffectiveness of the insecticide on the syntheses of the macromolecules suggests that we have to investigate other toxic effects which may be exerted by the high binding ability of this chemical to cellular proteins.

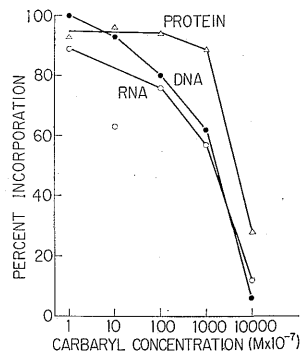

Fig. 1 Effects of varying concentrations of carbaryl on the syntheses of DNA, RNA and protein in cultured human embryonic lung cells.
The percentage incorporation shown is expressed relative to the controls.

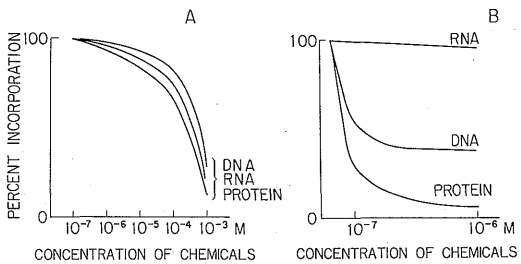

Fig. 2 Comparison of effects of varying concentrations of two insecticides (A) and three inhibitors of protein biosynthesis (B) on the syntheses of DNA, RNA and protein in cultured cells.
Figure 2A is drawn schematically from the results obtained in the experiments of chlordimeform[9] and carbaryl (this note). Figure 2B is drawn schematically from the results obtained in the experiments of anisomycin,[10] tubulosine[11] and emetine.[12]

REFERENCES

1) C. Declume & P. Benard: *Toxicology* **8**, 95 (1977)
2) M. Murakami & J. Fukami: *Bull. Environ. Contam. Toxicol.* **28**, 500 (1982)

3) J. Mitsuhashi, T. D. C. Grace & D. F. Waterhouse: *Entomol. Exp. Appl.* **13**, 327 (1970)
4) C. L. Litterst, E. P. Lichtenstein & K. Kajiwara: *J. Agric. Food Chem.* **17**, 1199 (1969)
5) B. C. Myhr: *J. Agric. Food Chem.* **21**, 362 (1973)
6) M. Yoshida, M. Onaka, T. Fujita & M. Nakajima: *Pestic. Biochem. Physiol.* **10**, 313 (1979)
7) M. Murakami & J. Fukami: *Bull. Environ. Contam. Toxicol.* **15**, 425 (1976)
8) M. Murakami & J. Fukami: *Bull. Environ. Contam. Toxicol.* **21**, 478 (1979)
9) M. Murakami & J. Fukami: *Bull. Environ. Contam. Toxicol.* **11**, 184 (1974)
10) A. P. Grollman: *J. Biol. Chem.* **242**, 3226 (1967)
11) A. P. Grollman: *Science* **157**, 84 (1967)
12) A. P. Grollman: *J. Biol. Chem.* **243**, 4089 (1968)

要　　約

ヒト培養細胞における核酸およびタンパク質生合成に対する carbaryl の影響

村上　誠，深見順一

ヒト胎児肺2倍体細胞 (HEL 299) の単層培養を殺虫剤 carbaryl を含む培地で培養し，細胞の核酸およびタンパク質生合成に対する影響を調べたところ，DNA，RNA およびタンパク質生合成のそれぞれに対するこの殺虫剤の効果には有意の差は見いだされなかった．10^{-3} M の濃度で，これら高分子生合成はいずれも強く阻害されたが，10^{-7} M～10^{-5} M のより低い濃度ではこれらの生合成はあまり影響を受けなかった．すでに著者らは carbaryl がこの細胞のタンパク質に強く結合することを報告しているが [*Bull. Environ. Contam. Toxicol.* **28**, 500 (1982)]，この結合から由来することが予想される carbaryl の毒性機構としては，生体高分子生合成阻害以外の機構を検討しなければならない．