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Synergistic Modulation of Lymphocyte Mitogenesis by Carcinogenic Xenobiotics

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The degree of danger posed by the production and dispersion of new substances into the environment is a constant source of concern. A considerable research effort has already identified a number of potential hazards, and assessment of safe levels of exposure are under constant review by various public health authorities.

Identified hazards include soluble arsenic compounds (product of metal smelting, cement manufacture etc) for which the risks of chronic subacute exposure in man appear to relate to As as a carcinogen (Yeh et al, 1968) and an immune modulator (McCabe et al, 1983). Similar risks are recognised for prolonged subacute exposure to chlorinated hydrocarbon insecticides, both as carcinogens (Epstein, 1977) and immune modulators, both in vivo (Street & Sharma, 1975) and in vitro, (Lee et al, 1979). McCabe et al (1984) have shown an increased incidence in leukemias in sugar cane farmers in Queensland, an occupation with sigificantly high exposure to these chemicals.

Modulation of cell division by potentially carcinogenic xenobiotics is of considerable interest as it may relate to the more subtle changes which stem cell populations must be presumed to undergo as a consequence of chronic low level exposure, and which manifest themselves eventually as an increased cancer frequency. Additionally the modulation of cells of the immune system in particular may indirectly contribute to an increased cancer frequency either through a reduction of immune surveillance as proposed by Burnet (1957), or through an increased incidence of viral infection.

Because the biochemical events leading up to and accompanying mitogenesis are multiple, the opportunity for xenobiotic subversion is similarly large. Also it is known that several significant biochemical events must occur synchronously or sequentially and within carefully prescribed limits before the cell can successfully complete mitogenesis. For these reasons the modulation of lymphocyte mitogenesis by very low levels of xenoiotics is of considerable interest.

Because of the possibility for synergic interaction between

different xenobiotics upon lymphocyte mitogenesis, and the potential importance of this cell system as an indicator for safe levels of xenobiotics in the environment, we have investigated the effects of combinations of xenobiotics upon in vitro lymphocyte mitogenesis.

MATERIALS AND METHODS

The UV lamp was a "Mineralite UVS-11" (Ultra-violet Products Inc. Calif). Chlorinated hydrocarbons were donated by Montrose Chemical Corp. Calif. Stock solutions were prepared in ethanol and appropriately diluted (at least x 10^3) with Eagles minimal essential medium (MEM) immediately prior to use. For the isolation of lymphocytes and the transformation asssays the conditions and methods were exactly as described previously (McCabe et al, 1983). Briefly, bovine lymphocytes were separated from whole blood by standard techniques using Ficoll-Paque. Mitogenesis in response to either phytohaemagglutinin(PHA) or concanavalin A (con A) was measured by the uptake of ^{3}H-thymidine, both in the presence and absence of varying concentrations and combinations of sodium arsenite, carbaryl, dieldrin, pp-DDT, lindane, 2:4D, chlordane, and UV light.

The assays were carried out using 6 replicate samples of 100 µl of lymphocyte suspensions which were incubated with the appropriate concentrations and mixtures of xenobiotics for an hour at room temperature prior to the addition of mitogen. Exposure to the UV was performed 75 mins prior to mitogen addition. The results were expressed as the mean ± standard deviation. For each agent the ratio of response of test/control was calculated, and where 2 agents were simultaneously incorporated, a synergy index (SI) calculated. SI was defined as the observed ratio of response, divided by the predicted response from an assumption that action of the two agents was additive. Thus synergy occurs when SI varies significantly from unity.

RESULTS AND DISCUSSION

The figure shows the effects of a range of concentrations of sodium arsenite, (which alone are suppressive of mitogenesis) in combination with UV radiation at 75 ergs/mm^2. The corresponding expected values (assuming no synergy) are also included. It can be seen that significant synergic interaction occurs between the two immunosuppressive treatments. The results were also analysed for interaction by analysis of variance, and a significant interaction effect (p 0.01) was found for these two modulators of lymphocyte cell division.

The table shows results for the assays of individual xenobiotics and various combinations of them. It can be seen that synergic interaction can express itself as hormesis (the stimulatory effect of sub inhibitory concentrations of a potential toxin) as well as inhibition. Synergy was found to be a frequent occurence among the random combinations of xenobiotics tested.

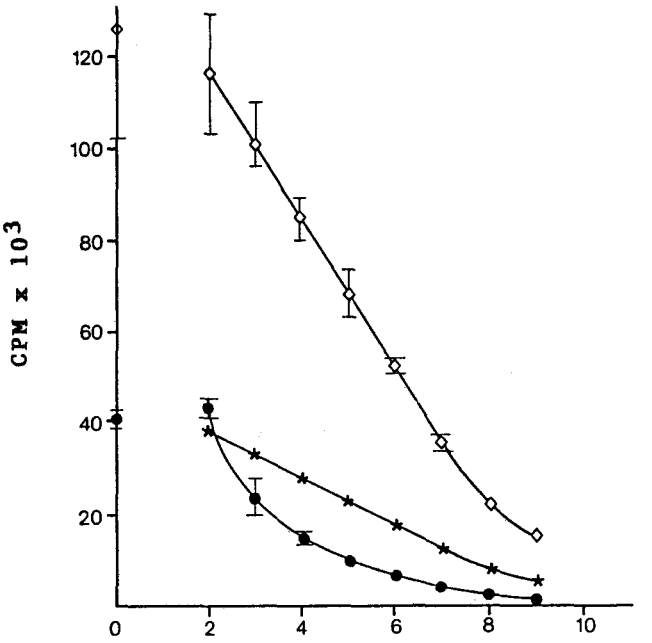

Figure 1. Combined effect of sodium arsenite and ultraviolet radiation (75 ergs/mm^2, 254nm) on lymphocyte mitogenesis stimulated by PHA.

—◇—◇— cells not treated with UV

—●—●— cells pretreated with UV, 15mins prior to the addition of sodium arsenite.

—*—*— values predicted for cells subjected to both UV and arsenite if the effect were additives.

Hormesis has been reported previously for lymphocyte mitogenesis in the presence of either sodium arsenite or arsenate (McCabe et al, 1983), and the results shown here additionally indicate that several of the herbicides and insecticides used will similarly show a stimulus of cell division at lowest concentrations with inhibition at higher concentrations. A similar effect can also be demonstrated for UV light (Castellanos et al, 1982).

Laughlin et al (1981) considers hormesis to be a "generalised aspect of environmental stress aetiology". Generally the results obtained here indicate that for any combination of agents each at an inhibitory concentration, the two acting together are more inhibitory than can be predicted from their independent effects (ie they are synergic). For combinations where one of the agents is at a stimulatory concentration, while the other is at an inhibitory level, the two together can show either an excessive stimulation or depression, however the magnitude of the effect is

Table 1. Effects of xenobiotics and agents alone and in combination on the ^{3}H-thymidine uptake of bovine lymphocytes

Xenobiotics and agents	^{3}H-thymidine Uptake (CPM)	Ratio of test/ control	Synergy index
(i) Concanavalin A, 120µg/ml			
Control (no additives)	13,696 ± 2436	-	-
Carbaryl ($2x10^{-5}M$)	23232 ± 3141	1.70	-
Sodium arsenite ($5x10^{-7}M$)	14105 ± 2418	1.03	-
Carbaryl ($2x10^{-5}M$) + Sodium arsenite ($5x10^{-7}M$)	29521 ± 3723	2.16	1.23
Carbaryl ($4x10^{-5}M$)	2172 ± 198	0.16	-
Sodium arsenite ($10^{-6}M$)	9467 ± 696	0.69	-
Carbaryl ($4x10^{-5}M$) + Sodium arsenite ($10^{-6}M$)	242 ± 31	0.02	0.16
(ii) PHA 1 mg/ml			
Control (no additives)	33723 ± 5714	-	-
Carbaryl ($5x10^{-6}M$)	29418 ± 4983	0.87	-
UV light;75ergs mm^{-2} for 5secs	30274 ± 4706	0.90	-
Carbaryl + UV light(as above)	17657 ± 2384	0.52	0.67
(iii) PHA 0.5 mg/ml			
Control	29611 ± 4083	-	-
Carbaryl ($5x10^{-6}M$)	54822 ± 6371	1.85	-
UV light 75ergs mm^{2} for 10secs	6085 ± 795	0.21	-
Sodium arsenite ($5x10^{-7}M$)	36281 ± 4113	1.23	-
UV + Carbaryl (as above)	31678 ± 4074	1.07	2.81
UV + Sodium arsenite(as above)	1785 ± 249	0.60	2.39
(iv) Concanavalin A 120µg/ml			
Control	85521 ± 9941	-	-
UV alone(75ergs $mm^{-2}$for 5secs)	90086 ± 14371	1.05	-
Carbaryl ($2x10^{-6}M$)	105,009 ± 16811	1.23	-
Dieldrin ($10^{-5}M$)	84955 ± 9390	0.99	-
UV + carbaryl(as above)	120752 ± 21311	1.41	1.09
UV + dieldrin (as above)	102243 ± 15104	1.20	1.14
(v) PHA 1 mg/ml			
Control	14941 ± 2061	-	-
UV light(75 ergs mm^{-2} 5secs)	17173 ± 2283	1.15	-
PP-DDT ($10^{-5}M$)	25737 ± 2894	1.72	-
Lindane ($5x10^{-6}M$)	22831 ± 2810	1.53	-
2.4D ($10^{-5}M$)	29453 ± 3010	2.03	-
Chlordane ($10^{-5}M$)	26029 ± 3418	1.74	-
Chlordane ($5x10^{-5}M$)	8566 ± 1016	0.57	-
UV + PP-DDT (as above)	11828 ± 1513	0.79	0.40
UV + 2:4D (as above)	13866 ± 1019	0.43	3.94
UV + Chlordane ($10^{-5}M$)	15420 ± 2170	1.03	0.52
UV + Chlordane ($5x10^{-5}M$)	6986 ± 1103	0.47	0.71

generally not additive. Finally where the concentrations of both agents is selected so that each is independently stimulating, then the two together can again show either an excessive stimulation or even a suppression.

A lack of predictibility supports the concept of Gilbert (1974) that cell proliferation is dependent upon the successful integration of several biochemical oscillations within the cell. The unpredictibility of xenobiotic effects when used in combination may relate to modulation of more than one system within the cell, each of which may be rate limiting for mitogenesis.

The results suggest that synergy between mitogenic modulators may be commonplace, and that considerable caution should be exercised in proposing permissible levels of metabolically active xenobiotics. Compounds accepted as innocuous at one concentration, may become less so when in combination with other substances. This should be considered as new xenobiotics are added to the environment.

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