Vitamin C Mediated Minimisation of Malathion and Rogor Induced Mitoinhibition and Clastogeny

Q. Hoda, S. Bose and S. P. Sinha

University Department of Zoology, Bhagalpur University, Bhagalpur-812007, India

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The roles of ascorbic acid (Vitamin C) as antimutagen, anticlastogen and anticarcinogen have been evaluated in various test-systems (Shamberger 1984). Under *in vitro* condition, it has been found to decrease the incidence of carcinogen-induced gene mutations (Guttenplan 1977, Raina and Gurtoo 1985), sister-chromatid exchanges (Galloway and Painter 1979), and chromosomal breakages (Gebhart *et al.* 1985, Shamberger *et al.* 1973). In humans, ascorbic acid prophylaxis has been reported to decrease chromosomal changes in peripheral lymphocytes of coal-tar workers occupationally exposed to polycyclic aromatic hydrocarbons and benzene (Sram *et al.* 1983). It (Vitamin C) has been shown to be anticarcinogenic in rodents previously treated with UV radiation, benzopyrene and nitrosocompounds (Ames 1983). The anticlastogenic property of this vitamin has been also found in *in vivo* mammalian systems (Sinha and Bose 1989, Hoda and Sinha 1990).

The present work is an attempt to evaluate such a role of ascorbic acid against pesticide-induced mitotic inhibition and clastogeny in the common tissue, i. e., onion-root tip cells.

Materials and methods

The role of ascorbic acid was checked when administered concurrently, as well as before and after the pesticide treatment. The doses of the pesticide ranged from the one to which the population is generally exposed to and upto that considered as maximum tolerable dose. (A) *Pesticide used*: Two commonly used formulations of organophosphorous pesticide, viz., Malathion (O-O-dimethyl phosphorodithioate of diethyl mercaptosuccinate, Cyanamid Company, India, which contains the active principle and emulsifiers in equal volume) and Rogor (O-O-dimethyl-S (N-methyl carbomoyl methyl) phosphorodithioate, Rallis-Tata, India, which contains 30% of the active principle and rest of emulsifiers and other surface active agents) were selected for the present work. The concentrations selected for both the pesticides were 0.01%, 0.02%, 0.04%, 0.08% and 0.16% (V/V) of the marketed formulation which in the case of Malathion amounts to 50, 100, 200, 400 and 800 ppm and in the case of Rogor to 30, 60, 120, 240 and 480 ppm.

(B) *Vitamin used*: The source of vitamin C was synthetic injectable preparation of the sodium salt of L-ascorbic acid manufactured by Roche India Ltd, with trade name "Redoxon". The general recommended dose of the preparation for adult human being is 500 mg/day. The dose of the vitamin used in the present study was 0.005%, which is proportional to the normal human dose.

Onion (*Allium cepa*) bulbs were grown over jars in six groups, each containing five bulbs. When healthy young roots could come out to a length of about 1 cm, they were subjected to treatment in two changes each of 24 hr duration (Table 1). At the end of the second treatment in each group, three roots per bulb were cut, fixed in aceto-methanol (1:3) for 24 hr and then preserved in 70% ethanol. Acetocarmine-stained squash preparations were made and the slides were screened for counting the number of dividing cells and locating abnormalities

in them. In each set of experiment, the effect was observed in the count of at least three thousand cells in foci selected randomly following the method suggested by Bhalla *et al.* (1973).

Results

(A) *Effects on mitotic-index:*

This index was represented in the form of relative division rate (RDR) calculated by the formula given below:

$$\text{RDR (\%)} = \frac{\frac{\% \text{ of dividing cells in treated bulbs}}{100 - \% \text{ of dividing cells in control bulbs}} \times 100}{\% \text{ of dividing cells in control bulbs}} \times 100$$

Negative value of RDR indicated mitosis inhibition.

Table 1. Mode of treatment with Vitamin and Pesticide

	1st Treatment for 24 hours	2nd Treatment for 24 hours	Abbreviated in the text
6 groups (I to VI) of bulbs grown on tap water	Groups I to III on fresh tap water	I. Tap water	Control (C)
		II. Pesticide	P*
		III. Pesticide + Vitamin C	P+VC (concurrent treatment)
	Group IV on Vitamin C	IV. Pesticide	VC→P (Pre-treatment)
	Groups V and VI on pesticide	V. Vitamin C	P→VC (Post-treatment)
		VI. Tap water	REC (Recovery)

P* was either Malathion (M) or Rogor (R) as the case may be.

(i) *Effect of Malathion:*

Significant decreases in RDR were noted (Fig. 1) in pesticide treated bulbs, and mitosis completely halted at 800 ppm dose of the pesticide. In cases where pesticide and vitamin were administered concurrently (M+VC), decreases in RDR were still observed but their intensity was much less. Pre-treatment with vitamin could not produce any significant protective effect against the mitoinhibitory effect of the pesticide (VC→M). However, at the lower doses (50 and 100 ppm) it showed minimisation of the inhibition, though the decrease in RDR was still significantly lower than in the group treated with the pesticide only. When vitamin C was given at the end of the pesticide-treatment (M→VC), the pesticide-induced inhibition markedly decreased at the three lower doses; and surprisingly at 400 ppm dose of the pesticide, the RDR value showed increases over the control value (RDR=+5.16). Even the mitosis which had completely ceased at 800 ppm dose of the pesticide, got started in M→VC group. The improvement in the division rate (as observed in M→VC group) may be the result of the withdrawal of the pesticide and/or of some protective effect of the vitamin itself. The recovery experiments (REC), no doubt, showed some improvement in division rate but its impact was not enough to discard the protective role of the vitamin.

(ii) *Effect of Rogor:*

Like Malathion, Rogor (R) also decreased the division rate at all its concentrations and the decrease appeared to be dose-dependent (Fig. 2). In the R+VC group, the RDR at the two lower concentrations (30 and 60 ppm) was almost nearer to the control value (RDR=

Fig. 1. Relative Division Rate (RDR in %) in the root-tip cells of onion grown over different concentrations of Malathion with various modes of treatment with Vitamin C.

Fig. 2. Relative Division Rate (RDR in %) in the root-tip cells of onion grown over different concentrations of Rogor with various modes of treatment with Vitamin C.

−0.73, −0.92). However, at the two higher doses, significant decrease in RDR still persisted. Post-treatment with vitamin C (R→VC) was less effective in checking the inhibition at the three lower doses, but more so at the two higher ones (RDR=0.00, −2.03). In the VC→R group, the cell division completely ceased at the three higher doses of the pesticide, but at the two lower doses (30 and 60 ppm) some improvement over the mitotic inhibition was found which was well marked at 60 ppm dose of the pesticide. Data from recovery experiment (REC) also confirm the protective role of the vitamin against the pesticide induced mitoinhibition.

Fig. 4. Relative abnormality Rate (RAR in %) in the root-tip cells of onion grown over different concentrations of malathion with various modes of treatment with Vitamin C.

Thus, it appears that the vitamin C produces some protective effect against pesticide induced mitoinhibition in root-tip cells and also that its concurrent treatment is more efficacious than any other mode of its supplementation. Results from analysis of variance (ANOVA) also confirm to this (at $P < 0.05$).

(B) Effect on mitotic chromosomes:

Pesticide-induced chromosomal abnormalities can be broadly put into two categories: (a) chromosome/chromatid breaks and their subsequent re-arrangements manifested as fragmentation, laggard, clumping and anaphase bridges, and (b) miscellaneous types such as multipolar spindles, polyploidy, disturbed cytokinesis, multinucleated cells and precocious separation of sister chromatids (Fig. 3).

Fig. 3. Chromosomal abnormalities induced by the pesticides in onion root-tip cells. A-D, fragmentation, E-G, laggards, H-J, anaphase bridge, K, L polyploidy, M-O, multipolar spindle, P-S, disturbed cytokinesis ($\times 1000$).

(i) *Effect of Malathion:*

The incidence of chromosomal abnormalities significantly increased upon Malathion treatment and was directly proportional to the dose of the pesticide used (Fig. 4). When the vitamin was administered concurrently with the pesticide, no abnormalities could be seen at the 50 ppm (lowest) dose of the pesticide; at the higher doses also significant decreases in the relative abnormality rate (calculated by the formula analogous to that used for RDR values) were observed. Pre- and post-treatments with vitamin also minimise the abnormality rate. In the recovery experiment, increase in the abnormality rate was observed, which suggests that the vitamin C, at its own, is capable of minimising pesticide-induced clastogeny and other related abnormalities.

Fig. 5. Relative Abnormality Rate (RAR in %) in the root-tip cells of onion grown over different concentrations of Rogor with various modes of treatment with Vitamin C.

(ii) *Effect of Rogor:*

The pattern of chromosomal abnormalities produced by Rogor was similar to that observed in the case of Malathion (Fig. 5). A definite trend of dose-dependence of abnormality rate was found. The various modes of supplementation of vitamin C and the recovery experiments showed a minimising effect as observed in the case of Malathion.

In order to understand the relative minimisation rate of break-related and miscellaneous types of abnormalities by the vitamin, the sum total of incidence of these abnormalities scored at all the doses of pesticide as well as vitamin treated bulbs were compared (Table 2). It appears that most (around 80 to 90%) of the abnormalities caused by the pesticide are break-related, and as expected, most of the vitamin mediated (concurrent, pre- or post-treatment) decreases in their frequency were mainly due to decrease in the incidence of these very (break-related) damages. The result is strongly suggestive of the anticlastogenic nature of vitamin C.

Relationship between Mitotic index and RAR:

The mitotic-index and abnormality rate as observed during the various modes of treatments with pesticide and vitamin showed negative correlation with each other (Table 3) except in the VC→R variant. This value was much higher in concurrent and pre-treatment variants with Malathion, but with Rogor in the concurrent variant only. The absence of correlation was noted only in those eventualities where the vitamins were used for post-treatment.

Discussion

The result presented suggest that mitotic inhibition as well as clastogeny induced by the pesticide can be appreciably minimised by the vitamin C and the concurrent treatment with the vitamin is more effective than any other mode of its supplementation.

Table 2. Cumulative abnormality rate (%±SE) for all concentrations of pesticide and vitamin administration in root-tip cells of onion

Experimental groups	Abnormality Types (%±SE)			Experimental groups	Abnormality Types (%±SE)		
	Total	Break-related	Miscellaneous		Total	Break-related	Miscellaneous
M	4.68±0.91	4.11±0.85	0.57±0.32	R	7.32±1.10	6.60±1.04	0.72±0.35
M+VC	1.56±0.37	1.32±0.34	0.24±0.14	R+VC	4.47±0.61	3.07±0.51	1.40±0.34
VC→M	2.52±0.60	2.52±0.60	nil	R→VC	8.61±0.73	4.44±0.53	4.17±0.52
M→VC	5.95±0.57	3.27±0.43	2.70±0.43	VC→R	4.16±0.75	2.87±0.63	1.29±0.42
REC	9.35±0.99	5.38±0.77	3.97±0.77	REC	11.80±1.07	7.57±0.88	4.23±0.67

Table 3. The coefficient of correlation (r) between the mitotic-index and relative abnormality rate as observed in different variants of experimentation

Treatment type	r	Treatment type	r
M	-0.65	R	-0.88*
M+VC	-0.99*	R+VC	-0.99*
M→VC	-0.49	R→VC	-0.66
VC→M	-0.99*	VC→R	+0.98*
REC	-0.88*	REC	-0.84*

* indicates significant correlation at 5% level of significance.

A successful completion of mitosis is a result of integrated harmonious functions of the cell as a whole. Presence of pesticide molecules or their derivatives may disturb the synthesis or the structure of DNA (Mitchison 1977) or may cause inhibition of cell's entry into mitosis (Kihlman 1966) either by delay in onset of G₂ (Gelfant 1963, Evans and Scott 1964, Waechter and Baserga 1982) and/or S-phases (Mitchison and Creanor 1971). Arrest of division would take place in most of these eventualities.

Pesticide-mediated alkylation of not only guanine but also of thymine, cytosine and uracil (Datta and Chedda 1980) and subsequent base-replacements is perhaps the most common mechanism of mutagenesis in micro-organisms, cells in culture or in experiments carried out in *in vitro* where almost unmodified pesticide molecules are available for causing the damage. In the higher organisms, the pesticides undergo a series of enzyme-mediated biotransformations and it is these biotransformed forms that have to be mutagenic. According to Hutson (1982), the fats and lipids present in the body/cells combine with pesticide metabolites to produce a conjugate type of chemical and it is believed that these conjugates have the real mutagenic nature.

Vitamin C is well known to hydrolyse lipids and cholesterol (Rees and Slaten 1987, Fuzimoto *et al.* 1988, Wafer and Sies 1988, Koul *et al.* 1989). Hence the antimitoinhibitory and anticlastogenic effect of the vitamin C, as observed in the present case, may be due to this vitamin C-mediated hydrolysis of the lipid/cholesterol, thus minimising the chances of the formation of mutagenic conjugate of pesticide with them.

Summary

Administration of vitamin C, either concurrently or as pre-and post-treatment to the pesticide exposure, was very much helpful in minimising mitoinhibition and clastogeny induced by two organophosphorus pesticides, Malathion and Rogor. The concurrent treatment with vitamin C was more effective than the two other modes of its supplementation.

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