Hyperthermic Modulation of the Seedling and Genetic Damage Produced by a Chemical Mutagen in Plants

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Hyperthermia alone and in combination with ionizing radiations has in recent years been shown to be useful in selective killing of tumour cells. A considerable amount of work is presently being carried out to find out its efficacy as an adjuvant to current therapeutic techniques in cancer therapy. Hyperthermic treatment of mammalian cells within the range of 42–48°C causes cell killing (Westra and Dewey 1971, Plazer and Heidelberger 1973); blockage of cell progression through cell cycle (Gerweck and Dewey 1975); chromosomal aberrations (Dewey *et al.* 1971) and an increased sensitivity to X-irradiation (Ben-Hur *et al.* 1974, Henle and Leeper 1976). There is, however, no information whether response of cells to chemical mutagens is also modified in the same way nor has it been verified whether these observations can hold true in the case of higher plant systems also. The present investigations were conducted to provide information and possible insight into the mode of action of hyperthermia alone and in combination with a chemical mutagen in barley seeds.

Materials and methods

Dormant caryopsis of barley (*Hordeum vulgare* L.) var. Himilaya formed the test system. Solution of mutagen 1, 3 propane sultone (PS), Sigma, USA, was freshly prepared in distilled water and 4 and 8 mM solutions were generally used in the experiments. For hyperthermic treatments, seeds were placed in preheated ovens at 35°C or 45°C for specific periods of time either immediately before, concomitant or immediately following PS treatments. Details have been given in each experiment.

Treatments were carried out in three replications. In each case 50 seeds were soaked in 25 ml of the test solution and treatments terminated by giving them a quick rinse in running water, planted on moist filter papers in sterile plastic petri dishes and allowed to germinate. Seedling height was measured when primary leaf had stopped further growth. Root tips about 1 cm long were fixed in acetic acid: alcohol (1:3) and squash preparations prepared according to Feulgen technique. At least 100 cells in each treatment were scored to assess cytological damage.

Results

Data presented in Table 1 show that a hyperthermic pretreatment (45°C) reduces the seedling injury from 36% to a mere 8%. Similarly 20.9% root injury

Table 1. Effect of hyperthermic pretreatments on the biological activity of propane sultone

Treatment		Seed germination % of control	Seedling height		Seedling injury %	Root length		Root injury %
Pre	Post		cm	% of control		cm	% of control	
Control		100	13.35	100	0	5.4	100.00	0
45°C 3 hr	PS, 4 mM 3 hr	100	12.25	91.76	8.24	5.6	104.07	0
PS, 4 mM 3 hr	45°C 3 hr	97	7.60*	56.92	43.07	3.6*	67.96	32.04
PS, 4 mM 3 hr	—	89	8.55*	64.04	36.02	4.3*	79.07	20.93
45°C 3 hr	—	95	8.64*	64.72	35.28	2.9*	54.07	46.29

* Significantly different from the control at 0.05 probability.

Table 2. Effect of hyperthermic pretreatments on the radiomimetic activity of propane sultone in barley root meristematic cells

Treatment		Cells scored at metaphase	Abnormal cells scored	Aberrations per cell		Total I and II	Mitotic index
Pre	Post			Breaks (I)	Exchanges (II)		
PS, 45°C 1/2 hr., 4 mM 3 hr	—	100	16	0.18	0.105	0.285	6.3
45°C 3 hr	—	100	4	0.04	—	0.040	4.7
45°C 3 hr	PS, 4 mM 1/2 hr 45°C 3 hr	100	12	0.08	0.060	0.140	3.4
PS, 4 mM 1/2 hr 35°C, 1/2 hr	—	100	13	0.10	0.12	0.220	3.1
35°C 3 hr	—	100	21	0.234	0.214	0.448	7.1
35°C 3 hr	PS, 4 mM 1/2 hr 35°C 3 hr	100	7	0.025	0.074	0.099	7.9
PS, 4 mM 1/2 hr	—	100	13	0.096	0.104	0.200	6.0
	35°C 3 hr	100	17	0.146	0.244	0.390	5.9

produced by PS was totally eliminated by hyperthermic pretreatment. However, hyperthermic post treatment did not have similar effects either with respect to seedling injury or root injury.

To study the effect of hyperthermic pretreatment on the chromosome breaking activity of PS, two hyperthermic treatments (35°C and 45°C) for 3 hours were administered. It can be seen from Table 2 that total aberrations per cell were reduced by almost one half and there was also a significant reduction in number of abnormal cells. Thus, it is clearly brought out by the data in Tables 1 and 2 that hyperthermic pretreatments provide a strong protection against the mutagenic damage produced by PS in terms of seed or seedling injury, root injury as well as chromosomal aberrations.

Table 3. Effect of hyperthermic treatments on the biological activity of propane sultone (8 mM, 2 hr, 30°C)

Treatment		Seedling height, cm	Increase over control %	Root length cm	Increase over control %	Seed germination %	Increase over control %
Pre	Post						
PS	—	3.65	—	0.81	—	48	—
45°C 1 hr	PS	5.76*	57.26	2.06	155.1	72.5*	51.4
45°C 2 hr	PS	5.71*	56.44	1.95	140.7	78.4*	63.7
45°C 3 hr	PS	6.04*	65.48	2.03	150.6	71.2*	48.6
45°C 4 hr	PS	4.80*	31.51	1.68	107.4	61.3	27.9

* Significantly different from the control at 0.05 probability.

Table 4. Comparative effect of various durations of hyperthermic pretreatments on the radiomimetic activity of propane sultone (8 mM, 3 hr, 30°C)

Treatment		Cells scored at meta-phase	Abnormal %	Aberrations %		Total aberrations I and II %	Reduction over control %	Mitotic index
Pre	Post			Breaks (I)	Exchanges (II)			
PS	—	100	32	0.975	0.025	1.010	—	7.4
45°C 1 hr	PS	100	38	0.596	0.016	0.612	39.40	5.6
45°C 2 hr	PS	100	26	0.274	0.016	0.290	71.29	4.9
45°C 3 hr	PS	100	11	0.237	0	0.237	76.53	4.1
45°C 4 hr	PS	100	23	0.435	0	0.435	56.93	3.9

To estimate the period of exposure to hyperthermia necessary to give protection, seeds were pretreated for 1 to 4 hours at 45°C before exposing them to PS treatment. Data are presented in Tables 3 and 4. Protection was evident following all the hyperthermic pretreatments, a 3 hour pretreatment showing the maximum protection. Prolonged posttreatments (5 hr) also seem to offer protection (Table 5).

Weather hypothermic treatments could also have similar effects an experiment was conducted treating seeds to 5°C for three hours period prior to or following PS treatments. Low temperature treatment by itself had no effect nor did it increase or decrease the activity of PS (Table 6).

Table 5. Effect of hyperthermic post-treatments on the PS induced seed lethality and seedling injury

Treatment		Seed germination %	Seedling height		Protection %
Pre	Post		cm	% of control	
PS, 8 mM, 2 hr 45°C	—	48.0	3.65	27.34	0
PS, 8 mM/2 hr	—	40.4	3.17	23.74	—
PS, 8 mM/2 hr	45°C, 1 hr	24.4	2.15	16.10	—
PS, 8 mM/2 hr	45°C, 2 hr	54.3	4.47	33.48	22.45
PS, 8 mM/2 hr	45°C, 5 hr	64.6	5.15	38.57	41.07

Table 6. Effect of hypothermic pretreatment on the biological activity of propane sultone

Treatment		Seed germination % of control	Seedling height		Seedling injury %	Root length		Root injury %
Pre	Post		cm	% of control		cm	% of control	
Control	—	100	13.35	100.00	0	5.40	100.00	0
5°C, 3 hr	PS 4 mM, 3 hr	85	9.35*	70.03	29.96	3.03	56.11	44.44
PS, 4 mM 3 hr	5°C	80	5.64*	42.25	57.75	2.31*	42.77	57.22
PS, 5°C 4 mM, 3 hr	—	70	7.11*	53.26	46.74	2.66*	49.26	49.26
5°C 3 hr	—	93	12.91	96.70	3.29	4.46	82.59	17.41

* Significantly different from control at 0.05 level of probability.

Discussion

The data gathered through these experiments show that hyperthermic pretreatments reduce the biological damage produced by PS, which is reflected in various parameters like seed lethality, seedling injury, root injury as well as chromosomal aberrations. Both hyperthermia as well as PS when applied alone produced considerable genetic damage. However, to understand this paradoxical situation we may have to explain the mechanism of PS action first. The activity of PS is a result of a specific chemical reaction between the mutagen and DNA (Osterman-Golkar and Wachtmeister 1976). It has been clearly shown that PS reacts with cellular macromolecules producing complexes made of nucleic acid and proteins (Zeldin *et al.* 1975) and S-phase of cell division is the most sensitive stage for these reactions (Kaul 1969). For these reactions to take place it is also necessary that cells are in an active metabolic state.

It has been reported by many workers that the progression of cell cycles and duration of its various phases are highly sensitive to temperature stress. Both at low temperature (3°–4°C) or high temperature (30°C) the duration and the mitotic cycle time decreases (see Rost 1977). Obviously, at such high temperature (e.g. 45°C) the cell proliferation is reduced to almost zero (Burholt and Van't Hof 1971). The key to explaining the protective effect of temperature may lie in this fact, as at higher temperature the mitotic index falls very rapidly and target molecules for mutagenic reaction are not available. Hence, no real genetic damage takes place.

In an analogous situation it was shown by Caldecot and Smith (1952) that dry barley seeds exposed to heat shock of about 80°C for half an hour either before or immediately after X-irradiation reduced induced chromosome breakage frequency by about one half. This observation was later on confirmed by many independent studies using dry seed system (Caldecot 1961, Konzak *et al.* 1961) though no satisfactory explanation for thermal restoration was provided. It was also shown that storage effect in dry seed irradiation was strongly temperature dependent (see Caldecot 1961).

Summary

Experiments were conducted to find out the influence of hyperthermic treatments on the biological activity of 1,3-propane sultone. Hyperthermic (35–45°C) pretreatments for 1–4 hours gave significant protection against the biological damage produced by the mutagen. Seedling injury was reduced from 36 to 8% and root injury totally eliminated. Chromosome aberrations in root tip cells were reduced by one half. Hyperthermic post-treatments, however, were ineffective.

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