Inducible protective processes in animal systems. IX. Potentiality of adaptive response by nicotinamide in MMS adapted meiotic cells of grasshopper Poecilocerus pictus

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Introduction

The organisms as diverse as bacteria, animals and plants, when exposed to low doses of DNA damaging agents, respond by adaptive response, which renders them resistant to mutagenic, clastogenic and cell killing impacts of high doses of the same agents or different agents. This in-
ducible DNA repair pathway was discovered by Samson & Cairns (1977) in *E. coli*. There are many reports on adaptive response using physical and chemical agents in prokaryotes (Athir et al., 1984; Hadden et al., 1983) *in vitro* eu-karyotes (Ikushima et al., 1996; Kaina, 1982; Kleczkowska & Althaus, 1996; Lankinen & Vilpo, 1997; Mudigal-Bujaidar et al., 1994; Nikolai et al., 1998; Nikolova & Huttner, 1996; Olivere & Bosi, 1990; Olivere et al., 1984; Samson & Schwartz, 1980; Sankaranarayanan et al., 1989; Shadley & Wolff, 1987; Wolff, 1996; Zhang, 1995) and plants (Baranczewski et al., 1997; Rieger et al., 1982; 1990). We also have reported such cytogenetic adaptive response in *in vivo* grasshopper *Poicilocerus pictus* and mouse (Riaz Mahmood & Vasudev, 1990; 1991; 1992; 1993; Riaz Mahmood et al., 1996; Vasudev et al., 1997) by using alkylating agents viz. ethyl methane sulfonate and methyl methane sulfonate.

In spite of vast literature on adaptive response, the molecular mechanism of adaptive response remains obscure. Many DNA repair enzymes such as 0^6^-methyl guanine methyl transferase, poly (ADP-ribose) polymerase (PARP) etc. have been implicated. Much attention has been laid on PARP which is involved in DNA plasticity related phenomena viz. DNA repair, carcinogenesis, cell proliferation, gene expression, etc. (Così & Marien, 1998). In response to DNA damage PARP synthesizes poly (ADP-ribose) polymers by utilizing cellular NAD+ as substrate (Così & Marien, 1998; Così et al., 1996) and PARP is also activated by DNA strand breaks (Chatterjee & Berger, 1994; Cleaver & Morgan, 1991; Cleaver et al., 1983; De Murcia & De Murcia, 1994; Kleczkowska & Althaus, 1996).

In view of these evidences, we wanted to test the involvement of PARP in adaptive response in *in vivo* grasshopper *P. pictus*. Hence, nicotinamide as an inhibitor of PARP (Purnell & Whish, 1980) has been employed in the present to study the results of the test.

**Material and methods**

**Animals**

*Poicilocerus pictus*, a short horned grasshopper (Acrididae: Orthoptera) that feeds on *Calotropsis* leaves was employed in the present studies. Male individuals weighing 2.5 g were collected from the localities of Mysore city and maintained in the laboratory for 2-3 days until use.

**Chemicals**

The monofunctional-alkylating agent MMS (CAS NO:66-27-3) and nicotinamide (CAS NO 98-92-0) were obtained from Sigma Chemical Company (USA). MMS and Nicotinamide were dissolved in 0.4% NaCl and distilled water respectively to obtain required concentrations. 50 µL of the fixed concentration was injected into the abdomen of the animal between the third and forth segments. Each time freshly prepared solutions of agents were used. The conditioning and challenging concentrations of MMS were established in the previous experiments with *P. pictus*, where the used concentrations were 0.012 M nd 0.048 M MMS as conditioning (L) and challenging (H) doses respectively (Vasudev et al., 1997; 1998). From the pilot toxicity experiments 5 mM nicotinamide was selected.

**Treatment schedule**

**Nicotinamide inter-treatment**

Nicotinamide inter-treatment was given between conditioning and challenging treatment. A 2 h time lag has been used as in the earlier experiments (Vasudev et al., 1997). Nicotinamide was given 1 h after conditioning dose and 1 h later they were challenged with challenge dose of MMS (conditioning-1 h-nicotinamide-1 h-challenging).

**Nicotinamide pre-treatments**

The animals were treated with Nicotinamide 2 or 4 h earlier to conditioning dose; 2 h later they were challenged with challenge dose (nicotinamide-2 or 4 h-conditioning- 2 h-challenging).

**Nicotinamide post-treatments**

Nicotinamide was given 2 or 4 h after the combined treatment (conditioning-2 h-challenging-2 or 4 h-nicotinamide).

In all these experiments, the control group received 0.4% NaCl solution only. After challenge treatment animals were sacrificed at 12, 24, 36 and 48 h recovery times. Regular Heidenhain’s iron haematoxylin testes squash chromosome preparations were made following the procedure of Riaz Mahmood & Vasudev (1990). Frequencies of individual anomalies with respect to the stages of meiosis (Metaphase I, Anaphase I, Metaphase II, Anaphase II) were tabulated by scoring chromosome stickiness, stickiness and clumping, fragments, bridges, pseudo-bridges and laggards. A minimum of two experiments was conducted using four animals in each treatment schedule. Analysis of variance was used to compute the data.
Results and discussion

The results of present studies were similar those observed in the earlier experiments wherein, the conditioning and challenging dose of MMS significantly induced \((p < 0.05)\) chromosomal anomalies in meiotic cells of grasshopper \(P.\) pictus (Vasudev et al., 1997; 1998) when compared to controls. In the combined treatment (conditioning-2 h-challenging) there was reduction in the yield of chromosomal anomalies compared to challenge treatments at all recovery times tested (Figs 1–3). This decrease in the yield of chromosomal anomalies was due to induction of adaptive response by low dose of clastogen viz. MMS, which might be caused by induction of certain DNA repair enzymes responsible for the repair of alkylation damage. PARP is one of the DNA repair enzymes implicated in adaptive response. Hence, in the present studies when nicotinamide an inhibitor of PARP has been employed to understand the involvement in MMS treated grasshopper meiotic cells, one would expect increased chromosome anomalies after inter-treatment. This is because of the fact that nicotinamide inhibits the activity of PARP to an extent of 89% (Sim et al., 1982) and in turn interferes in DNA repair. Contrary to this, in the present investigations, the inter treatment of nicotinamide has resulted in significant reduction in chromosomal anomalies compared to challenge and combined treatment (Fig. 1). Similar results were observed in both pre- and post-treatment experiments of nicotinamide (Figs 2, 3). Further the same levels of chromosomal anomalies were observed within 48 h of post-treatment. This may occur because of the same amount of repair enzyme/s available at that particular duration. Albeit, from these results it can be said that nicotinamide enhanced the MMS-induced adaptive response. Consistently, we have recently reported the enhancement of adaptive response by nicotinamide in EMS-treated grasshopper meiotic cells and mouse bone marrow cells ( Guruprasad et al., 2000; Guruprasad & Vasudev, 2000; Vasudev et al., 1999). The results of pre-treatment experiments are an example of cross-adaptation as has been reported earlier (Vasudev et al., 1999). The decreased chromosomal anomalies due to different nicotinamide treatments may be caused by non-involvement of PARP in adaptive response or some other pathways through NAD+. It has been shown that the presence of NAD+ causes inhibition of \(Ca^{++}/Mg^{++}\) endonuclease, which fragments DNA (see Kladman et al., 1996). The data with EMS (in press) and the results with MMS (unpublished) show constancy of NAD+ in the cellular pool. This constancy of NAD+ may re-
result in the non-activity of endonuclease, and enhanced the adaptive response. In agreement with this nicotinamide and other PARP inhibitors are reported to prevent NAD+ depletion (De Múrcia & De Múrcia, 1994; Lindahl et al., 1995). Nicotinamide also acts as anti-oxidant (Kamat & Devasagayam, 1999), anti-inflammatory (Pero et al., 1999), anti-diabetic (Kolb & Burkart, 1999), and anti-carcinogenic (Ludwig et al., 1990) agent. Furthermore, there are reports on enhancement of DNA repair by inhibitors of PARP including nicotinamide (Bohr & Klenow, 1981; Cleaver et al., 1985). The potential of adaptive response may be another role of nicotinamide.

There are evidences in literature to show that PARP is not involved in adaptive response. The prokaryotes and lower eukaryotes devoid of PARP (Rhun et al., 1998), or cell extracts depleted of PARP or the PARP knock out mice (Wang et al., 1995) carry out DNA repair process efficiently. Ding et al. (1992) and Caria et al. (1997) have shown the existence of DNA repair process in the absence of PARP. The evidence for the latter hypothesis is that there is a synthesis of poly (ADP-ribose) polymers in the absence of PARP in DNA repair (Melissa et al., 1998).

Contrary to these, there are evidences of induction of chromosomal aberrations, SCEs (Cate-


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