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Suppression of alkylating agent induced cell transformation and gastric ulceration by low-dose alkylating agent pretreatment

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ABSTRACT

Exposure to mild stress by chemicals and radiation causes DNA damage and leads to acquired stress resistance. Although the linear no-threshold (LNT) model of safety assessment assumes risk from any dose, evidence from radiological research demonstrates a conflicting hormetic phenomenon known as the hormesis effect. However, the mechanisms underlying radiation hormesis have not yet been clarified, and little is known about the effects of low doses of chemical carcinogens. We analyzed the efficacy of pretreatment with low doses of the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) on the subsequent induction of cell transformation and gastric ulceration by high-dose MNNG. We used an *in vitro* Balb/3T3 A31-1-1 cell transformation test and monitored the formation of gastric ulcers in 5-week-old male ICR mice that were administered MNNG in drinking water. The treatment concentrations of MNNG were determined by the cell survival rate and past reports. For low-dose *in vitro* and *in vivo* experiments, MNNG was used at 0.028 μM, and 2.8 μg/mL, respectively. The frequency of cell transformation induced by 10 μm MNNG was decreased by low-dose MNNG pretreatment to levels similar to that of spontaneous transformation. In addition, reactive oxygen species (ROS) and mutation frequencies induced by 10 μm MNNG were decreased by low-dose MNNG pretreatment. Importantly, low-dose MNNG pretreatment had no effect on cell proliferation. *In vivo* studies showed that the number of gastric ulcers induced by 1 mg/mL MNNG decreased after low-dose MNNG pretreatment. These data indicate that low-dose pretreatment with carcinogens may play a beneficial role in the prevention of chemical toxicity under specified conditions.

1. Introduction

A fundamental misconception in toxicology is that no matter how low the dose, carcinogens can cause DNA damage and mutations that increase the risk of cancer. This concept is established by toxicity tests in bacteria, cultured cells, and animal no-threshold models and is based on linear dose–response relationships that are extrapolated to the origin for use in human safety assessments. However, previous reports have demonstrated the efficacy of low-dose treatments, which conflicts with these concepts. These observations are widely recognized as radiation hormesis or hormetic effects [1–3]. The hormetic effect is defined as "high dose is harmful, but low dose stimulates biological activity" or "low dose pretreatment leads to resistance to subsequent high doses" and it has been reported since the 1980s [4]. Clinical applications of this knowledge used single low-dose treatments or low-dose pretreatments [5]. Mechanisms of radiation hormesis have also been reported, and several lines of evidence show activation of antioxidants, DNA repair systems, and bystander effects [6–8]. Although these observations make it increasingly difficult to refute radiation hormesis, verification of hormesis is hampered by varied physicochemical properties of radiation and species differences. As such, the mechanisms underlying radiation hormesis have not yet been clarified, and safety assessments of radiation and carcinogens continue using the linear no-threshold (LNT) model.

Problems defining such a "threshold" have been demonstrated using a carcinogen with clear mechanisms of toxicity. Although research on the radiation hormesis-like effects of chemical carcinogens is scarce, we previously reported the specific efficacy of low doses of heavy metal [9]. Moreover, at low doses, the nongenotoxic carcinogens phenobarbital and alpha-benzene hexachloride exerted hormetic effects in N-diethylnitrosamine-initiated hepatocarcinogenesis. Indeed, the thresholds of 2-amino-3,8-dimethylimidazo

Abbreviations: MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; LNT, linear no-threshold; 6-TG, 6-thio-guanine; GST-P, glutathione S-transferase placental; ROS, reactive oxygen species; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; DMSO, dimethyl sulfoxide.

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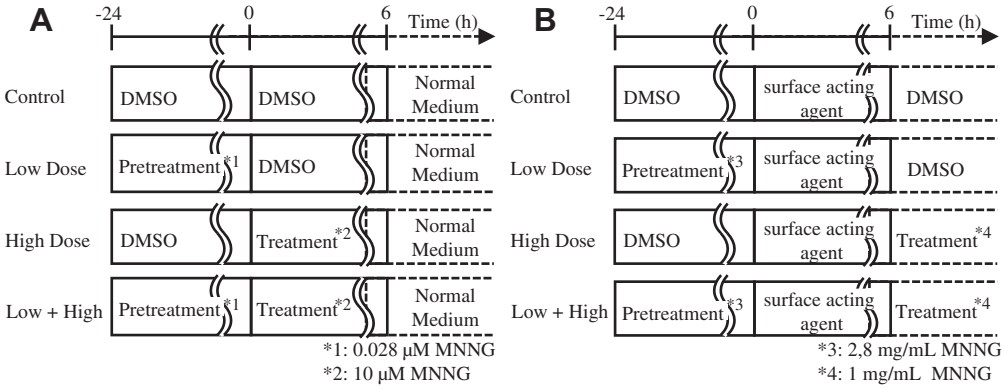

Fig. 1. Schematic representation of the MNNG pretreatment protocol.

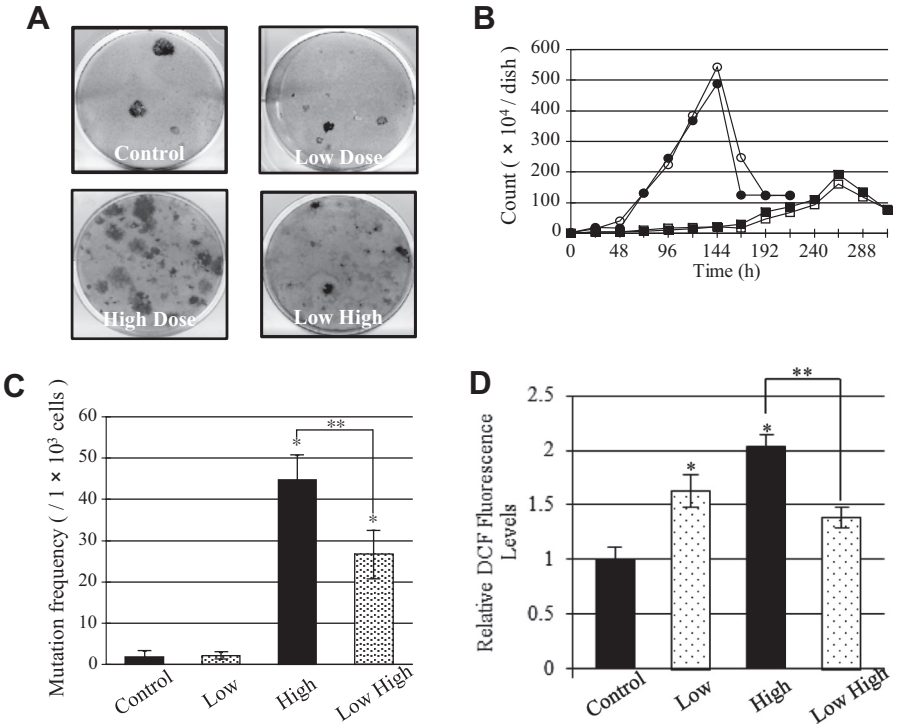

Fig. 2. Typical images of cell transformation show that low-dose MNNG mitigates induction of mutations and ROS by high-dose MNNG but does not affect cell proliferation. (A) Cells were treated with low- and high-dose MNNG according to experimental schema in Fig. 1A. After 4–5 weeks, the cells were stained with Giemsa and scanned, (B) the effect of low-dose MNNG on cell proliferation was indicated by the trypan blue dye exclusion test. For details refer to Materials and methods in Section 2.6. Control Group, open circle (○); Low Group, closed circle (●); High Group, open square (□); and Low High Group, closed square (■). (C, D) The effects of low-dose MNNG on induction of mutations and ROS by high-dose MNNG were assessed using 6-TG mutation resistance and DCFH-DA assays. For details refer to Materials and methods section 2.7–8. $^{*}P < 0.001$ vs control; $^{**}P < 0.001$ vs indicated sample (ANOVA and Tukey's HSD tests).

[4,5-f] quinoxaline and 2-amino-1-methyl-6-phenolimidazo [4,5-b] pyridine were demonstrated using aberrant crypt foci, glutathione S-transferase placental (GST-P)-positive foci, and 8-hydroxy-2′-deoxyguanosine levels as indexes [10–13].

Toxicology based on the carcinogenic threshold concept has frequently prompted reconsideration of the fundamental LNT concept [14]. In terms of human safety assessments, application of the LNT model might be suitable for extrapolation to humans. However, given the successes of radiation hormesis, full disclosure of its benefits is still required to serve the spirit of toxicological inquiry.

To analyze low-dose chemical effects, this report demonstrates the efficacy of low-dose MNNG, similar to radiation hormesis, using the *in vitro* transformation assay, the 6-thioguanine (6-TG)-resistant mutation assay, and assessments of gastric ulceration *in vivo* as indexes.

2. Materials and methods

2.1. Cell culture

Cryopreserved BALB/3T3 A31-1-1 cells were obtained from the RIKEN Bioresource Center (Ibaraki, Japan). The cells were thawed and used for experiments after varying periods. The cells were maintained in a state of continuous subconfluent growth by subculturing 3 times per week using trypsin. They were cultured in Eagle's minimum essential medium (Wako Chemical) containing 5% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 in air.