

RESEARCH COMMUNICATION

Aspirin Intake Suppresses MMC-induced Genotoxicity in Mice

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Abstract

The genotoxicity induced by mitomycin C (MMC) was found to be decreased by aspirin on alkaline single cell gel electrophoresis (SCG) assay in multiple organs of mice. Aspirin at doses of 0.5, 5 and 50 mg/kg and MMC at 2 mg/kg were administered and then liver, lung, kidney, spleen, colon and bone marrow were sampled after 3 h. Significant protective effects of aspirin against MMC-induced genotoxicity was observed in all but the bone marrow, where no change was evident. The results suggest that the radical scavenging ability of aspirin prevents damage by MMC-induced reactive oxygen species (ROS) in multiple organs.

Key Words: Alkaline single cell gel electrophoresis assay - aspirin - Mitomycin C - radical scavenger

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Introduction

Cancer is the highest cause of death in Japan and death rates from cancer have increased in 2005, thirty thousand persons died of cancer. Colorectal cancer is the fourth highest incidence of cancer among men and the first incidence of cancer among women in 2005 in Japan as a cause of cancer-related mortality. Recent studies, nonsteroidal anti-inflammatory drugs (NSAIDs), especially aspirin, can reduce the incidence of colorectal adenomas and carcinomas (Baron and Sandler, 2000; Gwyn and Sinicrope, 2002). Regular aspirin use at low doses may reduce the risk of fatal colon cancer when used 16 or more times per month for at least one year (Thun et al., 1991). Furthermore, aspirin may reduce the risk of fatal cancer of the esophagus, stomach, colon and rectum (Thun et al., 1993). Aspirin had a protective effect against non-cardia gastric cancer but did not affect the risk of cancer of the gastric cardia (Zaridze et al., 1999). In addition, NSAIDs have been demonstrated to inhibit chemically-induced cancer in various animal models and several cell strains. Sulindac reduces the number and size of colorectal adenomas in patients with familial adenomatous polyposis, but its effect is incomplete (Giardiello et al., 1993). Aspirin prevented azoxymethane (AOM)-induced colon cancer and aberrant crypt foci and reduced prostaglandin E2 levels. And aspirin and α -difluoromethylornithine prevented colon cancer induced by AOM (Li et al., 1999). Aspirin and sulindac inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (by tobacco-specific nitrosamine)-induced lung tumorigenesis in A/J mice (Duperron and Gastonfuay, 1997).

Recently, many different anticancer drugs have been used for therapeutics of cancer. There have been many

reports that anticancer drugs have mutagenicity (Benerjee and Benedict, 1979; Singh and Gupta, 1983; Pedersen-Bjergaard et al., 1988; Rossi et al., 1997) and/or carcinogenicity (Marselos and Vanio, 1991; Dedrick and Morrison, 1992; Sanderson et al., 1996) to generate secondary cancers, and this has become a serious problem. It is suggested that carcinogenicity of anti-cancer medicines such as mitomycin C (MMC), adriamycin and bleomycin may damage the DNA of cancer cells and normal cells to further act as cancer initiators.

Several previous studies showed aspirin has hydroxy radical scavenging ability (Aruoma and Halliwell, 1988). Reactive oxygen species (ROS) are critically involved in multistage carcinogenesis. NSAIDs scavenged the hydroxyl radical directly in a dose-dependent manner (Kataoka et al., 1997). It is hypothesized that aspirin may protect against anti-cancer agent induced carcinogenesis by ROS induced DNA damage.

In our previous paper, we reported aspirin suppressed MMC-induced chromosomal aberration in mice (Niikawa et al., 2001). In the present paper, we attempted to elucidate why aspirin acts as a cancer preventive agent. We used MMC which is widely used in cancer treatment as a potent anticancer agent with an antibacterial spectrum. We investigated the antigenotoxicity potential of aspirin using an alkaline single cell gel electrophoresis (SCG) assay.

Materials and Methods*Chemicals*

MMC (CAS No.50-07-2) was purchased from Kyowa Hakko (Tokyo, Japan) and aspirin (CAS No.50-78-2) was purchased from Nakarai Tesque Co., Ltd. (Tokyo, Japan).

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Alkaline single cell gel electrophoresis assay (SCG assay) and animal treatment

Animals: Seven-week-old male ICR mice were purchased from SLC (Shizuoka, Japan) and used for the experiments at the age of 8 weeks. Each group in the SCG assay consisted of four animals.

Treatment and organ preparation: MMC was dissolved in saline. MMC was administered once to the mice at a dose of 2 mg/kg by intraperitoneal injection. Aspirin was suspended in 10% arabic gum solution. Aspirin was given orally once to the mice at doses of 0.5, 5, 50 mg/kg. The mice were administered MMC and aspirin at the same time. They were sacrificed 3 h after the administration and 4 organs, that is, liver, lung, kidney and spleen, were removed. Each organ was minced, suspended at concentration of 1 g/ml in chilled homogenizing buffer (pH 7.4) containing 0.075 M NaCl and 0.024 M Na₂EDTA, and then homogenized gently using a Potter-type homogenizer in ice. To obtain nuclei, the homogenate was centrifuged at 3,400 rpm for 10 min at 0°C, and the precipitate was re-suspended in chilled homogenizing buffer at 1 g organ weight per ml. Bone marrow cells were sampled from femora. The colon was taken, opened and rinsed and then homogenized in 100 µl chilled homogenizing buffer. The homogenate was centrifuged at 3,400 rpm for 10 min at 0°C, and the precipitate was re-suspended in 100 µl chilled homogenizing buffer.

SCG assay: SCG assays were carried out using the procedure of Sasaki et al. (1997a and 1997b) with a few modifications. Two hundred µl of 1.5 W/V% normal melting point agarose (NMA) was quickly layered on a fully frosted slide (Matsunami Glass Industries, Ltd., Osaka, Japan) and covered with cover glass. The sandwiched slides were placed on ice to allow the agarose to gel. The bone marrow cells prepared as above were mixed 1:1 with 1.5%, 45°C low melting point agarose (LMA), and 30 µl of the nucleus mixture was quickly layered in the same manner after removal of the covering slide. Finally, 200 µl of NMA was quickly layered on again. The slides were placed immediately to a chilled lysing solution (pH 10) of 2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris, 1% sodium methylglycine, 10% DMSO, and 1% Triton X-100 and 0°C in the dark for 60 min.

The slides were placed on a horizontal gel electrophoresis platform and covered with chilled alkaline solution made up 300 mM NaOH and 1 mM Na₂EDTA (pH 13). In an experiment to ascertain the amount of time required for DNA unwinding and expression of alkali-labile sites, the slides were left in the solution in the dark at 0°C for 10 min. The power supply was set at 25 V. To ascertain suitable electrophoresis time, the DNA was electrophoresed at 0°C in the dark for 15 min at 25 V and approximately 250-300 mA. The slides were rinsed gently 2 times with 400 mM tris-HCl (pH 7.4) to neutralize the excess alkali. Each slide was stained with 50 µl of 20 µg/ml ethidium bromide (Nakarai Co. Ltd.) and covered with a cover-slip.

Fifty cells on one slide per organ were examined at 200x magnification using a fluorescence microscope equipped with a green filter. The length of the whole comet

(length) and the diameter of the head (diameter) were measured. Migration was calculated as the difference between total length and diameter of nuclei. We did not score the comets having a head and tail separated clearly. The average length of DNA migration from the 50 nuclei was analyzed for statistical significance using Student's *t*-test.

Biochemical assays

The experimental animals were killed 3 h after administration of aspirin and MMC. Capiject™ (Terumo Co., Ltd., Tokyo, Japan) was used to collect a blood sample from the heart, which was centrifuged at 15,000 rpm for 30 min to obtain the plasma. T-Bil (total bilirubin), LDH (lactic dehydrogenase), GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), GGT (γ-glutamyl transpeptidase) and ALP (alkaline phosphatase) were analyzed using a SPOTCHEM SP-4420 (Kyoto Daiichi Kagaku Co., Ltd., Kyoto, Japan).

Results

MMC itself produced an increase of DNA damage 3 h after treatment in liver, lung, kidney, spleen and colon. But DNA damage in bone marrow was not observed (Fig. 1-A-F). T-Bil, LDH, GOT, GPT, GGT and ALP valued in the blood sample of all animals used for the experiments, were determined and all were in the normal range (data not shown). DNA damage at 3 h after treatment in liver, lung, kidney, spleen and bone marrow was observed. In this report, aspirin was seen to have significant protective effects at all concentrations in a dose-dependent manner in liver, lung, kidney and colon. Furthermore, in spleen, the protective effect of aspirin was observed at dose of 5 and 50 mg/kg.

The clastogenicity of aspirin was measured by the micronucleus test, and aspirin at a dose of 0.5, 5 and 50 mg/kg was administered orally at 24 h after administration of 10% Arabic gum solution instead of MMC as a negative control. No clastogenicity of aspirin was observed (data not shown).

Discussion

The effect of aspirin on the clastogenicity of MMC in the SCG assay has been studied. The SCG assay is a rapid and sensitive test for the detection of DNA damage and their repair. Sasaki et al (1997c) reported when MMC was administered to the male CD-1 mice at a dose of 2 mg/kg intraperitoneally, DNA damage at 3 h after treatment in liver, lung, kidney, spleen, and bone marrow was increased. But for bone marrow, the DNA damage is weak. Although each chemical mutagen has different targeted organs, the *in vivo* SCG assay can evaluate the genotoxicity for multiple organs.

From our results, DNA damage was observed in liver, lung, kidney, spleen and colon, but not in bone marrow. For liver, lung, kidney, spleen and colon, MMC-induced DNA damage was decreased by aspirin in the SCG assay.

Many anticancer agents have been shown to be carcinogenic and mutagenic in experimental animals and

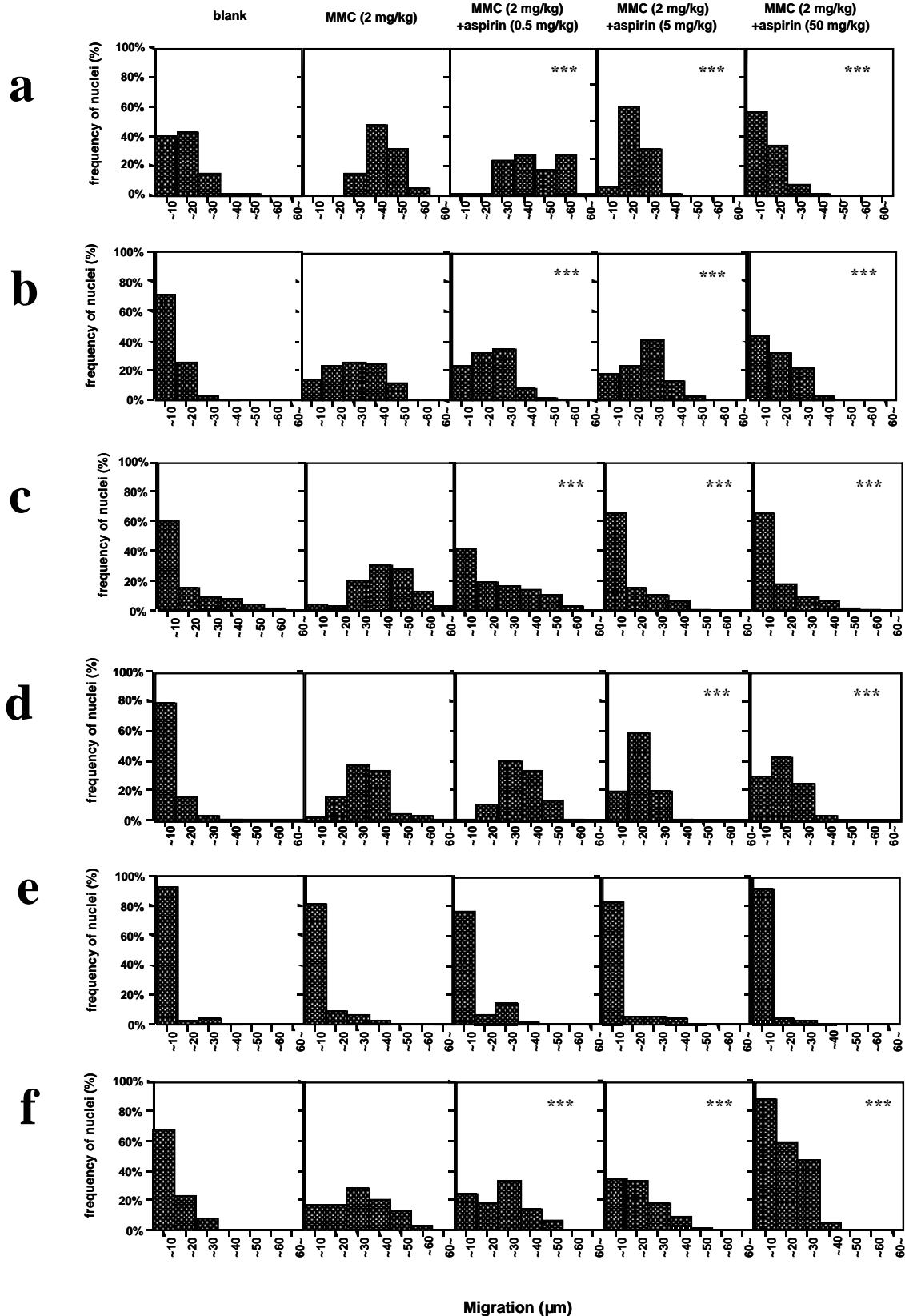


Figure 1. Effects of Aspirin on DNA damage in MMC-treated Mouse Organs in the SCG Assay. a) Liver, b) Lung, c) kidney, d) spleen, e) bone marrow, f) colon. *** Significant different from the MMC treatment value $p < 0.001$

in vitro test systems. Although several publications (Doroshov, 1986) have described the formation of oxygen radicals from MMC and its analogs, and there exists an excellent correlation between MMC cytotoxicity and the formation of oxyradicals, many researchers believe that alkylation and subsequent cross-linking of DNA is the

major mechanism of action of this drug.

Aspirin, as is well known, has the ability to scavenge or quench various oxygen free radicals (Aruoma and Halliwell, 1988). Therefore, we have remarked the radical scavenging ability of aspirin as a mechanism. The ability of the hydroxyl radical-scavenging properties of aspirin

at pharmacologically relevant concentrations markedly inhibited oxidative DNA damage induced by either H₂O₂/Cu(II) or hydroquinone/Cu(II) system (Hsu and Li, 2002). Saini et al. (1998) reported that aspirin showed a concentration-dependent inhibition of the radiation-induced production of ROS in cultured J774A.1 macrophage cells. But it has not been reported whether aspirin inhibited the MMC-induced DNA-DNA interstrand cross-links. So, we speculated that radical scavenging ability of aspirin prevents the MMC-induced ROS for the multiple organs.

MMC is used in clinical cancer chemotherapy against a variety of solid neoplasm. The primary exposure to MMC is from their use in therapy of cancer. Oxygen toxicity of MMC generated secondary cancer for normal cells in cancer patients. So we considered that aspirin intake is effective to protect against secondary cancer. Also, aspirin can be expected to be applicable to the chemopreventive agents for carcinogenesis by occupational MMC exposure to persons, such as doctor, pharmacist and worker in manufacturing industry.

From our results, it is not clear whether or not aspirin decreases the medicinal effectiveness (anti-tumor activity) of MMC. We think there are effective doses and/or administration timings of aspirin and MMC to protect against secondary cancer without losing the anti-tumor activity. Therefore, more intensive research is required to determine the dose and/or timing of aspirin and MMC to protect against secondary cancer.

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