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Effects of curcumin on methyl methanesulfonate damage to mouse kidney

G Cuce[1], S Cetinkaya[2], N Isitez[3], S Kuccukturk[4], ME Sozen[1], S Kalkan[1], IH Cigerci[3], HH Demirel[5]

[1]*Necmettin Erbakan University, Meram Medical Faculty, Department of Histology and Embryology, Konya,* [2]*KTO Karatay University, Medical Faculty, Department of Histology and Embryology, Konya,* [3]*Afyon Kocatepe University, Faculty of Science and Arts, Department of Moleculer Biology and Genetics, Afyon,* [4]*Necmettin Erbakan University, Meram Medical Faculty, Department of Medical Biology, Konya, and* [5]*Afyon Kocatepe University, Bayat Junior Technical College, Department of Laboratory and Veterinary Health, Afyon, Turkey*

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Abstract

Methylmethane sulfonate (MMS) is an alkylating agent that may react with DNA and damage it. We investigated histological changes and apoptosis caused by MMS and the effects of curcumin on MMS treated mouse kidneys. Twenty-four mice were divided into four equal groups: controls injected with saline, a group injected with 40 mg/kg MMS, a group injected with 40 mg/kg MMS and given 100 mg/kg curcumin by gavage, and a group given 100 mg/kg curcumin by gavage. MMS caused congestion and vacuole formation, and elevated the apoptotic index significantly, but had no other effect on kidney tissue. Curcumin improved the congestion and vacuole formation caused by MMS and decreased the apoptotic index. Curcumin administered with MMS appears to decrease the deleterious effects of MMS on the kidney.

Key words: curcumin, histopathology, kidney, methylmethane sulfonate, TUNEL

Alkylating agents are ubiquitous reactive chemicals that transfer alkyl carbon groups to a wide range of biological molecules. In this way, they alter the structure and potentially disrupt the functions of these molecules (Fu et al. 2012).

Alkylating agents exhibit cytotoxic, teratogenic and carcinogenic effects, and therefore pose significant threats to human health. Despite this, certain toxic alkylating agents commonly are administered systemically as chemotherapeutic drugs to kill cancer cells in patients (Fu et al. 2012, Kaina et al. 2007).

Methylmethane sulfonate (MMS) is a widely used solvent that catalyzes polymerization, alkylation and esterification (Vrzoc and Petras 1997). It is an SN2 type alkylating agent that methylates predominantly the nitrogen atoms of purines (Grzesiuk 1998). The genotoxic and cellular effects of MMS are caused by damage to DNA (Clarkson and Mitchell 1979, Schmid et al. 1978, Sega et al. 1976). Horvathova et al. (1998) reported that DNA strand breakage was evident immediately after application of MMS to hamster V79 cells.

Curcumin (diferuloyl methane) is a natural yellow pigment found in turmeric. It is isolated from the rhizomes of the plant, *Curcuma longa* (Joe et al. 2004). Curcuma contains 60–70% carbohydrate, 8.6% protein, 5–10% fat, 2–7% fiber, 3–5% curcuminoids (50–70% curcumin) and 5% essential oils and resins. The turmeric curcuminoid content varies from 2 to 9% depending on geographical conditions (Trujillo et al. 2013). Anti-inflammatory (Ueki et al. 2013), antidiabetic, antioxidative (Anto et al. 1996, Arcaro et al. 2014) and anti-carcinogenic (Johnson et al. 2009, Krishnaswamy et al. 1998) effects of curcumin have been described.

Correspondence: Gokhan Cuce, Necmettin Erbakan University, Meram Medical Faculty, Department of Histology and Embryology, Konya, Turkey. Phone: +90-3322237145, e-mail: gokhancuce@gmail.com

The literature concerning histological changes in the kidney caused by MMS and the effects of curcumin on these changes is limited. Therefore, we investigated the histological and apoptotic changes caused by a single dose of MMS with and without curcumin treatment in mouse kidneys.

Material and methods

Our study was approved by the ethics committee of the Research and Training Center of Afyon Kocatepe University. We used 24 20–40 g 5–7-week-old Swiss mice for our study. All mice were maintained in a room at 20–22° C with controlled ventilation, humidity and a 12 h light/12 h dark cycle. All animals were provided commercial mouse chow and water *ad libitum*. General clinical examinations were conducted and the experiment was begun after 1 week of adaptation.

Experimental groups

The mice were divided into four groups of six: group 1, control group given 40 mg/kg saline intraperitoneally (i.p.); group 2, MMS group given 40 mg/kg MMS i.p.; group 3, MMS + curcumin group given 40 mg/kg MMS i.p. plus 100 mg/kg curcumin by gavage 24 h after MMS; group 4, curcumin group given 100 mg/kg curcumin by gavage. All mice were sacrificed by intramuscular injection of 30 mg/kg ketamine HCl 48 h after administration of agents.

Histopathology

Left kidneys were fixed in 3% (v/v) formaldehyde and prepared for routine histology. The kidneys were dehydrated through 70% (2 h), 85% (1 h), 95% (1 h) and 100% (2 h) alcohol. After clearing in two baths of xylene (2 h each) the kidneys were embedded in paraffin. Serial longitudinal sections 4 µm thick were cut and evaluated after staining with hematoxylin and eosin (Fischer et al. 2008). Vacuole formation, desquamation (brush border degeneration), dilation and congestion were scored as follows: (0) negative, (1) weak, (2) moderate (3) severe.

TUNEL

Apoptotic cells were labeled using an ApopTag In Situ Apoptosis Detection Kit (Millipore, Billerica, MA) according to the supplier's instructions. The TUNEL assay was performed on 4 µm sections and examined by light microscopy. Twenty × 400 fields were selected randomly and the apoptotic index (AI) for each group was calculated by counting apoptotic cells/100 tubule cells (Estakhri et al. 2013). We did not assess other cells.

Statistics

All data were compared using one-way ANOVA and the Tukey test. Histological changes were compared using the Mann-Whitney U-Test. Values for $p \leq 0.05$ were considered significant.

Results

We examined the microscopic structure of mouse kidney to determine whether MMS caused damage and whether curcumin was protective against any damage. Normal histological structure was observed in the control and curcumin groups (Fig. 1A, B).Vacuolization of tubule cells was significantly increased in group 2 (Fig. 1C−F) compared to the other groups (Table 1); however, vacuolization was significantly less in group 3 (Fig. 1G, H) than in group 4 (Table 1). No significant differences in dilation or desquamation of brush borders were evident among the groups. Congestion increased significantly in group 2, decreased in group 3, but it was significant (Fig. 1C−E); groups 1, 3 and 4 showed no differences (Table 1).

The apoptotic index was significantly greater for group 2 (Fig. 2B) than for the other groups (Fig. 2A, C, D). The apoptotic index was significantly less in group 3 than in group 2. Curcumin significantly decreased kidney damage caused by MMS (Table 1).

Discussion

We observed significant increases in vacuolization and congestion in kidney sections prepared 48 h following a single dose of MMS. No significant difference was evident in dilation or desquamation (brush border degeneration) between the MMS and control groups. The TUNEL assay showed that the number of cells undergoing apoptosis in kidney tissue exposed to MMS was significantly greater than for the control group.

Mitogen-activated protein kinases (MAPKs) help organize cell proliferation, differentiation and apoptosis. One of the MAPKs, p38, is activated by stress stimuli; therefore, it is a stress activated protein kinase. Stress activated protein kinases cause apoptosis and prevent growth. Application of MMS activated p38 MAPKs in the kidneys of young and old rats (Suh and Park (2001). We found

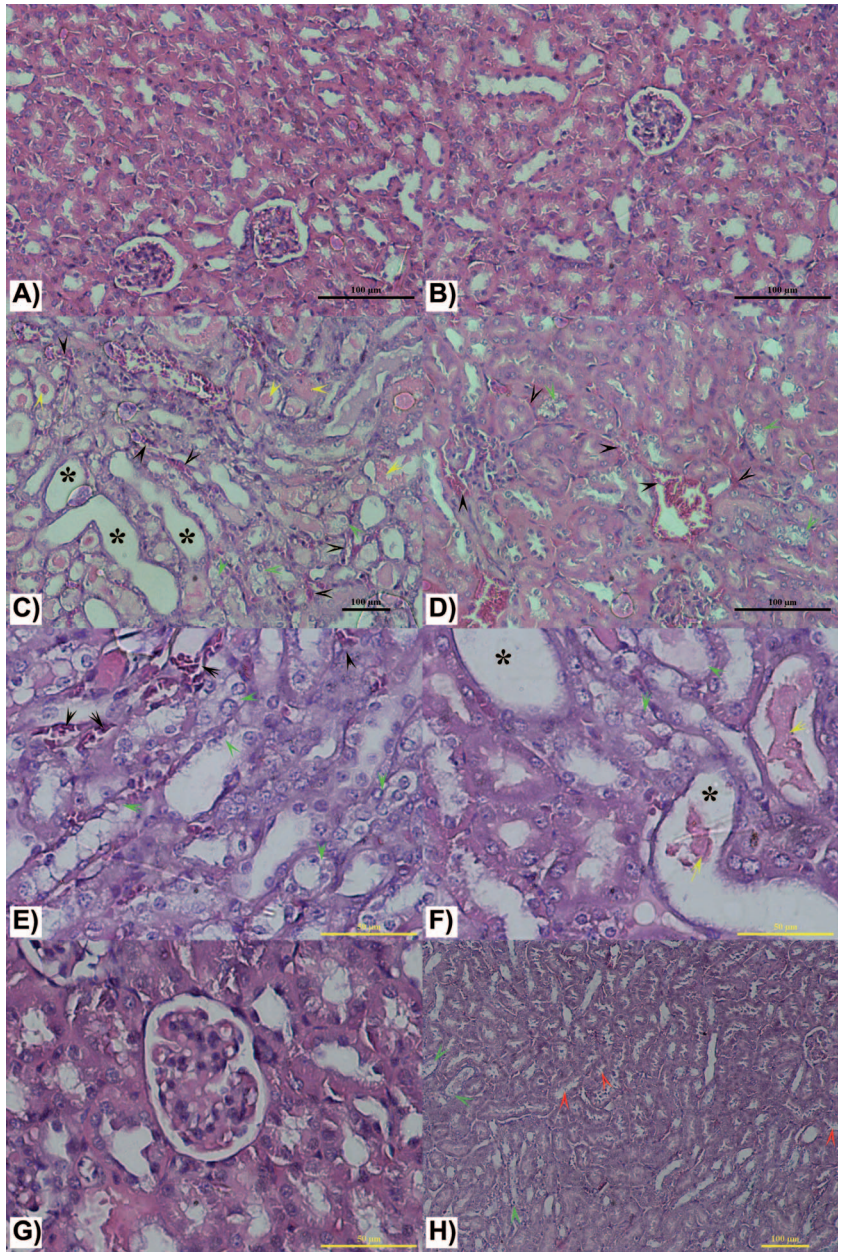

Fig. 1. A) Control group. B) Curcumin group (Group 2). C – F) MMS treated group (Group 3). G, H) MMS + curcumin group (Group 4). Green arrows, vacuolization and tubule degeneration; black arrows, congestion; yellow arrows, tubule secretion; *, tubule dilation; red arrows, desquamation or degeneration of brush border.

that the apoptotic index was greatest in the MMS treated group; the elevated apoptotic index that we observed may be due to increased p38 MAPK.

Significant increases in DNA damage to kidneys were evident 4 h after administration of 50, 100 and 150 mg/kg MMS, and damage still was apparent 24 h after administration of the 150 mg/kg dose; no significant damage was observed after either 50 or 100 mg/kg doses at 24 h. DNA damage characteristics were used to evaluate the genotoxicity (Oshida et al. 2008). These characteristics include tailed nuclei, tailed length, percent DNA in the tail and the tail moment, which were detected using the comet assay (Lee and Steinert 2013). Oshida et al. (2008) reported that MMS caused a significant increase in tailed nuclei, percent DNA and tail moment. Nephrotoxicity has been associated with necrosis, degeneration of tubular structure, tubular congestion and swelling (Rehman et al. 2012). Nephrotoxicity caused by MMS was evident from our histopathology results. At the same time, a significant increase in serum levels of BUN and creatinine causes nephrotoxicity and renal damage (Rehman

Table 1. Histopathological assessments

	Group 1	Group 2	Group 3	Group 4
Congestion	0	1.33 ± 0.2^a	0.5 ± 0.2	0
Dilation	0	0.33 ± 0.21	0.16 ± 0.16	0
Vacuolization	0	2.1 ± 0.3^a	0.6 ± 0.2^b	0
Desquamation	0.16 ± 0.1	0.83 ± 0.3	0.5 ± 0.2	0
TUNEL	1.15 ± 0.14	8.15 ± 0.87^a	3.13 ± 0.51^b	1.05 ± 0.14

Data are means ± SE
$^a p < 0.05$ compared to group 1 (control); $^b p < 0.05$ compared to group 2 (MMS)

et al. 2012). Oshida et al. (2008) reported that increased BUN was not toxicologically significant and might not indicate nephrotoxicity of MMS; no changes in plasma creatinine levels were observed, however, presumably because genotoxicity rather than nephrotoxicity was in play (Oshida et al. 2008).

Curcumin inhibited apoptosis triggered by p53 by stimulating p53 disruption and the NQO1-dependent pathway (Tsvetkov et al. 2005). Also, curcumin is a powerful inhibitor of nuclear factor-kB (NF-kB) (Goel et al. 2008) and therefore inhibited apoptosis by suppressing NF-kB-dependent gene transcription; curcumin also stimulates cell proliferation, cellular invasion and angiogenesis (Aggarwal and Harikumar 2009).

Ueki et al. (2013) reported that the anti-inflammatory effects of curcumin decreased the nephrotoxicity of cisplatin in mice. Curcumin also increased superoxide dismutase, catalase and gluthatione peroxidase levels, all of which are antioxidant enzymes, and decreased the levels of malondialdehyde and protein carbonyl groups, which are oxidative stress biomarkers, in rats with diabetes, (Arcaro et al. 2014). Curcumin also is a promising therapeutic agent for preventing renal epithelial damage, inhibiting lipid degradation, lipid oxidation and cytolysis caused by oxidative damage (Cohly et al. 1998). Curcuminoids inhibited lipid oxidation in cultured L929 cells and eliminated superoxide and hydroxyl radicals (Anto et al. 1996). Biological damage caused by free radicals is called oxidative stress (Valko et al. 2007). Reactive oxygen species play an important role in apoptosis caused by drugs. Curcumin acts as an antioxidant and free radical scavenger to block the apoptotic effect of chemotherapeutic drugs (Somasundaram et al. 2002). We administered curcumin 24 h after administering MMS which decreased the AI in Group 3.

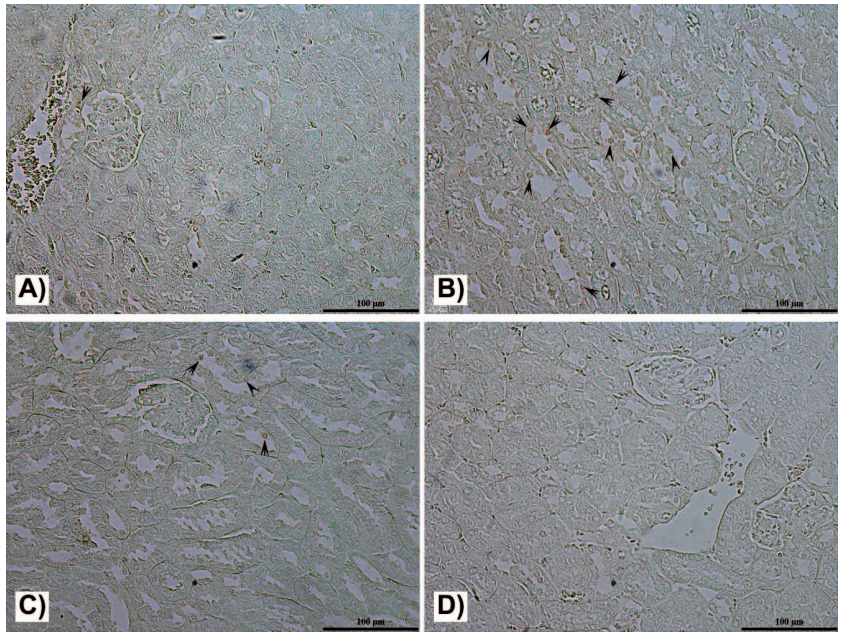

Fig. 2. A) TUNEL-negative cells in a section from control group. B) TUNEL-positive cells from group 2 (arrows). C) TUNEL-positive cells from group 3 (arrows). D) TUNEL-negative cells from group 4.

It has been reported that 80 mg/kg MMS significantly damaged DNA of isolated kidney cell nuclei by 3 and 8 h after administration (Sekihashi et al. 2001, Tsuda et al. 2000) and that 160 mg/kg MMS significantly damaged DNA by 24 h (Sekihashi et al. 2001). We used a low dose of MMS and sacrificed the animals 48 h after MMS application, which may explain the limited kidney damage that we observed. We observed the antioxidant effect of curcumin in group 3. Administration of curcumin after MMS may be useful for cancer treatment to prevent histopathological changes in kidneys.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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