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Evaluation of dose-related genotoxicity of desflurane by SCE human lymphocytes

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Aim: To evaluate dose-related genotoxicity of desflurane. In the literature, there are studies with equivocal data regarding the potential genotoxicity of inhalational anesthetics. However, it is important to know about the genotoxic safety of an anesthetic agent.

Materials and methods: Two groups were enrolled into the study. The first group was maintained with a desflurane minimum alveolar concentration (MAC) of 0.5 and the second was maintained with 1 MAC of desflurane. Blood samples were obtained and studied at the baseline and after 3 h of anesthesia via sister chromatid exchange (SCE) assay.

Results: In both groups, SCEs per cell were noted to increase after 3 h of anesthesia, though the increase was only significant in the 1 MAC group.

Conclusion: There are limited data on the genotoxicity of desflurane. It seems that 1 MAC of desflurane has a genotoxic effect, as shown by SCE assay.

Key words: Anesthesiology, desflurane, SCE

Desfluran genotoksisitesinin insan lenfositlerinde SCE ile değerlendirilmesi

Amaç: İnhalasyon anesteziklerinin potansiyel genotoksisitesi literatürde farklı sonuçları bulunan ve çalışmaları halen devam eden bir konudur. Yaygın kullanılan inhalasyon ajanlarının genotoksik güvenliğini belirlemek hastalarda ajan seçiminde önemlidir.

Yöntem ve gereç: Bu çalışma desfluranın doza bağlı genotoksisitesini araştırmak amacıyla planlandı. Çalışmaya 2 grup dahil edildi, 1 grup 0,5 MAC, diğer grup 1 MAC desfluran ile idame edildi. Bazal ve anestezinin 3. saatindeki kan örnekleri SCE assay test ile çalışıldı.

Bulgular: Her iki grupta da bazal SCE oranı anestezinin 3. saatinde artmış olarak bulundu. Fakat 1 MAC grubundaki bazale göre anestezinin 3. saatindeki artış istatistiksel olarak anlamlıydı.

Sonuç: Desfluranın genotoksisitesiyle ilgili sınırlı bilgi mevcuttur. SCE frekanslarını çalışarak 1 MAC desfluranın genotoksik etkilerini belirledik.

Anahtar sözcükler: Anestezi, desfluran, SCE

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Introduction

Inhalation anesthetics are volatile substances and are found at the liquid state at room temperature. The induction rates of these anesthetics differ according to their anesthetic, analgesic, and muscle relaxation effects. The strengths of inhalation anesthetics are determined by their minimum alveolar concentrations (MAC). MAC is determined by the minimal alveolar drug dose that is required to prevent the reflex formation against a painful stimulant like skin incisions in 50% of the subjects. The individual MAC rates differ between 0.5 and 1.5 MAC (1).

The mutagenic effects of anesthetic gases have been studied (2,3). Both experimental and epidemiologic studies demonstrated that not only the patients but also the operation staff exposed to anesthetic gases are at risk (4,5). Cobett et al. demonstrated fetal mortality in rats exposed to low doses of nitrous oxide during pregnancy (6). Nitrogen protoxide may affect DNA synthesis by inhibiting methionine synthase. Negative effects on reproductive functions have also been demonstrated. Epidemiologic studies indicated that exposure to nitrogen protoxide at lower doses and at longer periods increased the spontaneous abortion rate (7).

Sardas et al. showed the genotoxic effect of isoflurane in human lymphocytes (8). Lüleci et al. indicated the reversible mutagenic effect of sevoflurane in human lymphocytes (9). Desflurane is a halogenated methyl ether, soluble in blood and other tissues. The structure and pharmacodynamics of desflurane is similar to that of isoflurane (10). Regarding the medical literature, there are few studies demonstrating the dose-dependent genotoxic effect of desflurane. We therefore aimed to study the potential genotoxic effect of desflurane at 2 different doses, 0.5 and 1 MAC.

Materials and methods

After the obtaining of informed and written consent, 30 patients of physical status I-II according to the American Society of Anesthesia (ASA), who underwent gastroenterological and urological major surgery, were enrolled in the study. The study was approved by the hospital ethics committee. All patients were randomized into 2 groups with 15 patients in each group: Group 1, 0.5 MAC (desflurane 3%), and Group 2, 1 MAC (desflurane 6%). Patients with diabetes mellitus, alcohol abuse, and cigarette usage were excluded. Patients received 0.07 mg kg^{-1} of midazolam intramuscularly as premedication 30 min prior to anesthesia induction. Noninvasive blood pressure, ECG, oxygen saturation by a pulse oximeter, end-tidal anesthetic agent, and end-tidal CO_2 (Capnomac Ultima, Datex, Helsinki, Finland) were used for routine patient monitoring in the operating room. After insertion of a peripheral venous catheter, 3 mL of blood was withdrawn into a heparinized syringe for the sister chromatid exchange (SCE) assay test. Genotoxicity was determined via SCE assay because of its low cost and availability. The comet assay, mitotic index, and chromosome aberration techniques are other alternative methods that could be used for this purpose (5,8). Anesthesia was induced with 1 μg kg^{-1} fentanyl, 3-7 mg kg^{-1} thiopental sodium, and 0.5-0.6 mg kg^{-1} rocuronium bromide. Anesthesia was maintained with 0.5 MAC (Group 1) or 1 MAC (Group 2) desflurane in an O_2/air mixture. N_2O was not used in any of the patients, but fentanyl was added for pain relief if required. As midazolam, thiopental, and fentanyl have relative genotoxicity but are safer than other anesthetics, we preferred these as standard medications (11,12).

Sister chromatid exchange assay test

Peripheral blood samples (3 mL) of the patients were also taken into heparinized tubes 180 min after the initiation of anesthesia. Blood samples were cultured at 37 °C for 72 h in RPMI-1640 medium (Biological Industries, Israel) supplemented with 1.5% phytohemagglutinin (Biological Industries), 20% fetal calf serum (Biological Industries,), 200 mM l-glutamine (Biological Industries), and antibiotics. At 24 h, 25 μM 5-bromodeoxyuridine (SERVA, Germany) was added to the culture. All cultures were incubated in the dark at 37 °C for 72 h. During the last hour of incubation, colcemid (Biological Industries) at a final concentration of 0.1 μg mL^{-1} was added to all cultures to arrest the dividing of lymphocytes in mitosis. After hypotonic treatment in 0.075 M KCl solution for 20 min at 37 °C, chromosomes were fixed in methanol and acetic acid (3:1 v/v). The fixed cells

were dropped onto clean microscope slides, which were treated as follows: the slides were immersed into Hoechst 33258 solution for 25 min in the dark and washed with phosphate-buffered saline (PBS). They were then placed in a glass tray containing PBS, 10 cm away from a UV source for 120 min, and cleaned with distilled water. The slides were stained with 2% Giemsa for 15 min (13-15). The samples were spread on glass slides and analyzed with a light microscope to determine the SCE frequency.

Statistical analysis

All results were expressed as mean ± standard error of mean (SEM). Because of the abnormal distribution, nonparametric statistics were utilized. Two independent groups were compared with the Mann-Whitney U test. (SPSS 10.0.1 for Windows) and $P < 0.05$ was considered statistically significant. The distribution of data is shown in box plot graphics in Figure 1.

Results

Regarding the 30 patients, 19 of them were male (63.3%) and 11 of them were female (36.7%). The mean ages for Groups 1 and 2 were 45.26 ± 11.69 and 41.06 ± 11.11 years, respectively. There was no difference in the distribution of gender between the 2 groups, with 10 males and 5 females in Group 1 and 9 males and 6 females in Group 2 (Table).

The mutagenicity of desflurane was evaluated based on lymphocytes obtained from peripheral blood in both groups, at the baseline and after 3 h of anesthesia. The baseline SCE in Group 1 increased from 6.48 ± 1.38 to 7.11 ± 1.55 SCE cell^{-1} after 3 h of anesthesia ($P > 0.05$). The baseline SCE in Group 2 increased from 6.89 ± 1.81 to 8.19 ± 1.95 SCE cell^{-1} after 3 h of anesthesia ($P < 0.05$) (Figures 1 and 2).

Table. Data of the patients included in the study.

	0.5 MAC	1 MAC
Male	10	9
Female	5	6
Age	41.06 ± 11.1	45.26 ± 11.7

Discussion

Anesthetic gases have genotoxic effects and also have negative effects on both the patients and the operation staff (16). As the genotoxic effects of anesthetic gases have been reported to begin at 1 h and peak after 2 h, the blood samples were drawn at 3 h after exposure to anesthetic gases in the current study (9,11). The SCE frequencies in Group 1 were noted to increase between the preoperative and intraoperative 3-h period, though it was not statistically significant ($P = 0.117$). SCE frequencies of Group 2 in the same period yielded a statistically

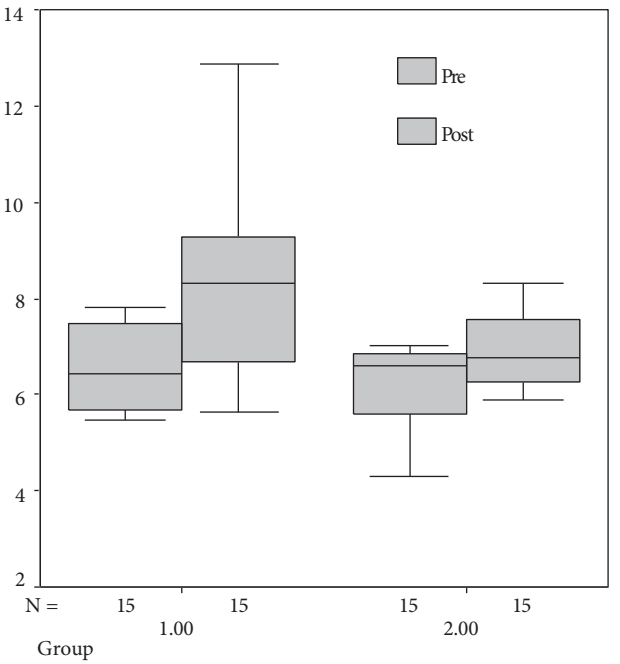

Figure 1. The distribution of data in box plot graphics.

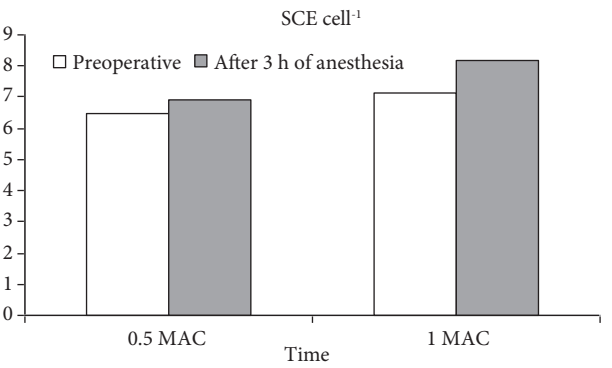

Figure 2. The pre- and postoperative mean SCE values of Groups 1 and 2.

significant increase (P = 0.002). There are limited studies related to the genotoxicity of desflurane in the literature, though various studies have been published regarding such effects of other anesthetic gases. Szyfter et al. reported that sevoflurane had no genotoxic effect in vivo or in vitro, and halothane was reported to be more genotoxic than isoflurane (17). Jaloszynski et al. showed that halothane and isoflurane increased DNA damage (18). Karabıyık et al. also indicated similarly high genotoxic effects of both isoflurane and sevoflurane (11). Lüleci et al. reported the mutagenic effect of sevoflurane on DNA (9). In our study, we also found an increase in the SCE frequencies after exposure to desflurane. Although Lüleci et al. used sevoflurane both in the induction and the maintenance of anesthesia, we only used desflurane in the maintenance of anesthesia, as it was not suitable for induction. We preferred thiopental and fentanyl for the induction of anesthesia, as no risk of genotoxic effects was reported previously (11). Husum et al. reported that isoflurane exposure for a short period did not have a mutagenic effect based on the SCE frequencies (19). Akin et al. used 1 MAC of desflurane in 15 female patients and found an increase in SCE frequencies after both 1 and 2 h (12). Our study revealed similar results at 1 MAC, and 0.5 MAC seemed relatively safer.

White et al. studied the SCE ratios after induction with inhalation anesthetics. They indicated that exposure to 1 MAC of halothane, enflurane, isoflurane, methoxyflurane, and nitrogen protoxide for 1 h did not change SCE frequencies, but exposure to divinyl ether, fluroxene, and ethyl vinyl ether was found to increase the SCE frequencies (3). Although White et al. used inhalation anesthetics for 1 h, an increase in SCE frequencies was reported to begin after 1 h of exposure (9,11). We also did not use nitrogen protoxide for induction, as teratogenic and carcinogenic effects were reported (6,7). Bozkurt et al. studied the SCE frequencies in operational staff and indicated that there may not be a genotoxic effect.(20). Hoerauf et al. studied the genotoxic effects of inhalation anesthetics with SCE frequencies and showed that nitrogen protoxide (11.8 ppm) and isoflurane (0.5 ppm) increased the SCE frequencies (5,21).

Bilban et al. reported an increase in the SCE frequencies of operational staff after extended exposure to anesthetic gases (22). Husum et al. indicated that halothane and nitrogen protoxide did not induce the SCE frequencies of the operational staff exposed to anesthetic gases (23). In conclusion, we showed the genotoxic effect after exposure to desflurane at 1 MAC by studying the SCE frequencies. It is very important to use lower doses of desflurane or the less genotoxic inhalation anesthetics for the safety of patients, particularly those with a predisposition to genetic diseases or malignancy. The potential risks for the operational staff exposed to desflurane or other anesthetic gases also have to be considered. It is also very important to determine whether the genotoxicity of desflurane is reversible over time or not. As there are limited reports related to the genotoxicity of desflurane, it might be very important to study its potential reversible effect.

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