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Micronucleus evaluation of remifentanil exposure

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ABSTRACT: Remifentanil is an analgesic used in clinical settings. However, its potential genotoxic effects on lymphocytes have not been extensively investigated. This research aimed to assess the dose-dependent impact of remifentanil on micronucleus formation in healthy human lymphocytes after exposure of 24 and 48 hours, comparing them with spontaneous and positive controls. Lymphocytes from healthy individuals were exposed to remifentanil at concentrations of 50, 150, 250, and 350 µg/mL for 24 and 48 hours. Micronucleus formation incidence was evaluated and compared with both spontaneous control and positive control groups. Remifentanil elevated the formation of micronucleus in a dose dependent manner as compared to the spontaneous control values, however, this increse was not significant statistically. This increase was significant at only the highest (350 µg/mL) concentration. Micronucleus (MN) frequency was statistically significant at only the 350 µg/mL dose of remifentanil when compared with the spontaneous. The value of cell proliferation index (CPI) was not decreased by remifentanil when compared to spontaneous control. Decrease in CPI values provide information about the genotoxicity of the doses. Evaluation of remifentanil research should extend beyond in vitro methods to include in vivo approaches applied to individuals with frequent exposure, particularly concerning chromosomal abnormalities.

KEYWORDS: Micronucleus assay; remifentanil; lymphocyte culture; toxicity.

1. INTRODUCTION

Remifentanil is a short-acting opioid analgesic, commonly used in anesthesia and pain management during surgical aplications. Its remarkable pharmacological properties, such as rapid start and end of action, have made it a preferred choice among healthcare professionals for achieving and maintaining intraoperative analgesia. However, the use of remifentanil is not without concerns, as its pharmacological properties and potential toxicity have raised questions among healthcare professionals and researchers [1-3].

The CBMN (Cytokinesis-Block Micronucleus) technique is a valuable tool employed in toxicology and genotoxicity studies to assess the potential of chemical compounds to induce genetic damage. Micronuclei are small, extra-nuclear structures that arise from the mis-segregation of chromosomes or acentric chromosome fragments during cell division. Detection of micronuclei (MN) serves as a reliable indicator of genotoxicity, as it reflects damage to the genetic material within a cell. Human lymphocyte cells are frequently used in in vitro tests due to their effectiveness in evaluating the effects on human [4, 5]. This assay has been widely utilized in evaluating the genotoxicity of various substances, including pharmaceuticals, to ensure the safety of people and living organisms [6, 7]. Presently, the CBMN assay stands as the most commonly employed technique for assessing the frequency of micronucleus formation in lymphocytes of healthy individuals [8-10]. Micronuclei occurance serve as indirect indicators of chromosomes [11]. MN arise from fragments of chromosomes or entire chromosomes that lag behind during anaphase in nuclear division. Agents with clastogenic effects on spindle fiber function or formation can be identified through the induction of micronuclei [12-15]. The human peripheral blood lymphocyte cells highly responsive to chromosomal damage induced both in vivo and in vitro [8].

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Such toxicity research will also contribute to assessing the safety profile of remifentanil, understanding toxicity mechanisms, and developing strategies for mitigating potential adverse effects. This research enables the exploration of remifentanil toxicity through the micronucleus assay, highlighting the importance of assessing the genotoxic potential of this widely used opioid analgesic. It emphasizes the necessity for ongoing research to better understand the effects of exposure to remifentanil on genetic stability, ultimately aiming to contribute to safer and more informed clinical practices.

2. RESULTS and DISCUSSION

The Cytokinesis-Blocked Micronucleus Test (CBMN) was conducted to determine the clastogenic effects of remifentanil. As the clastogenic and aneugenic effects increase in cells, micronucleus formation increases as a result of damage. With an increasing cytotoxic effect, the proliferation rate in cells decreases due to damage. Mitomycin was employed as a clastogenic control at positive control concentrations of 3µg/mL. Following a 24 and 48-hour incubation, the positive control (mitomycin) led to statistically significant differences in the percentages of micronuclei (MN) formation and cell proliferation index (CPI) values compared to the experimental groups. The positive controls resulted in decreased proliferation, attributed to mitomycin's role as an alkylating agent. This means that it induces breaks in the form of the DNA that are not repaired, leading to an apparent reduction in proliferation [17]. When we considered the CPI results, no meaningful reduction in the cell proliferation was observed. The cytotoxic effect on lymphocytes is associated with a decrease in the rate of CPI. The CPI values of mitomycin C, applied as a positive control, exhibited a statistically significant decrease with varying doses of remifentanil. However, when compared to the positive control (MMC), remifentanil doses did not show a significant decrease, indicating no observable impact on the vital functions or cell division cycle of lymphocytes.

Cell Proliferation Index (CPI) serves as appropriate indicator for cell proliferation, particularly in genotoxicity studies, due to its sensitivity in assessing the cytotoxic and cytostatic effects of diverse environmental pollutants and therapeutic agents. Concerning CPI, it is well-established that neoplastic cells exhibit high values for this biomarker. This phenomenon is attributed to the disorder or loss of control in cellular proliferation. Based on this, a decrease in CPI is expected in cells exposed to agents or drugs with cytotoxic and cytostatic effects [18].

A total of 2000 cells were counted for each identified dose (50 µg/mL, 150 µg/mL, 250 µg/mL, 350 µg/mL). The effects of remifentanil on MN formation and Cell proliferation index (CPI) values in healty human lymphocytes are summarized in Table 1.

In healthy human lymphocytes, after 24 hours of incubation, doses of 50, 150, and 250 µg/mL remifentanil have shown an increase in micronucleus (MN) formation (Figure 2); however, when compared to the spontaneous control, they did not demonstrate a statistically significant difference. On the other hand, the remifentanil dose of 350 µg/mL has revealed a significant difference in terms of MN formation frequency when compared to the spontaneous control ($P \leq 0.01$) (Table 1).

After a 48-hour incubation period, when compared to the spontaneous control in healthy human lymphocytes, a significant difference in micronucleus formation was observed only at the dose of 350 µg/mL remifentanil ($P \leq 0.01$) (Table 1). However, it has been determined that Remifentanil has no diminishing effect on the CPI value (Figure1- Table 1). The effect of remifentanil on the frequency of micronucleus formation did not show a significant difference at the end of incubation periods (24 and 48 hours). Remifentanil elevated the formation of micronucleus in a dose dependent manner as compared to the spontaneous control value, however, this increse was not significant statistically except .350 µg/mL This increase in MN frequency was significant at only the 350 µg/mL dose of remifentanil. The value of CPI was not decreased by remifentanil when compared to spontaneous control (Table 1). CPI values provide information about the genotoxicity of the doses, while micronucleus values indicate the extent of breakage in the DNA chain. These identified values were analyzed using the Tukey test in the GraphPad Prism 8 program, comparing them to negative control (spontaneous) values, as the data showed a normal distribution.

The formation of micronuclei is based on the occurrence of DNA damage. Exposure of the organism to various mutagenic, clastogenic, and carcinogenic agents leads to DNA damage. It has become one of the most economical and practical techniques for determining the rate of DNA damage both in vivo and in vitro [16, 19-21].

Table 1. The micronucleus (MN) and Cell proliferation index (CPI) of cultured human lymphocytes treated with Remifentanil.

24 Hours				
Test substances	**Doses**	**İnvestigated cell numbers**	**Mean MN (±SD)**	**Mean. CPI (±SD)**
spontaneous	-	2000	2±0,81	1,4320±0,00
MMC	0,3 µg/mL	2000	15,66±2,62***	1,1861±0,02**
Remifentanil	50 µg/mL	2000	1,66±0,47	1,1441±0,03***
	150 µg/mL	2000	4±0	1,3862±0,04
	250 µg/mL	2000	5±0,81	1,6259±0,05*
	350 µg/mL	2000	7,33±1,24**	1,6676±0,08**
48 Hours				
Test substances	**Doses**	**İnvestigated cell numbers**	**Mean MN (±SD)**	**Mean CPI (±SD)**
spontaneous	-	2000	4±0,81	1,3074±0,01
MMC	0,3 µg/mL	2000	26,66±1,69***	1,1397±0,00
Remifentanil	50 µg/mL	2000	3,66±0,47	1,2192±0,01
	150 µg/mL	2000	4,66±0,47	1,3954±0,08
	250 µg/mL	2000	5,66±1,24	1,5798±0,04*
	350 µg/mL	2000	6,66±1,69**	1,4992±0,14

MN: Micronucleus, MMC: Mitomiycin-C, CPI: cell proliferation index
± SD: standard deviation values, P: Statistically significant difference
* Significantly different from the control P≤ 0.05
** Significantly different from the control P≤ 0.01,
*** Significantly different from the control P≤ 0.001 (Tukey testi)

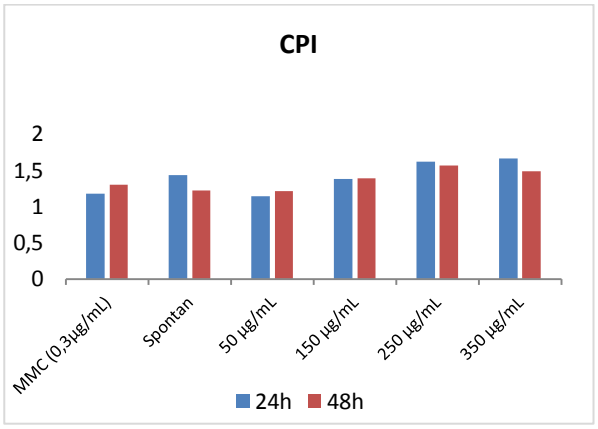

Figure 1. Effects of remifentanil doses on CPI value

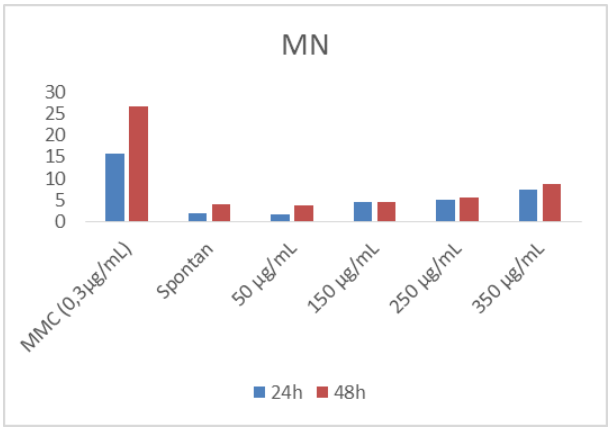

Figure 2. Effects of remifentanil doses on micronucleus (MN) formation

3. CONCLUSION

In this research, results demonstrated the inability of remifentanil to increase the frequency of MN formation significantly. Only the remifentanil dose of 350 µg/ml caused a statistically significant difference in micronucleus formation. Studies in recent years, the in vitro micronucleus test has begun to be recognized as a promising method for genotoxicity assessment. Research on remifentanil should be evaluated not only through in vitro methods but also through in vivo methods on individuals frequently exposed to it, regarding chromosomal abnormalities and micronucleus formations. The impact of frequent exposure should be investigated in a comprehensive manner, considering the dose and duration-dependent results that may arise.

4. MATERIALS AND METHODS

Experiments were performed by using peripheral blood samples obtained from non-smoking health donor aged 25-30 who had any drug usage. The drug (remifentanil) used in this study is an opioid analgesic drug known by the trade name Ultiva. CBMN assay was performed asdescribed by Fenech (2000) [16]. This study was conducted because no literature records demonstrating the genotoxic effects of Remifentanil on healthy human lymphocytes could be found. To determine the concentration of Remifentanil to be used, high concentrations were tested, and based on the observed cytotoxic effects, four concentrations (50, 150, 250, 350 µg/mL) were selected.

Blood samples were transferred to 2.5 mL of Chromosome Medium B and then The growth culture tubes were incubated at 37°C for 72 hours. Remifentanil doses (50, 150, 250, 350 µg/mL) were prepared and lymphocyte cultures were treated with for 24 and 48 hours. 50 µL of cytochalasin B (6 µg/mL) was added into the growth medium at the 44th hour of incubation. Following the incubation period, the tubes were centrifuged at 1200 rpm for 15 minutes, and the supernatants were aspirated.

After a 72-hour incubation period, cells were collected, treated with a hypotonic solution (0.4% KCl), and subjected to fixation through three rounds of methanol: glacial acetic acid fixative. Subsequently, the slides were air-dried and stained using 5% Giemsa. The frequency of micronuclei was determined by analyzing 1000 binucleated cells for each donor and treatment. Statistical differences in micronucleus numbers between treated cells and their respective solvent controls were assessed using the Dunnett test in ANOVA.

The Cell Proliferation Index (CPI) was computed using the formula: (B + 2P) / (M + B + P), where M represents the number of mononucleated cells yet to enter the first mitosis, B is the number of cells that have undergone one division (binucleated), and P is the number of cells that have undergone two divisions (plurinucleated). The sum of (M + P + P) accounts for a total of at least 1000 cells scored.

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