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Effects of concomitant use of THC and irinotecan on tumour growth and biochemical markers in a syngeneic mouse model of colon cancer

Suzana Žunec[1], Irena Brčić Karačonji[1], Martin Čatalinac[2], Andreja Jurič[1], Anja Katić[1], Goran Kozina[3], Vedran Micek[1], Marijana Neuberg[3], and Ana Lucić Vrdoljak[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb Faculty of Science, Zagreb, Croatia
[3] University North, University Centre Varaždin, Varaždin, Croatia

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Clinical treatment with the antineoplastic drug irinotecan (IRI) is often hindered by side effects that significantly reduce the quality of life of treated patients. Due to the growing public support for products with Δ^9-tetrahydrocannabinol (THC), even though relevant scientific literature does not provide clear evidence of their high antitumour potential, some cancer patients take unregistered preparations containing up to 80 % THC. This study was conducted on a syngeneic colorectal cancer mouse model to test the efficiency and safety of concomitant treatment with IRI and THC. Male BALB/c mice subcutaneously injected with CT26 cells were receiving 60 mg/kg of IRI intraperitoneally on day 1 and 5 of treatment and/or 7 mg/kg of THC by gavage a day for 7 days. Treatment responses were evaluated based on changes in body, brain, and liver weight, tumour growth, blood cholinesterase activity, and oxidative stress parameters. Irinotecan's systemic toxicity was evidenced by weight loss and high oxidative stress. The important finding of this study is that combining THC with IRI diminishes IRI efficiency in inhibiting tumour growth. However, further studies, focused on more subtle molecular methods in tumour tissue and analytical analysis of IRI and THC distribution in tumour-bearing mice, are needed to prove our observations.

KEY WORDS: antitumour activity; cannabinoid-based preparations; oxidative stress; systemic toxicity

Cannabinoids, the active compounds of the cannabis plant, have become popular alternative medicine, especially in alleviating the adverse effects of cancer treatment such as pain and nausea (1). However, they can cause side effects and interact with the metabolism of other drugs, antineoplastic drugs with a narrow therapeutic window in particular. One such drug is irinotecan [7-ethyl-10-(4-(1-piperidino)-1-piperidino) carbonyloxycamptothecin] (IRI), indicated to treat metastatic colorectal cancer and different solid tumours (2). The antitumor activity of IRI is often associated with clinically important side effects like the acute cholinergic syndrome, myelosuppression, and late-onset diarrhoea (3), so it often happens that cancer patients instead of taking conventional supportive therapy resort to cannabis products in order to alleviate the side effects of chemotherapy (4). Although there are preparations with purified or synthetic cannabinoids approved for medical use, many patients decide to buy cannabis products on the illegal market (5). Considering that illegal preparations may contain very high levels (>80 %) of the main psychoactive cannabis component Δ^9-tetrahydrocannabinol (THC) (6), this can diminish the therapeutic efficiency of the antineoplastic drug or cause unacceptable toxicities.

Corresponding author: Suzana Žunec, Institute for Medical Research and Occupational Health, Toxicology Unit, Ksaverska cesta 2, 10000 Zagreb, Croatia; E-mail: *suzana@imi.hr*, ORCID: 0000-0002-7710-8250

We already know that the metabolic pathways of IRI and THC overlap in the first two phases of metabolic biotransformation, regulated by the enzymes from the cytochrome P450 family, uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronyltransferase), and β-glucuronidase (7–10). Furthermore, both compounds and their metabolites are transported to cells by ATP-binding cassette (ABC) transporters, undergo enterohepatic recirculation, bind to plasma proteins, and affect oxidative phosphorylation in mitochondria and fatty acid metabolism (7, 10–12).

Therefore, it is reasonable to assume that high concentrations of concomitantly administered THC could impair the efficiency of IRI chemotherapy. Concerns about pharmacological interactions between cannabis or its derivatives with chemotherapy have already been voiced in the literature (13, 14), but there is no experimental evidence from either preclinical or clinical studies to support them. Recently, our group conducted a pilot study on healthy rats, which suggests that concomitant use of a high dose of THC (comparable to doses found in illicit preparations) results in a significant synergistic increase in IRI toxicity (15, 16). With this study we wanted to further elucidate systemic and biochemical changes resulting from

concomitant treatment with IRI and THC in an experimental model with a tumour.

MATERIALS AND METHODS

Chemicals and reagents

Irinotecan (CAS No. 100286-90-6, in the form of hydrochloride trihydrate salt) was purchased from LC Laboratories (Woburn, MA, USA) and prepared as a 20 mg/mL solution in Milli-Q water by mixing at 325 *g* for 5 min and heating at 70 °C. Δ^9-tetrahydrocannabinol (Dronabinol; CAS-No. 1972-08-3) was obtained from THC Pharm GmbH (Frankfurt, Germany) and dissolved in sesame oil (Bio Primo, Ulm, Germany) to a 7 mg/mL concentration. Other chemicals and reagents were bought from Sigma-Aldrich Laborchemikalien GmbH (Steinheim, Germany).

Experimental design

The study was carried out in compliance with national and international animal welfare standards (17–19). The experimental protocol was approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb, Croatia (approval number: 100-21/16-16 of 30 June 2016) and the Croatian Ministry of Agriculture (approval number: 525-10/0543-19-6 of 29 October 2019).

Ten-week-old male BALB/c mice were obtained from the breeding colony at the Ruđer Bošković Institute, Zagreb, Croatia. The mice were maintained under pathogen-free conditions in a steady-state microenvironment with a 12 h light/dark cycle, 22 °C, and free access to standard GLP-certified food (4RF21, Mucedola, Settimo Milanese, Italy) and tap water. After seven days of acclimatisation, the mice were weighed and injected subcutaneously with $5x10^6$ CT26.WT colon cancer cells (ATCC® CRL-2638™) in the right flank. When the average tumour size reached 150 mm^3, the mice were randomised to four groups of fifteen animals with minimal weight variation (30 g ± 10 %), as follows: Control – tumour control (injected with CT26.WT and left untreated); IRI group – receiving IRI intraperitoneally (60 mg/kg) on days 1 and 5 of treatment; THC group – receiving THC by gavage (7 mg/kg a day) for seven days; and IRI+THC group – receiving the combination of IRI and THC as described above.

The choice of the IRI dose and intraperitoneal administration are based on the studies of Hardman et al. (20, 21), who showed that 60 mg/kg of IRI every four days effectively suppresses lung, colon, and breast cancer growth in xenografts and on the fact that intraperitoneal administration is more effective and less toxic to mice (22) than intravenous. Furthermore, intraperitoneal administration leads to the absorption of the compound through the portal circulation and passage through the liver before reaching systemic circulation (23). This is important because IRI is a pro-drug that has to metabolise into its active form SN-38 (2, 3). IRI plasma concentration in mice peaks about 1 h after intraperitoneal administration, while its and its active metabolite's SN-38 half-life is about 6.4 h (22). There are arguments that tumour xenografts may influence irinotecan and SN-38 availability (24), but these have been declined by Guichard et al. (22), who reported no changes in the pharmacokinetic profile of IRI and its metabolites in a tumour model.

The choice of 7 mg/kg of THC and oral administration was based on the available literature reports of oral doses of various illicit preparations rich in THC (25) and usual oral intake of illicit and approved forms (26–28). Most pharmacokinetic studies of THC have been conducted in humans and show that it peaks in the plasma between 1 and 6 h (10, 29, 30). Schwilke et al. (31) reported the accumulation of THC metabolites but not parent compound in the plasma after a seven-day oral administration (2–7 daily oral doses of 20 mg Marinol capsules). This is why we opted for the seven-day treatment, considering that oral THC absorption and metabolism varies and is relatively slow, which can lead to its prolonged activity (32).

The seven-day treatment was also based on the recommendation that animals should be euthanised when the tumour reaches the volume of 1500 mm^3 (33) and followed the Institutional Animal Care and Use Committee (IACUC) guidelines (34).

Animal body weight and tumour size were measured on days 1, 3, 5, and 7. Tumour was measured with callipers by the same operator and tumour volume was calculated from two-dimensional measurements as follows: tumour volume (mm^3) = $(a \times b^2) \times 0.5$, where a and b are the tumour length and width (mm), respectively (35).

Animals were sacrificed four and 24 hours after the first IRI dose and on day 7 (Figure 1) under an anaesthetic cocktail of 80 mg/kg ketamine and 12 mg/kg xylazine (Narketan, Vetoquinol UK Ltd, Towchester, UK) delivered intraperitoneally.

Brain and liver weights were recorded immediately after the sacrifice and relative organ weights calculated as described earlier (36).

Blood was drawn directly from the heart into heparinised vacutainers (Becton Dickenson, Plymouth, UK). One portion was stored at -20 °C while the other centrifuged (at 976 *g*, and 4 °C for 10 min) to separate and remove plasma. The remaining erythrocytes were resuspended in cold saline (0.9 % NaCl) and centrifuged at 976 *g* for another 10 min. Washing and centrifugation were repeated three more times. Plasma and erythrocytes were stored at -20 °C until analysis.

Cholinesterase activity assay

Blood samples were analysed for total cholinesterase (ChE), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) activities using the Ellman's spectrophotometric method (37). Enzyme activity was measured in a 0.1 mmol/L sodium phosphate buffer, pH 7.4, at 25 °C using acetylthiocholine (ATCh) (1.0 mmol/L)

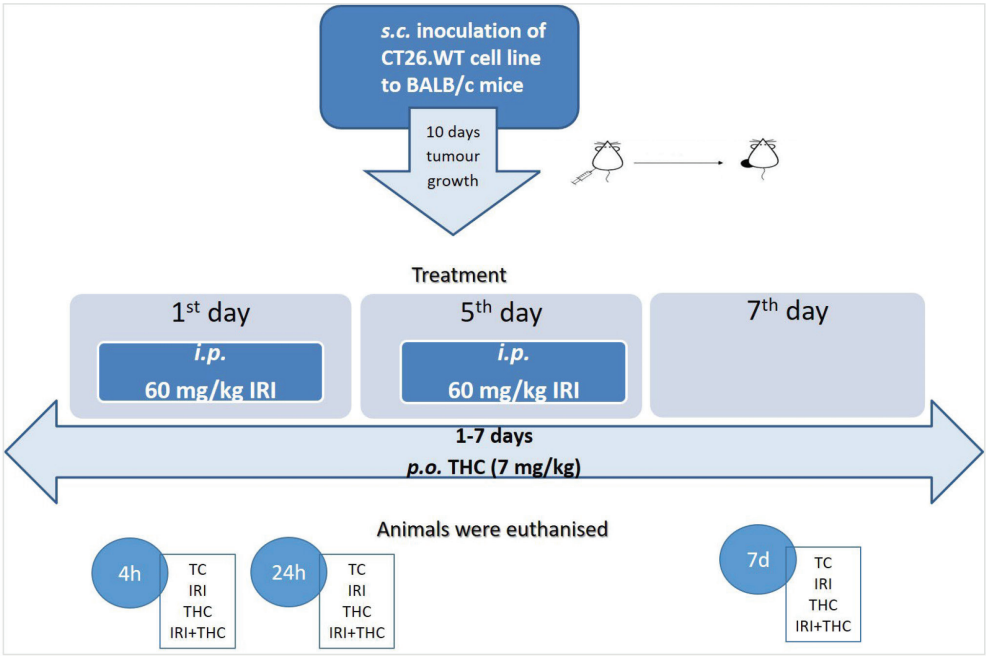

Figure 1 Experimental design. *s.c.* – subcutaneous; *i.p.* – intraperitoneal; *p.o.* – per os; TC – tumour control; IRI – irinotecan; THC – Δ^9-tetrahydrocannabinol

and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) (0.3 mmol/L). Final sample dilutions were 400 times. AChE and BChE activities were determined using selective inhibitors 4,49-(3-oxo-1,5-pentanediyl)bis(N-allyl-N,N-dimethylanilinium) (10 µmol/L) and ethopropazine (20 µmol/L), respectively, as described previously (38). Increase in absorbance was monitored at 436 nm over 4 min. All measurements were taken with a Cecil 9000 spectrophotometer (Cecil Instruments Limited, Cambridge, UK). Enzyme activity is expressed as µmol/min/mL.

Determination of oxidative stress parameters

Lipid peroxidation

Lipid peroxidation in plasma was measured using the thiobarbituric acid reactive substances (TBARS) assay as described by Ohkawa et al. (39) with some modifications. In brief, plasma samples were treated with 2 % (v/v) sodium lauryl sulphate (SDS). After protein precipitation, 24.1 mmol/L of thiobarbituric acid (TBA; dissolved in acetic acid, pH 3.5) was added and samples incubated at 95 °C for 60 min. Absorbance of each sample was measured on a Tecan Infinite 200 PRO plate reader (Tecan Trading AG, Männedorf, Switzerland) at 532 nm. The results are expressed as µmol/L TBARS according to a standard curve prepared with serial dilutions with 1,1,3,3-tetramethoxypropane.

Reactive oxygen species

The amount of reactive oxygen species (ROS) in plasma was measured using 2',7'-dichlorofluorescin diacetate (DCFH-DA) (40). Briefly, plasma samples diluted to 10 % with ice-cold PBS (pH 7.4) and 0.12 mmol/L DCFH-DA dye were incubated at 37 °C for 30 min. The control for dye autofluorescence was prepared without adding the dye. All samples were analysed on a Tecan Infinite 200 PRO plate reader at an excitation wavelength of 485 nm and emission wavelength of 535 nm. Data are expressed as fluorescence arbitrary units (AU).

Glutathione

Glutathione (GSH) levels were measured with a fluorogenic bimane probe monochlorobimane (MBCl), which reacts specifically with GSH to form a fluorescent adduct (41). Plasma samples [10 % (v/v) in PBS, pH 7.4] were incubated with 0.24 mmol/L of MBCl dye at 37 °C for 20 min. The amount of GSH in plasma samples was determined on a Tecan Infinite 200 PRO plate reader at an excitation wavelength of 355 nm and emission wavelength of 460 nm. Data are expressed as fluorescence arbitrary units (AU).

Catalase activity

Catalase (CAT) activities in erythrocytes (diluted 1000 times) were determined with a Catalase Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Superoxide dismutase activity

Superoxide dismutase (SOD) activities in erythrocytes (diluted 100 times) were determined with Superoxide dismutase Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Glutathione peroxidase activity

Glutathione peroxidase (GPx) activities in erythrocytes (diluted 100 times) were determined using a Glutathione peroxidase Assay

Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Haemoglobin concentrations

Haemoglobin (Hb) in erythrocytes was measured spectrophotometrically at 540 nm using the standard cyanmethaemoglobin method with the haemiglobincyanide standard (Mallinckrodt Baker B.V., Denver, Holland).

Statistical analysis

Statistical calculations were run on the Dell™ Statistica™ software v 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA, USA). The normality of data distribution was tested with the Shapiro-Wilk test. Normally distributed data (whole body and relative brain and liver weights and tumour volumes) were compared between the groups using the parametric one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey's HSD test. For the comparison of data not normally distributed (cholinesterase activities and biochemical markers of oxidative stress) we used the non-parametric Kruskal-Wallis test followed by the median test. Variable changes over time were tested with the Friedman test. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Systemic effects

During the experiment, no animal exhibited signs of diarrhoea and none died. Figure 2 shows time-dependent changes in mouse body weight. None were significant across the time points or between the groups. However, IRI treatment led to weight loss, most notably on day 3. These findings confirm our pilot study results in healthy rats (15, 16) and point to IRI systemic toxicity. Despite the absence of diarrhoea, body weight loss following the treatment with IRI could be a consequence of its adverse effect on intestinal absorption as reported elsewhere (42). Mice receiving THC alone maintained body weights across all time points, but these were about 2 % lower than control. However, it seems that combined treatment enhanced acute IRI toxicity, as mice body weights dropped 6 % compared to THC alone. This suggests a synergistic THC boost to IRI toxicity.

Although body weight of treated mice dropped somewhat, we found no significant changes in relative brain and liver weights between the groups (Table 1).

Effects of irinotecan and THC on tumour growth

Figure 3 shows mean tumour volume changes. The tumours grew significantly in all groups between days 3 and 7 (Friedman ANOVA: $p=0.00832–0.0150$). IRI reduced tumour growth by 35 % on day 3, 22 % on day 5, and 27 % on day 7 compared to control. These findings are consistent with earlier evidence that the chemotherapeutic effect of IRI is easily reversed if therapy is stopped and that prolonged administration is essential for inhibiting tumour growth (43, 44).

THC, in turn, lowered the efficacy of IRI, as the tumour in the IRI+THC group shrank by 10 %, 15 %, and 14 % on respective days 3, 5, and 7 compared to control. The highest tumour volumes were determined in the THC group, but these values do not significantly differ from control. For instance, Zhu et al. (45) reported that intraperitoneal THC administration (5 mg/kg four times/week for 4 weeks) promoted tumour growth in two murine lung cancer models. That effect depended on the production of immunosuppressive cytokines and was not detected in immunodeficient SCID mice, suggesting that THC promotes immunosuppression and thereby enhances tumour growth.

Biochemical changes following IRI and THC treatment

Syngeneic mouse models are used in preclinical studies whose aim is to develop cancer therapy, establish drug mechanisms of action, and/or determine biomarkers that best explain these mechanisms. Our intention was to evaluate biochemical markers that help explain the mode of action of the tested compounds by analysing them in the blood of syngeneic colorectal mice over three time points, most notably cholinesterase activities and markers of oxidative stress. Taking into account known facts about IRI and THC pharmacokinetics, we selected 4 and 24 h as the first two time points in order to detect early changes in these biochemical markers. The reason to include the third day time-point (day 7) was that oral THC absorption varies a lot, is generally relatively slow, and its active metabolites exert prolonged activity (32).

As regards cholinesterases, scientific evidence supports their involvement in functions such as cellular proliferation and differentiation, which implies their potential involvement in

Table 1 Relative brain and liver weights in syngeneic colorectal cancer mice treated with IRI, THC, their combination (IRI+THC), and in respective control (tumour control - TC)

	Relative weight (%)			
	TC	**IRI**	**THC**	**IRI + THC**
Brain	1.42±0.05	1.48±0.12	1.36±0.04	1.35±0.09
Liver	5.37±0.11	5.36±0.21	5.24±0.09	5.66±0.24

Results are given as means ± SEM of five mice per group

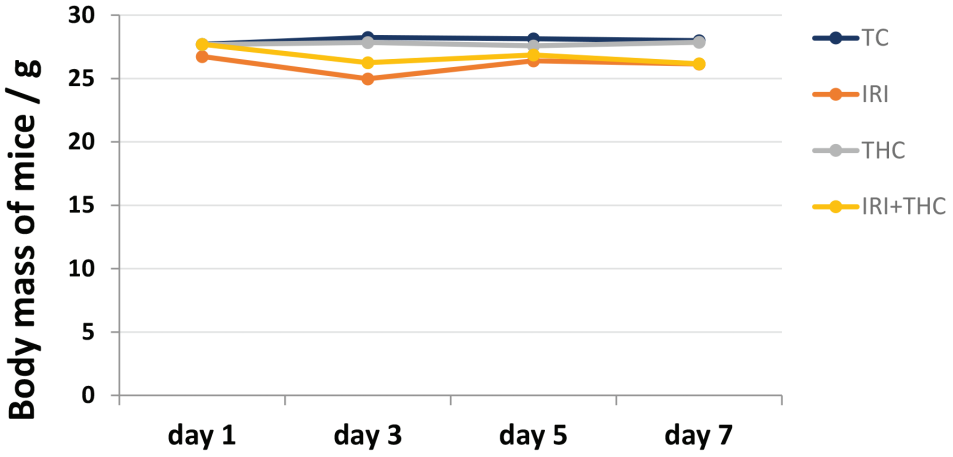

Figure 2 Changes in mouse body weight following treatment with IRI, THC, their combination (IRI+THC), and in respective control (tumour control – TC). Values represent the mean of five mice per group.

tumorigenesis (47–49). Variations in ChE activity and profile in cancerous tissues seem to depend on the kind of tumour cell. For instance, colon cancer has been characterised by lower AChE and BChE activities and their lower mRNA expression (47). Moreover, Battisti et al. (49) also reported lower whole blood AChE and plasma BChE activities in patients with advanced prostate cancer. Figure 4 shows changes in whole-blood ChE, AChE, and BChE activity. Total cholinesterases activities (0.994±0.134 IU/mL at 4 h, 1.335±0.039 IU/mL at 24 h, 1.261±0.207 IU/mL on day 7) increased with time and tumour size (Figure 3), but the changes were not significant (according to Friedman test). However, they are obvious compared to the basal ChE activity of healthy non-tumour bearing mice (1.097±0.035 IU/mL, unpublished result). The same was noticed for BChE (0.436±0.052 IU/mL at 4 h, 0.570±0.060 IU/mL at 24 h, 0.593±0.175 IU/mL on day 7) vs healthy animals (0.449±0.083 IU/mL, unpublished result). AChE, in contrast, dropped with time (0.542±0.054 IU/mL at 4 h, 0.495±0.123 IU/mL at 24 h, 0.433±0.047 IU/mL on day 7) and remained close to the levels observed in healthy animals (0.552±0.057 IU/mL, unpublished result).

According to Kimura et al. (50), serum cholinesterase can predict several cancers (50), but its clinical predictive value is not clear yet. The main reason for us to measure cholinesterase activities in the whole blood of syngeneic colorectal mice was to determine how would particular treatment affect their activity. Irinotecan is a potent AChE inhibitor, while BChE plays an important role in its hydrolysis (51). Reports of THC effects on cholinesterases, in turn, are inconclusive. Some authors hypothesised that it could bind into the AChE active site (52), which suggests that it may act similarly with BChE, as it is a homologous enzyme with a similar active site. In our study, however, neither IRI nor THC significantly changed cholinesterase activities in mice blood. Irinotecan inhibited approximately 10 % of the AChE and BChE activities compared to control, and this rate of inhibition remained similar throughout the experiment. The 10 % drop in total ChE activity was observed only 24 h after the first dose of IRI was applied. THC, in turn, inhibited AChE activities by 12 % after 4 h and 19 % after 24 h compared to control, yet total ChE and BChE activities slightly increased (about 10 %). Similar to THC, combined IRI and THC treatment led to an increase in total ChE and BChE and a decrease in AChE activities. However, we did not determine a clinically relevant inhibition of 30 %, which is consistent with the absence of the cholinergic syndrome symptoms (salivation, cramps or diarrhoea).

Most cancer cells exhibit high oxidative stress, metabolic activity, and production of ROS (53). In fact, the mechanism of action of many chemotherapy drugs involves ROS-mediated apoptosis (53). Similarly, drugs of abuse are often associated with oxidative stress, in addition to other toxicity mechanisms (54).

Figure 5 shows no significant time-dependent changes for any parameter of oxidative stress in untreated tumour control mice. However, IRI given alone increased ROS levels on days 1 (24 h) and 7. Although this effect was not accompanied by an increase in the concentration of lipid peroxidation products, it resulted in the activation of antioxidant defence. Four hours after IRI treatment, GSH level rose significantly vs control, and then significantly dropped. SOD activity was higher following IRI treatment at all time points. CAT activity increased slightly, while GPx activity remained unchanged.

THC treatment resulted in higher ROS and LPO levels after 24 h, but not significantly, which dropped to below control after 7 days. Neither GSH levels nor CAT and GPx activities varied significantly. Only SOD activity dropped significantly 4 and 24 h after THC treatment.

Combined IRI+THC treatment produced the highest ROS and TBARS levels. GSH levels significantly rose after 4 h and significantly dropped after 24 h and 7 days compared to tumour control. SOD activity was significantly lower than control at all time points, while GPx activity increased significantly compared to IRI given alone. Combined treatment also produced fluctuations in CAT activity: an increase at 4 h, followed by a decrease. In some instances it seems

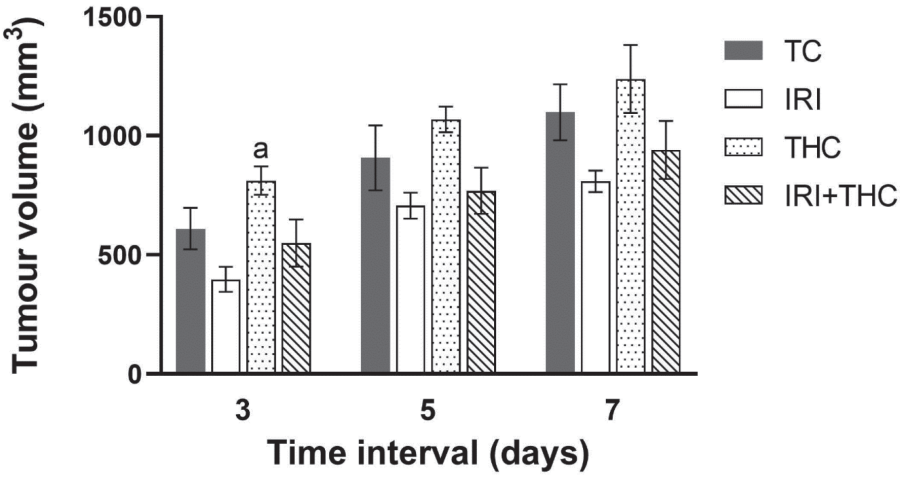

Figure 3 Changes in average tumour volume in syngeneic colorectal mice treated with IRI, THC, their combination (IRI+THC), and in respective control (tumour control – TC). Values are expressed as means ± SEM of five mice per group. [a] significantly different vs IRI ($p<0.05$).

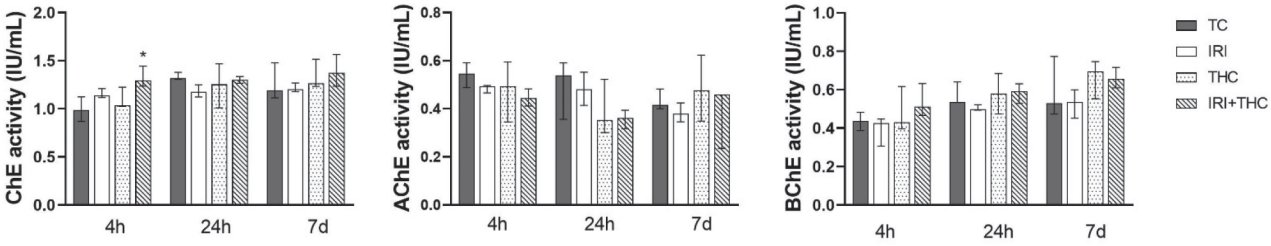

Figure 4 Changes in total cholinesterase (ChE), acetylcholinesterase (AChE), and butyrylcholinesterase activities (BChE) in the whole blood of syngeneic colorectal cancer mice after treatment with IRI, THC, their combination (IRI+THC), and in respective control (tumour control – TC). Results are presented as medians and interquartile ranges. * significantly different from control (Kruskal-Wallis test, $p<0.05$)

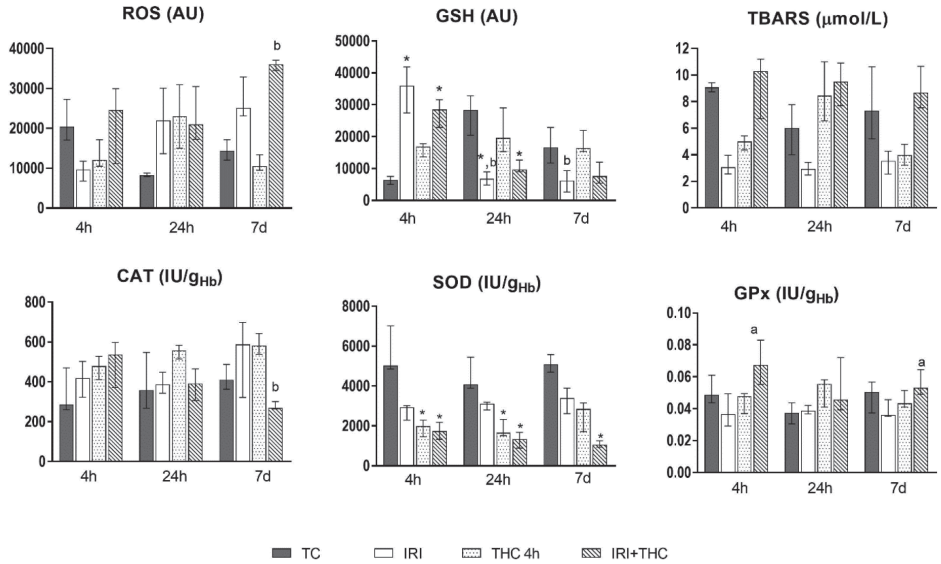

Figure 5 Changes in oxidative stress parameters in the plasma and erythrocytes of syngeneic colorectal cancer mice treated with IRI, THC, their combination (IRI+THC), and in respective control (tumour control, TC). (a) The levels of reactive oxygen species (ROS) in the plasma; (b) the levels of glutathione (GSH) in the plasma; (c) concentrations of thiobarbituric reactive substances (TBARS) in the plasma; (d) erythrocyte catalase (CAT) activity; (e) erythrocyte superoxide dismutase (SOD) activity; (f) erythrocyte glutathione peroxidase (GPx) activity. * significantly higher than TC ($p<0.05$); [a] significantly higher than IRI ($p<0.05$); [b] significantly higher than THC ($p<0.05$)

that combined treatment produced a synergistic effect (increase in ROS and TBARS levels).

High ROS levels contribute to many adverse effects that are common for IRI, such as gastrointestinal toxicity and mutagenesis. As oxidative stress causes imbalance in all cells, it is reasonable to assume that IRI should cause damage to other tissues during and after the treatment. Moreover, Rtibi et al. (55) reported that IRI caused oxidative stress-induced disturbances in water and electrolyte transport in the intestinal mucosa in rats, which suggests that such disorders are likely in the blood as well. As for THC given alone, even the early pharmacokinetic studies have established that oral administration results in erratic absorption, so the effects are sustained longer and are not as severe (10, 32).

CONCLUSION

Our study provides important evidence that high-dose THC reduces the efficiency of IRI as an anticancer drug. It also confirms systemic IRI toxicity, established through body weight loss and increased oxidative stress, which was even more pronounced in combined IRI+THC treatment.

As for other biochemical markers, fluctuations in AChE and BChE activities were treatment- and time-dependent, but statistically and clinically insignificant (about ±10 % vs control), as no animal manifested the cholinergic syndrome. However, since syngeneic models do not fully encompass the biology of tumours, our findings need to be confirmed by future studies using more advanced methods. One avenue of research should include the effects of THC on cytokine production in tumour-bearing mice and changes in ChE expression and activity in the tumour tissue. Valuable information could also be obtained from histopathological analysis and immunohistochemistry tests. Another direction would be to investigate if high cannabinoid doses interfere with the organism's ability to process IRI. To do that, it would be necessary to measure blood IRI levels before and after adding THC to treatment.

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Conflict of interests

None to declare.

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Učinci istodobne primjene THC-a i irinotekana na rast tumora i biokemijske markere na singeničnom modelu raka debelog crijeva u miševa

Kliničko liječenje antineoplastičnim lijekom irinotekanom (IRI) često je otežano nuspojavama koje značajno smanjuju kvalitetu života liječenih bolesnika. Zbog sve veće javne potpore proizvodima s Δ^9-tetrahidrokanabinolom (THC), iako relevantna znanstvena literatura ne daje jasne dokaze o njihovu visokom antitumorskom potencijalu, oboljeli od raka uzimaju neregistrirane pripravke koji sadržavaju i do 80 % THC-a. Ova studija provedena je na modelu singeničnoga tumora debelog crijeva u miševa kako bi se testirala učinkovitost i sigurnost istodobnog tretmana irinotekanom i THC-om. Mužjaci BALB/c miševa kojima su supkutano injicirane CT26 stanice primili su 60 mg/kg IRI-ja intraperitonealno prvi i peti dan i/ili 7 mg/kg THC-a oralno svaki dan tijekom sedam dana. Učinkovitost tretmana procijenjena je na temelju promjena u težini tijela, mozga i jetre, rasta tumora, aktivnosti kolinesteraza u krvi i parametara oksidacijskoga stresa. Sistemska toksičnost irinotekana potvrđena je smanjenjem težine miševa i povećanjem parametara oksidacijskoga stresa. Značaj je rezultata ove studije u smanjenoj učinkovitosti IRI-ja u inhibiciji rasta tumora tijekom istodobnog uzimanja s THC-om. Međutim, potrebna su daljnja istraživanja usmjerena na suptilnije molekularne metode u tumorskom tkivu i analitička analiza distribucije IRI-ja i THC-a u miševa s tumorom kako bi se dokazala naša opažanja.

KLJUČNE RIJEČI: antitumorsko djelovanje; oksidacijski stres; pripravci na bazi kanabinoida; sistemska toksičnost