Combined toxic effects of irinotecan and delta-9-tetrahydrocannabinol in rat liver: an introductory study using DNA integrity and oxidative stress markers

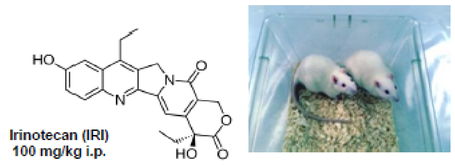
Ana Lucić Vrdoljak[1], Nino Fuchs[2], Anja Mikolić[1], Suzana Žunec[1], Irena Brčić Karačonji[1], Andreja Jurič[1], Vedran Micek[1], Radovan Fuchs[1], Nevenka Kopjar[1]
[1]Institute for Medical Research and Occupational Health, [2]University Hospital Centre Zagreb; Zagreb, Croatia
alucic@imi.hr

The use of cannabinoid-based preparations by cancer patients raises concern whether delta-9-tetrahydrocannabinol (THC) can modulate or even compromise the effectiveness of concurrently administered anticancer drugs.

Irinotecan (IRI) is a type of drug that causes various severe adverse effects such as diarrhoea, gastrointestinal toxicity and myelosuppression. Newer reports point to hepatotoxicity being an underestimated but important IRI side effect as well.

This study focused on the evaluation of toxic effects and potentially detrimental interactions of IRI and THC in the liver, with the complex overlapping between their metabolic pathways in mind. We used an experimental schedule similar to the first cycle of IRI chemotherapy with a concomitant intake of THC.

Experimental design



Adult 3-month-old male Wistar rats.
Each group comprised 5 animals with minimal weight variation (300 g ± 10%)

1-day treatment			3-days treatment				7-days treatment							
Groups	D_0	D_1	D_0	D_1	D_2	D_3	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7
CONTROL	-	↑	-	-	-	↑	-	-	-	-	-	-	-	↑
IRI	IRI	↑	IRI	-	-	↑	IRI	-	-	-	-	-	-	↑
THC	THC	↑	THC	THC	THC	↑	THC	THC	THC	THC	THC	THC	THC	↑
IRI+THC	IRI THC	↑	IRI THC	- THC	- THC	↑	IRI THC	- THC	- THC	- THC	- THC	- THC	- THC	↑

Estimation of changes in body and liver weight caused by the treatments

Oxidative stress biomarkers:
- lipid peroxidation (concentration of thiobarbituric reactive substances was determined using a modification of the method by Drury et al.[1]
- catalase activity (the method by Aebi[2])

Assessment of the primary DNA damage in hepatocytes using the alkaline comet assay
(the method by Singh et al.[3])
- scoring: Comet Assay IV™ software (Instem-Perceptive Instruments Ltd., Suffolk, UK) under an epifluorescence microscope (Olympus BX50, Japan), at 200× magnification.

METHODS

Liver to body weight ratio was calculated using the formula: liver weight (g) x 100/body weight (g).
The concentration of TBARS was calculated using standard curves of increasing 1,1,3,3-tetramethoxypropane concentrations, and expressed as μmol/L.
Catalase activity was calculated using the molar extinction coefficient (40.0 mM/cm) and expressed as $IU/g_{protein}$.
Tail length (presented in micrometres) and tail intensity (i.e., DNA% in tail) were chosen as indicators of DNA damage in the alkaline comet assay. A total of 200 comets per rat were measured on replicate slides in two independent evaluations.
Statistical calculations were done using the Dell™ Statistica™ 13.2 software (StatSoft, Tulsa, USA). The significance of the differences in body weight, liver weights, and the levels of DNA damage was tested using ANOVA and *post-hoc* Tukey HSD test. For biochemical markers of oxidative stress, Kruskal-Wallis test was used. The level of statistical significance was set at $p<0.05$.

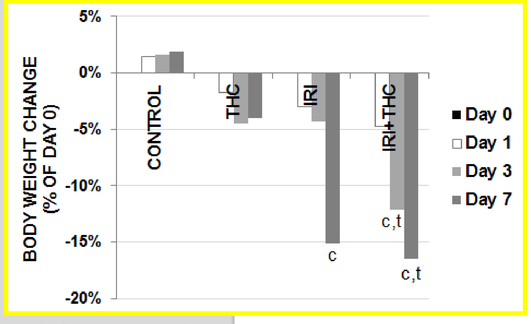


Figure1. Changes in body weight gains in male Wistar rats after the 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls. Values are expressed as mean ± SD (N=5). Significantly different ($P<0.05$) values were c: *vs*. control; t: *vs*. THC.

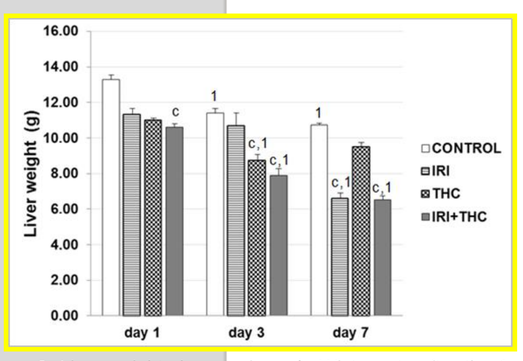


Figure 2. Liver weight changes in male Wistar rats after the 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls. Values are expressed as mean ± SD (N=5). Significantly different ($P<0.05$) values were c: *vs*. control; t: *vs*. THC; 1: *vs*. 1-day treatment.

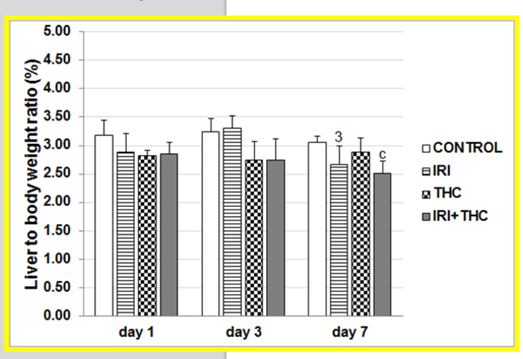


Figure 3. Liver to body weight ratio in male Wistar rats after the 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls. Values are expressed as mean ± SD (N=5). Significantly different ($P<0.05$) values were c: *vs*. control; 3: *vs*. 3-day treatment.

RESULTS

Table 1. The levels of primary DNA damage measured in hepatocytes of male Wistar rats using the alkaline comet assay after 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls

Parameter / Group		Mean	SD	SE	Min.	Max.	Lower quartile	Median	Upper quartile
Treatment – 1 day									
Tail length (μm)	Control	15.82[II]	3.28	0.10	9.17	29.17	13.33	15.42	17.50
	IRI	18.96[C,T,&]	5.40	0.17	10.83	48.75	15.42	17.92	20.83
	THC	15.77[II]	3.83	0.12	10.00	31.25	13.33	14.58	17.50
	IRI+THC	18.61[C,T,III]	6.01	0.19	9.58	47.92	14.58	16.67	22.08
Tail intensity (DNA%)	Control	0.80	1.37	0.04	0.00	8.61	0.00	0.05	1.04
	IRI	2.40[C,T]	3.95	0.12	0.00	37.31	0.04	0.77	3.07
	THC	0.85	1.34	0.04	0.00	10.64	0.00	0.14	1.19
	IRI+THC	2.57[C,T,III]	4.09	0.13	0.00	32.04	0.06	0.93	3.53
Treatment – 3 days									
Tail length (μm)	Control	14.80	3.01	0.10	9.17	28.75	12.50	14.58	16.25
	IRI	20.88[C,T,&,I,III]	6.03	0.19	10.83	68.75	16.67	20.00	23.75
	THC	14.63	3.37	0.11	8.75	29.58	12.50	14.17	15.83
	IRI+THC	18.83[C,T,I,III]	4.42	0.14	10.83	38.75	15.42	17.92	21.25
Tail intensity (DNA%)	Control	0.77	1.25	0.04	0.00	9.70	0.00	0.11	1.08
	IRI	2.97[C,T,&,I,III]	4.97	0.16	0.00	36.07	0.04	0.70	3.89
	THC	0.76	1.31	0.04	0.00	9.60	0.00	0.09	0.96
	IRI+THC	1.57[C,T]	3.17	0.10	0.00	28.72	0.00	0.14	1.71
Treatment – 7 days									
Tail length (μm)	Control	15.18[II]	3.37	0.11	9.17	29.17	12.92	14.58	17.08
	IRI	17.96[C,T]	5.10	0.16	10.00	57.50	14.58	16.67	19.79
	THC	16.27[I,II]	3.20	0.10	9.58	28.75	13.75	15.83	17.92
	IRI+THC	18.12[C,T]	4.35	0.14	10.42	34.58	15.00	17.08	20.63
Tail intensity (DNA%)	Control	1.25[I,II]	1.61	0.05	0.00	8.08	0.04	0.46	2.12
	IRI	2.73[C,T,&]	5.18	0.16	0.00	38.84	0.01	0.36	3.13
	THC	1.21	1.54	0.05	0.00	8.54	0.04	0.45	1.92
	IRI+THC	1.68	2.99	0.09	0.00	22.07	0.01	0.20	2.14

One thousand independent comet measurements per each experimental group were done.
SD – standard deviation; SE – standard error of the mean; Min. – minimum value; Max. – maximum value;
Significantly different ($p<0.05$, ANOVA with *post-hoc* Tukey HSD test) compared to: C – control; T – THC; & – combination of IRI and THC; I – 1 day treatment; II – 3 day treatment; III – 7 day treatment;

Literature:
[1]J.A. Drury, J.A. Nycyk, R.W.I. Cooke, Comparison of urinary and plasma malondialdehyde in preterm infants, Clin. Chim. Acta. 263 (1997) 177–185.
[2]H. Aebi, Oxygen radicals in biological systems, Methods Enzymol. 105 (1984) 121–126.
[3]N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.

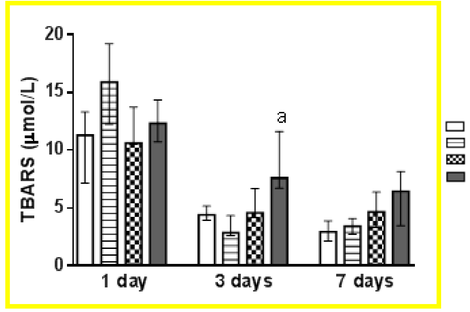


Figure 4. Changes in the thiobarbituric reactive substances (TBARS) concentration in the liver of rats after the 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls. The results are shown as median and interquartile range. The significantly increased values ($P<0.05$) were: (a) compared to rats treated with IRI.

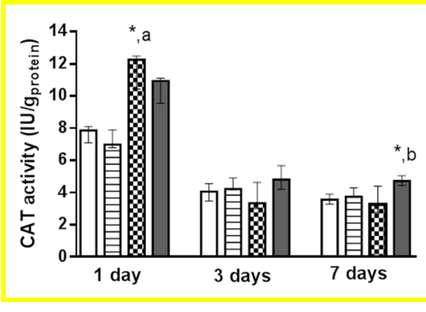


Figure 5. Changes in catalase (CAT) activity in the liver of rats after the 1-, 3-, and 7-day treatments with IRI, THC, their combination (IRI+THC), and in the respective controls. The results are shown as median and interquartile range. The significantly increased values ($P<0.05$) were: (*) compared to control, (a) compared to rats treated with IRI, (b) compared to rats treated with THC.

MAIN FINDINGS

The observed changes in body weights and liver weights suggest that all of the compounds produced acute toxicity and diminished the overall fitness of the exposed compared to control rats. It was most pronounced in rats administered a combined treatment.
DNA damage was the most pronounced after 3-day treatment, and its highest level was observed in hepatocytes of single IRI-treated rats, followed by those given combined treatment. In contrast to the 3-day, the 7-day treatment with single THC slightly impaired hepatocyte DNA integrity.
Rats given combined treatment had increased lipid peroxidation and higher CAT levels than those administered single IRI, at both time points, which may indicate that combined treatment induced more intense oxidative stress.

CONCLUSIONS

The overall reduced fitness and hepatotoxicity seen in the treated rats could be attributed to direct effects of both compounds, along with indirect effects mediated through free radicals, which result in DNA damage, altered oxidative/antioxidative balance in hepatocytes, and systemic toxicity. The results obtained in the present study mostly speak against the concomitant use of THC with IRI, due to the possible enhancement of liver damage.

Acknowledgement: This study was funded through the programme of cooperation between the Institute for Medical Research and Occupational Health (Zagreb, Croatia) and the University North (Varaždin, Croatia).