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Research article

The effects of topotecan on lipid peroxidation and antioxidant enzyme levels in rabbit liver tissue

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Background: To evaluate the possible influence of topotecan therapy on lipid peroxidation and antioxidant enzymes in rabbit liver tissue, the levels of thiobarbituric acid reactive substances (TBARS) and activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were investigated.
Methods: A total of 24 adult, healthy New Zealand rabbits were divided into three groups (n = 8). Topotecan was administered intravenously via the ear vein for 3 days at 0.25 mg/kg/day for the low-dose group and 0.50 mg/kg/day for the high-dose group; physiological saline was used for the control group. All animals were sacrificed on day 15. Livers were removed and homogenised. The homogenate supernatant was used for measurement of TBARS levels, and SOD, GSH-Px, and CAT enzyme activities (expressed as IU/mg protein).
Results: There were significant differences in the TBARS level and GSH-Px activity between control and the topotecan treatment groups. TBARS level of topotecan treatment groups was higher than control (89% and 126%, respectively, P = 0.001). It was also significantly higher in the high-dose group than the low-dose group (20%; P = 0.011). GSH-Px activity was lower in the low- and high-dose topotecan groups than the control (42% [P = 0.002] and 65% [P = 0.001], respectively). Enzyme activity was lowest in the high-dose group and the difference was also significant when compared with the topotecan groups (40%; P = 0.001). Although there was some decrease in SOD and CAT activities in the topotecan-treated groups, differences from the control group were not significant.
Conclusions: These findings suggest that topotecan treatment results in an increase in lipid peroxidation and a decrease in antioxidant enzyme activities in healthy liver tissue from rabbits. We believe that the addition of antioxidants to topotecan therapy may reduce the harmful effects of topotecan on liver tissue.

Keywords: Topotecan, lipid peroxidation, antioxidant enzymes

INTRODUCTION

The camptothecins are an effective class of anticancer agents that have shown significant clinical activity against a broad range of malignancies.[1] Topotecan, a semi-synthetic derivative of camptothecin, exerts its anti-neoplastic effects through the inhibition of topoisomerase-I (Topo-I).[2] Topotecan has demonstrated encouraging anti-tumour activity in human clonogenic assays against breast, non-small-cell lung, ovarian, stomach, cervical, colon and renal cancers as well as haematological malignancies.[2–5] Phase I and phase II clinical trials of topotecan both as a single agent and in combination with the other chemotherapeutics in adults and paediatric cancer patients have been performed.[1]

Topo-I is a nuclear enzyme, intimately involved in DNA replication.[5] Topo-I acts by transiently breaking one of the two DNA strands, rotating one of the ends about the

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unbroken strand, and rejoining the broken ends. Topotecan interacts with Topo-I and DNA, and forms a stable complex. As a result, Topo-I cannot rejoin the broken strand, which leads to apoptosis or cell death.[6–8]

Chemotherapy is one of the mainstays in the medical treatment of cancer.[9] The mechanism of anti-tumour action of cytotoxic agents involves interaction with specific cell components or metabolic pathways in cancer cells.[10] DNA synthesis inhibitors and topoisomerase inhibitors act during the S phase of the cell cycle.[7,11] Chemotherapeutic agents affect the cell cycle in a variety of ways and reduce the rate of cell proliferation.[11] Administration of some antineoplastic drugs leads to the generation of free radicals.[10] Lipid peroxidation is a chain reaction that involves the oxidation of polyunsaturated fatty acids (PUFAs) in membranes resulting in the production of free radicals and oxidative cell damage.[12] Cells protect themselves against oxidative damage by antioxidant systems, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT).

The possible influence of topotecan therapy on lipid peroxidation and antioxidant enzyme activity in rabbit liver tissue was studied. To this end, thiobarbituric acid reactive substances (TBARS) levels and the activities of the antioxidant enzymes SOD, GSH-Px, and CAT were investigated.

Materials and Methods

Twenty-four adult, healthy New Zealand rabbits weighing 2.7 ± 0.2 kg were used. All the animals were fed with standard laboratory chow and water *ad libitum* during the experiment. Rabbits were divided into three groups with eight rabbits in each.

Topotecan was diluted 1:10 with 0.9% physiological saline and protected from light using carbon paper. Topotecan (25 mg/kg/day) was administered intravenously via the ear vein for the low-dose group, and 0.50 mg/kg/day for the high-dose group, for 3 days. Physiological saline was administered for 3 days in the control group. The rabbits in all three groups were cared for and managed in the animal laboratory without any drug administration for 15 days. On the 15th day of study, livers were removed under anaesthesia with an intramuscular injection of ketamine hydrochloride (40 mg/kg; Ketalar, Parke-Davis, USA) and Xylazide (5 mg/kg; Rampun, Bayer, Germany) and then all animals were sacrificed. The livers were washed with physiological saline to discard blood and put into a tube. All tissue samples were preserved at –70°C until biochemical analysis.

The tissues were homogenised in 2 ml 0.9% NaCl using a Miccra D-8 homogeniser (Müllheim-Hügelheim) at 1000 *g* for ~2 min. After centrifugation at 1500 *g* for ~10 min, the supernatant was used for the study.

TBARS levels were measured using the method described by Dazhong *et al*.[13] A total of 50 µl of supernatant was added to 15-ml glass tubes containing 1 ml of DETBA (1,3-diethyl-2-thiobarbituric acid) solution (DETBA 10 mM + K_2HPO_4 75 mM, pH 7.0), 100 µl of EDTA solution (18.75 mM), and 100 µl of H_3PO_4 solution (3 mM) and mixed. The samples were placed in a water bath and heated for 45 min at 95°C. After the samples were cooled, 5 ml of *n*-butanol was added and the tubes vortexed. The butanol phase was separated by centrifugation at 1500 *g* for 10 min and the fluorescence of the butanol extract was measured with a Perkin-Elmer fluorimeter at wavelengths of 515 nm (excitation) and 553 nm (emission). A calibration curve was constructed using TEP (1,1,3,3-tetraethoxy propan). SOD, GSH-Px, and CAT enzyme activities were measured by the methods of Durak *et al*.,[14] Paglia and Valentine,[15] and Aebi,[16] respectively. Protein concentrations were measured by the Lowry method.[17] TBARS levels and enzyme activities were calculated as nmol/mg protein and IU/mg protein, respectively.

The results are presented as mean ± SD. Statistical analysis was carried out by the Kruskal Wallis test for the comparison of control, low- and high-dose groups. When a significant result was found, the Mann Whitney U-test was used for paired groups. The Spearman correlation test was used to evaluate the relationship between lipid peroxidation and antioxidant enzymes. The difference was considered to be significant when the probability was less than 0.05.

Results

The levels of TBARS and antioxidant enzymes activities are summarized in Table 1. There were significant differences in TBARS levels and GSH-Px activity between the control and the topotecan treatment groups. TBARS levels were higher in low- and high-dose topotecan treatment groups than the control (89% [P = 0.001,] and 126% [P = 0.001], respectively). It was also significantly higher in the high-dose group than the lower dose group (20%; P = 0.011). GSH-Px activity was lower in the low- and high-dose groups than the control (42% [P = 0.002 and 65% [P = 0.001], respectively). Enzyme activity was the lowest in the high-dose group and the difference between the high- and low-dose groups was also significant (40%; P = 0.001).

Although there was some decrease in SOD and CAT activities in the topotecan groups when compared with control, the differences were not significant in a dose-dependent manner (P > 0.05). On the other hand, we did not determine any significant correlation between TBARS levels and antioxidant enzyme activities in all three groups.

Table 1. Lipid peroxidation levels and antioxidant enzyme activities

Group (n = 8 for each group)	TBARS (nmol/mg protein)	GSH-Px (IU/mg protein)	SOD (IU/mg protein)	CAT (IU/mg protein)
Control	0.62 ± 0.13	24.6 ± 4.3	91 ± 7.5	119 ± 14
Low-dose topotecan (0.25 mg/kg/day)	1.17 ± 0.18[a]	14.3 ± 1.6[b]	88 ± 8.7	108 ± 10
High-dose topotecan (0.50 mg/kg/day)	1.40 ± 0.15[a,c]	8.6 ± 1.3[d,e]	82 ± 7.9	102 ± 10

Results are expressed as mean ± SD.

[a]Significantly higher than control (P = 0.001).

[b]Significantly lower than control (P = 0.002).

[c]Significantly higher than low-dose group (P = 0.011).

[d]Significantly lower than control (P = 0.001).

[e]Significantly lower than low-dose group (P = 0.001).

Discussion

In biological systems, when polyunsaturated fatty acids are attacked by free radicals they undergo lipid peroxidation. The elevated TBARS levels determined in the liver tissue of the rabbits treated with topotecan indicated an increased lipid peroxidation compared to the control group. This TBARS elevation was dose-dependent. Also, the activities of antioxidant enzymes measured in the liver tissue were decreased in the topotecan treatment groups compared with the control group. These decreases in all enzyme activities were dose-dependent. The enzyme activities were the lowest in high-dose group. While the decrease in GSH-Px activity was significant, the decrease in SOD and CAT activities was not (P > 0.05). GSH-Px activities between the high- and low-dose groups was also significantly different (P = 0.001; Table 1).

According to these results, topotecan treatment leads to an increase in lipid peroxidation and a decrease in antioxidant enzyme activities in healthy liver tissue. These effects may depend on a direct effect on Topo-I activity and cellular damage or unknown side-effects by topotecan or its metabolites. The decreased antioxidant enzyme activities may contribute to an increase in free radical production by some unknown factors. It is possible that increased lipid peroxidation may lead to oxidant stress in the affected liver tissue and this may cause peroxidation and cellular damage. It is obvious that topotecan exerts this oxidant effect on malign cells.

Although Rowinsky *et al*.[18] could not demonstrate hepatotoxicity of topotecan when used as a single agent and its toxic effect was seen particularly on bone marrow,[5,19] our results indicate that topotecan has an oxidant effect on the liver (Table 1). Topotecan sensitizes liver to the hepatotoxic effects of some agents, such as ifosphamide.[20] Ifosphamide potentially depletes glutathione thus decreasing the antioxidant capacity of the liver. The oxidant effect of topotecan on the liver as demonstrated here reaches hepatotoxic levels when it is used with another oxidant agent. This oxidant effect is important in the management of patients when topotecan is used for treatment, *e.g*. breast, non-small-cell lung, ovarian, stomach, colon, renal cancers, and haematological malignancies.

It has been reported that cancer patients have higher levels of both generalized oxidative stress and oxidative damage within tumour tissues as compared with normal tissues.[9] The source of these elevated oxidant molecules are activated phagocytes, tumour cells and the metabolism of some chemotherapeutic drugs. Such drugs are responsible for some detrimental side-effects as well as causing damage to healthy tissues and contributing to the development or progression of numerous different diseases.[21] These side-effects of anti-neoplastic agents play a crucial role in therapy. These agents impact all healthy proliferating cells such as bone marrow and the intestinal epithelium besides neoplastic cells.

Although chemotherapy is shown to increase the production of free radicals, the mechanism of cell cytotoxicity is likely not dependent on free radical formation.[10] These oxidants shift the form of cell death from apoptosis to pyknosis/necrosis by depleting ATP levels in the cell, which is required for apoptosis.[22,23] Antioxidant administration during chemotherapy may enhance the effectiveness of treatment and reduce or prevent the development of certain side-effects.[24] It has been shown that the co-administration of free radical scavengers did not reduce the anti-tumour effect of cytostatic agents, and the survival of animals that received chemotherapy alone.[25,26] However, the co-administration of cytostatic agents such as bleomycin or doxorubicin with antioxidant compounds can decrease their anti-tumour effect.[27,28]

Although there are some controversial effects of antioxidant agents on anti-tumour therapy, there is no doubt about the beneficial effects of these drugs on healthy tissues. The results of several clinical studies employing vitamin E decreased cardiac lipid peroxidation, delayed the lethality of a single dose of doxorubicin,[10] prevented cardiac damage and improved survival of rabbits. This was

related to the inhibition of doxorubicin-induced cardiac glutathione oxidation by vitamin E.[29] Vitamin C can prevent cardiac lipid peroxidation and structural damage, and does not affect the anti-tumour activity of doxorubicin in mice and guinea pigs.[10]

The optimal treatment regimen for topotecan is currently unclear.[23] Combined usage of topotecan with anti-oxidative agents requires further study in both preclinical and clinical settings. We believe that the addition of antioxidants to topotecan therapy may reduce the harmful effects of topotecan on liver tissue. However, whether this co-administration improves the anti-neoplastic effect of topotecan must be investigated.

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