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COMPARISON OF TOXIC AND ANTITOXIC EFFECTS OF CISPLATIN AND CAMP, AT CYP1A1 GENE EXPRESSION LEVEL OF WISTAR ALBINO TYPE RAT LIVER

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ABSTRACT. Throughout the course of cancer treatment, cisplatin has proven to be a successful chemotherapy agent. Several researches have been conducted in vivo and/or in vitro on gene expression changes occurring with the impact of this agent. The current study focuses on the alterations in CYP1A1 gene expression levels in liver cells by cisplatin and cAMP treatment. CYP1A1 gene which the expression levels were analyzed, encodes a member of the cytochrome P450 superfamily of enzymes. Throughout the study, liver tissues from Wistar-type albino rat, also known as the laboratory rat, were used. For normalization of the data, the housekeeping gene TATA box binding protein (TBP) was preferred. The results showed that; gene expression was reduced in tissues subjected independently to cisplatin and cAMP, however the tissues treated with cAMP showed further reduction in gene expression levels. In contrast, tissues subjected to both cisplatin and cAMP showed an increase in the level of expression. This study aims to provide an alternative perspective for comparative toxicology cases and will also serve as a guide for elucidation of cases connected with forensic toxicology.

1. Introduction

Some pharmacokinetic chemicals may produce changes on metabolism, especially on drug metabolizing enzymes (e.g. P450). In such cases, a multidisciplinary science called toxicogenetics assists to help to understand this phenomenon. Cytochrome P450 genes (CYP) are important variability source of drug pharmacokinetics and response [1]. Cisplatin is a highly effective, platinum-containing chemotherapeutic agent which is widely used in the treatment of various tumors. Despite the great success of the drug in treating various cancer malignancies, it is an important limiting factor in the wide use of induced toxicity [2]. Cisplatin-induced toxicities include nephrotoxicity, hepatotoxicity and ototoxicity. The primary target of cisplatin is to influence DNA, however, the mechanism of cell damage caused by cisplatin has not been fully elucidated [3].

Cyclic adenosine monophosphate (cAMP) is formed by the activation of G protein-

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dependent receptors with ligands, including hormones, autacoids, prostaglandins and pharmacological agents. cAMP is used as a common regulator of cellular function in organisms including amoeba, plants and humans. Intracellular cAMP is helpful with the innate immune functions such as production of inflammatory mediators, phagocytosis and killing microorganisms [4]. Studies on antioxidant enzymes; superoxide dismutase, catalase and glutathione peroxidase enzymes have shown that the activity of these enzymes decreased in liver cells exposed to cisplatin. It has been found that, in mouse liver cells that were treated with cAMP, the enzyme activities were inhibited to a lesser extent and the effect of cisplatin damage was decreased in the groups that were treated with cisplatin and cAMP [5]. Cytochrome P450s are heme containing enzymes found in extrahepatic tissues such as intestine, kidney, lung, brain, adrenal gland, skin and placenta. These enzymes have evolved over the years to metabolize foreign compounds and detoxify chemicals in the plant-animal paradox [6]. Cytochrome P450s have catalytic specificity towards various Phase I metabolism reactions, such as C-, N- and S_ oxidation and dealkylation [7, 19] and are widely examined in relation to its toxicological role. One of the three main members of the cytochrome P450s CYP1 is CYP1A1 found mainly in extrahepatic tissues and widely known for its extrahepatic function in the metabolism of xenobiotics and environmental chemicals. Exposure of the cells and tissues to chemicals and other environmental pollutants is thought to result in the increase expression of the CYP1A1 through activation of the aryl hydrocarbon receptor (AhR) [7,19]. The evidence of increased expression of CYP1A1 is presented in various researches from both i*n-vivo* and *in-vitro* experiments [8, 9, 10].

In cancer researches, numerous studies have focused on the expression patterns of CYP1A1 in extrahepatic tissues and results have shown that expression is largely influenced by induction of the enzyme by continual exposure to environmental chemicals [7].

In this study, the effects of cisplatin and cAMP were investigated on the CYP1A1 at mRNA level in rat liver cells.

2. Materials and methods

Albino type rats were killed by cervical dislocation, the abdomen was opened, and the livers were removed after perfusion with 0.9% NaCl. It was stored in 0.25 M sucrose solution before the chemical application process. Cisplatin (10 mg / kg) and cAMP (15 mg / kg) were applied on the liver samples that were prepared as

experimental groups. Injection has not been applied to the control group. The groups were given in the Table 1 below.

TABLE 1. Experimental groups, time and number of biological repetitions.

Group/Time	12 hours	24 hours	48 hours
Control	2	2	2
Cisplatin	2	2	2
cAMP	2	2	2
Cis+cAMP	2	2	2

2.1. Real Time PCR primer preparation

Cytochrome CYP1A1 gene expression levels in liver tissues were determined in order to compare the antitoxic effects of cisplatin and cAMP. The forward and reverse primers of the gene of interest were designed. The housekeeping TBP gene was preferred for normalization. The gene sequences of the CYP1A1 gene and the TBP gene were obtained from NCBI. The forward and reverse primers corresponding to the sequences are shown in Table 2.

TABLE 2. *TBP* and *CYP1A1* gene sequences (NCBI).

Gene Name	Forward Primers	Reverse Primers	NCBI Accession number
TBP (TATA binding protein)	5' TGCACAGGAGCCAAGAGTGAA-3'	5'-CACATCACAGCTCCCCACCA-3'	NM_003194
CYP1A1	5-GATGCTGAGGACCAGAAGACC GC	5 -CAG GAG GCT GGA CGA GAA TGC	NP_036672.2

2.2. Total RNA isolation and cDNA synthesis

Total RNA isolation was performed according to the Trizol protocol [11] with 2 biological replicates from liver samples. The amount and purity of the isolated

RNAs were measured with Thermo 2000 Nano spectrometer nanodrop (Figures 1, and 2) and RNAs were visualized with 1% agarose gel electrophoresis. Samples having suitable amount and purity were used for cDNA synthesis with kit (Transcriptor High Fidelity cDNA Synthesis Kit - Roche) according to the protocol provided by the producer.

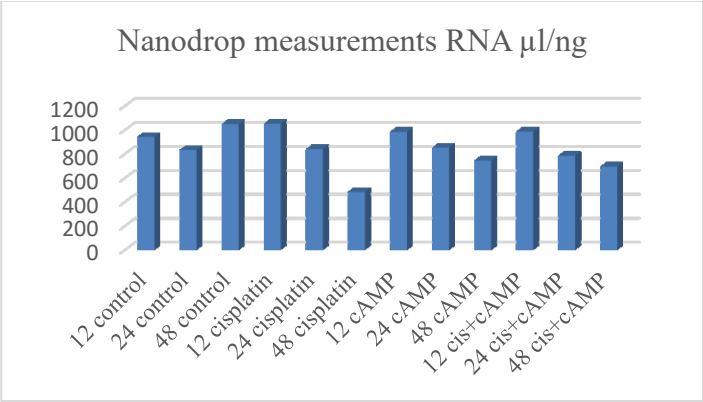

FIGURE 1. Nanodrop measurements.

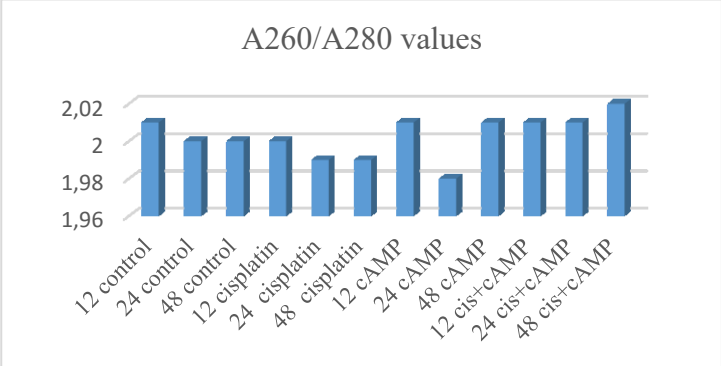

FIGURE 2. RNA purity values shown in A260/280.

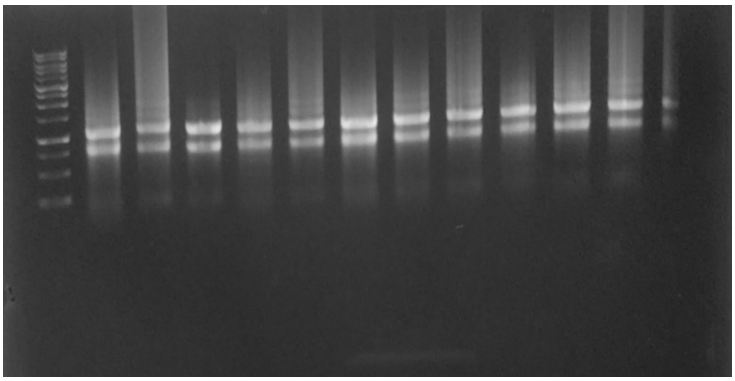

FIGURE 3. Agarose gel-electrophoresis image of the total RNAs.

In the study RNA concentration obtained from liver samples, according to spectrophotometric measurements varied between 483.7ng/µl and 1052.5 ng/µl. Results of the cDNA synthesis showed that A260/A280 ratio was ranged from 1.9 to 2.1 indicating the high purity levels of RNA. The RNA samples were also run through agarose gel electrophoresis on the ThermoElectron Cop-EC20-90 machine at 500A, 100V for 40 minutes. The results are shown in the image in (Figure 3).

2.3. Quantitative Real Time PCR (qRT-PCR) Studies and Normalization of Data

After the cDNA synthesis in appropriate quantity and quality (A260/A280 of between 1.9 and 2.1) (Figure 2); the expression levels of target CYP1A1 (accession number NP_036672.2) and TBP (TATA box binding protein; accession number NM_003194) genes were analyzed by qRT-PCR. Real Time PCR applications were performed with Light Cycle Nano (Roche) using the Go-Taq 2X Master Mix with SYBR Green I.

3. Results and discussion

The data obtained as a result of the analysis and normalization of the CYP1A1 and TBP gene in (Ct 2^Ct) values are as in the figure below. The Ct values below 29 are considered reliable.

TABLE 3. CYP1A1 and TBP (Ct and 2^Ct) values.

Work groups	**TBP cq**	**CYP1A1 cq**	**2^-(gencq-controlcq) normalization =**
12 Control	21.749	21.579	1.125058485
24 Control	21.522	21.564	0.971307496
48 Control	23.201	22.512	1.612165663
12 cisplatin	21.378	24.877	0.088449635
24 cisplatin	21.083	25.105	0.061554153
48 cisplatin	21.459	29.551	0.003664927
12cAMP	20.998	21.368	0.773782497
24cAMP	20.775	21.598	0.565265284
48cAMP	21.775	23.859	0.235859563
12cis+cAMP	21.831	25.473	0.080102995
24cis+cAMP	20.505	23.552	0.120993378
48sis+cAMP	20.882	23.469	0.166431452

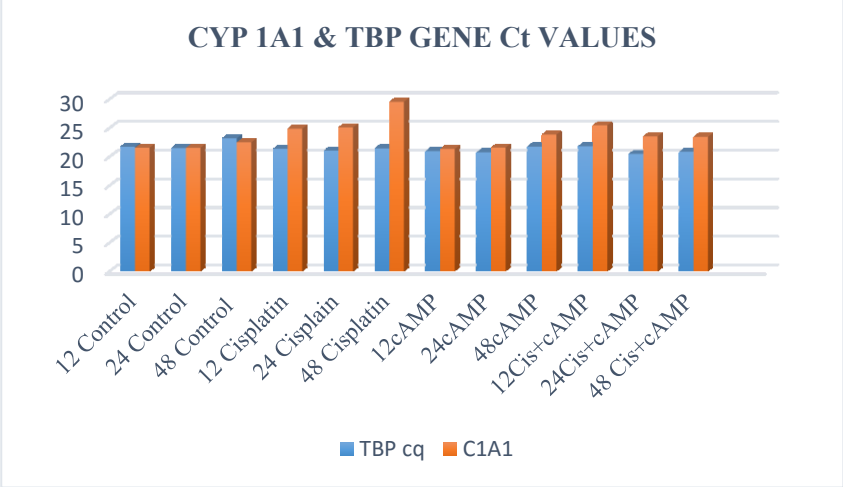

FIGURE 4. CYP1A1 and TBP (Ct and 2^Ct) values.

Figure 4 is a representation of CYP1A1 and TBP gene Ct values. Control group samples tended to show very less significant changes in Ct values. CYP1A1 gene samples tended to have higher Ct values to TBP across all treatment or exposure times. However, the highest Ct values were recorded on samples exposed to cisplatin, slightly linear increase with exposure time (12., 24. and 48. hrs.). An inverse correlation was detected on the combination treatment (cis+cAMP) with time exposure. According to the patterns of expression of the genes, it was apparent that cisplatin significantly reduced the expression levels of both CYP1A1 and TBP as compared to low cAMP effects. The values between 0 and 2 indicate decreased expression. Relatively low Ct values obtained in cisplatin treated samples indicated lower levels of mRNA of the target genes after treatment. cAMP treated samples displayed a slight increase in Ct values as compared to cisplatin treated samples with values above 0.2 despite still being a low expression figure. Although changes in expression levels were detected in each treatment, the average Ct values remained significantly low. Moderately low Ct values were detected on samples treated with combination treatment of cisplatin and cAMP. Cisplatin treated samples tended to be the most sensitive overall, as it manifested the lowest delta Ct values.

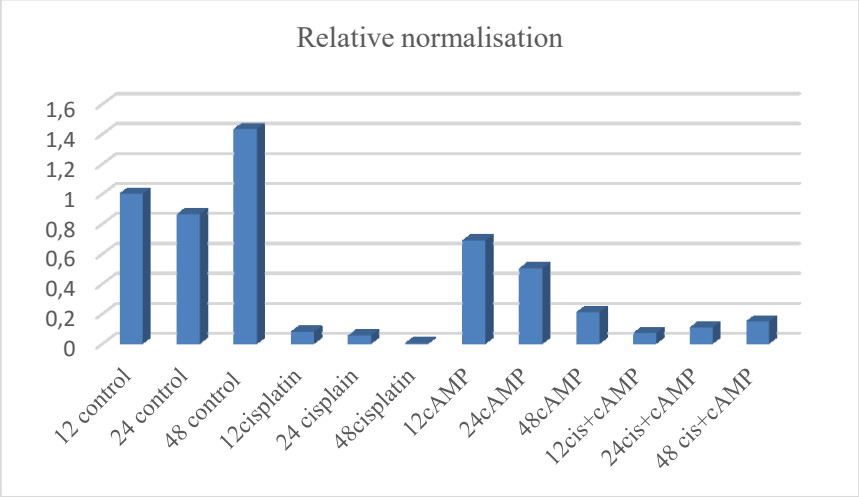

FIGURE 5. Normalization values in (ΔΔCt) Double delta Ct.

Inhibitory effects of both cisplatin and cAMP on SOD, catalase and glutathione peroxidase enzyme activities in liver tissues have been demonstrated in previous studies when they were administered independently [5]. In one of the studies, investigating the possible molecular mechanism of cisplatin hepatotoxicity and protective effects of cAMP and CoQ against free radical damage, MDA (Malondialdehyde) level and glutathione peroxidase activities were found to be higher in the cisplatin group compared to the control group. Also, in the same previous study, catalase, superoxide dismutase, glutathione peroxidase activities and MDA level were lower in the cAMP group, CoQ group, cAMP and CoQ groups compared with the cisplatin group [5].

In groups in which cisplatin was administered alone, gene expression was significantly reduced. There is also a reduction in gene expression in cAMP-administered groups, but to a lesser extent compared to the reduction level generated by cisplatin. However, groups to which cisplatin and cAMP were administered together showed a significant increase in gene expression. The results suggest that cAMP reduces the inhibitory effects of cisplatin on the expression of the enzyme CYP1A1 at transcriptional and/or posttranscriptional level, may be by due to protecting liver tissues from radical injury. P450 forms are not only expressed in the liver but also in extrahepatic tissues such as intestine, kidney, lung, brain, adrenal gland, skin and placenta [12, 13, 15]. Further studies on different tissues will help to clarify the efficacy of cisplatin and antioxidant cAMP on cancer treatment studies. In clinical trials and toxicogenetic studies, other related genes, effects of alternative chemicals should also be used with a comparative approach.

The expression of human CYP1A1 is considered to be largely among extrahepatic tissues such as lung and placenta [14]. Previous studies reported the presence of a possible protein that could be CYP1A1 in human liver (16,17,18). However, the idea of CYP1A1 protein being expressed in human liver is still debatable.

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