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Full Length Research Paper

Effect of cisplatin on glutathione redox status in isolated plasma and cytosolic fraction

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Cisplatin has been used therapeutically in the treatment of malignant tumors. Meanwhile, the major limitations associated with cisplatin are its side effects in the form of nephrotoxicity, neurotoxicity, emetogenesis and emerging resistance. Most of these problems are due to its adverse effects on the body’s endogenous cytoprotective molecules like glutathione (GSH). In current study, the effect of glutathione on the improvement of cisplatin therapy along with control on its growing problem of resistance was emphasized. The effect of cisplatin on the chemical and metabolic status of glutathione was evaluated in human venous blood after its separation in to plasma and cellular fraction using ultraviolet (UV)-visible spectrophotometer. The glutathione in isolated plasma and cellular fractions of the blood was exposed to different concentrations of cisplatin. It was found that there was a gradual depletion in the concentration of reduced glutathione. Similarly, time-dependent effect of cisplatin was also evaluated on the status of glutathione, in which positive correlation was found between exposure of glutathione to the given concentrations of cisplatin and the depletion of reduced glutathione as the time passed from 0 to 5 h. This depletion in the concentration of reduced GSH is either due to formation of Pt-SG complex or due to the conversion of this multifunctional molecule (glutathione) to its physiologically inactive disulfide form (GSSG). This study was carried out in vitro, which in principle depicts a model of in vivo reaction. This decrease in blood GSH levels after cisplatin treatment will result in decreased antioxidant capacity of the blood, which in turn will result in numerous pathological conditions.

Key words: Reduced glutathione (GSH), plasma and cellular fractions of blood, Ellman’s method, cisplatin.

INTRODUCTION

Glutathione is the most abundant cytoprotective thiol that maintains redox environment of the cell, and thus helps in the vitality of the body cells (Guoyao et al., 2004). In the human body, glutathione mostly exist in its reduced form (GSH), but it can be oxidized by many factors including free radicals and during intoxication reactions. When GSH/GSSG ratio shifts toward the oxidizing state, it results in the activation of several signaling pathways, thereby reducing cell proliferation and increasing apoptosis (Sen, 2000). Thus, oxidative stress acts as key factor in the pathogenesis of many diseases, including cancer, inflammation and diabetes mellitus (Turrens, 2003), which can be minimized by glutathione. Glutathione is formed from three constituent’s amino acids namely glutamic acid, cysteine and glycine. Most of the cellular GSH (85-90%) is present in the cytosol, with the remainder being in many organelles (including the mitochondria, nuclear matrix, and peroxisomes). With the
exception of bile acid, which may contain up to 10 mmol/L GSH, extracellular concentrations of GSH are relatively low (e.g., 2-20 μmol/L in plasma (Jones, 2002). There are three major thiol-containing molecules in the erythrocytes namely hemoglobin, glutathione and ergothione. The concentration of GSH found in erythrocytes is about 2-3 mM. In leukocytes, the concentration of GSH is 5-10 mM and maximum concentration exists in hepatocytes (7-14 mM) because metabolism and most of other cellular reaction occurs in the liver. Many investigators (Bashandy et al., 2011; Suhair and Hamdi, 2011; Etuk et al., 2009) have proven the protective role of GSH in poisoning caused by plant extracts.

In view of the fact that a vast majority of cytotoxic metal-containing compounds including cisplatin are administered intravenously, special consideration should therefore be given to interactions of these metal drugs with macromolecular blood components like glutathione that can be taken up and get accumulated in tumor tissue (Kratz, 1993). cis-Dichlorodiamineplatinum(II) or cisplatin is a frequently used and very effective chemo-therapeutic drug for the treatment of various malignancies (Rosenberg, 1985; Prasad and Giri, 1994). However, high-doses administered to patients produce dose-dependent nephrotoxic and hepatotoxic side effects (Jordan and Carmo-Fonseca, 2000; Yoshida et al., 2000; Pratibha et al., 2006). Formation of free radicals, leading to oxidative stress, has been shown to be one of the pathogenic mechanisms of these side effects (Jordan and Carmo-Fonseca, 2000). The treatment of tumor cells with cisplatin provokes several responses, including membrane peroxidation, dysfunction of mitochondria, inhibition of protein synthesis and DNA damage (Cohen and Lippard, 2001; Sadowitz et al., 2002). But the ability of cisplatin to react with DNA and the formation of cisplatin-DNA adducts are thought to be the main mechanisms underlying its cytotoxic action (Pinto and Lippard, 1985; Zamble and Lippard, 1995). Therefore, in current study the effect of cisplatin on the redox status of glutathione is emphasized because there is an increasing amount of evidence that cisplatin-induced cytotoxicity is due to oxidative damage resulting from free radical generation and that the administration of antioxidants is efficient in inhibiting these side effects.

### MATERIALS AND METHODS

The followings were used: L-Glutathione (GSH) (Fluka), cisplatin or cis-dichlorodiamineplatinum(II) (Korea United Pharm.Inc), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma), sodium hydroxide (Fluka AG), potassium dihydrogen phosphate (Merck), HCl 35% (Kolchligt), disodium edetate (Riedel Dehean AG Sizze Hannover), sodium chloride (Merck), chloroform (Merck), ethanol (Merck), distilled water (double distilled), pH 7.6 buffer solution. The instruments used included: ultraviolet (UV)/visible 1601 spectrophotometer (Shimadzu), pH meter: NOV-210 (Nova scientific company Ltd. Korea), oven: Memmert Model U-30.854 (Schwa-bach, Germany), magnetic stirrer, hot plate: 400 (England), sensitive Sartorius weighing balance.

### Preparation of stock solutions

Briefly, 100 ml of 0.9% NaCl solution was prepared by dissolving 90 mg of a pharmaceutical grade sodium chloride in sufficient quantity of water. Cisplatin injection (UNISTIN 10 mg/20 ml), containing 1.7 mM of cisplatin base was diluted to 1.0 mM by adding 14,483 ml of water for injection to make 35 ml of cisplatin (1.0 mM) isotonic solution. Glutathione (1.0 mM) standard solution was prepared by dissolving 30.75 mg of GSH in 100 ml of 0.1 N HCl. Also, 200 ml of phosphate buffer (0.2 M) having pH of 7.6 was prepared by mixing 50 ml of monobasic potassium phosphate (KH2PO4) solution (0.2 M) with 42.2 ml of NaOH (0.2 M) and making the volume up to 200 ml with distilled water. Finally, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) solution (1.0 mM) was prepared by dissolving 39.6 mg of DTNB in 0.2 M phosphate buffer (pH 7.6) to make 100 ml of DTNB solution.

### Isolation of plasma

Venous blood was taken from a healthy human volunteer in a heparinized plastic bag. About 1.8 ml of this blood was then transferred by means of a disposable syringe (dipped in 0.5 M disodium edetate solution) to each of 2.0 ml Eppendorf's test tubes. Subsequently, the blood was centrifuged at 3600 rpm for 15 min, resulting in the precipitation of red blood cells. The supernatant layer of plasma, about 0.5 ml from each of the Eppendorf's tubes was taken and mixed with 50 µL of 5 mM disodium edetate solution and then placed in refrigerator at 4°C till use.

### Isolation of cytosolic fraction

The red blood cell fractions left after isolation of plasma in the test tubes earlier mentioned was taken and washed three times with 0.9% NaCl solution. This fraction of blood was centrifuged at 3000 rpm softly for the next 5 min. The supernatant layer was discarded and 0.5 ml of red blood cell fraction thus obtained was then mixed with 0.5 ml of distilled water. It was then placed in a refrigerator at 4°C for 1 h to induce lyses of red blood cells. Afterward, 0.6 ml of chloroform: ethanol (3:5) mixture was added to each of the above test tubes, mixed thoroughly to precipitate hemoglobin, followed by the addition of 0.1 ml of water. These mixtures were then centrifuged hard for 10 min at 10000-12000 rpm. The pale yellow supernatant layer (cytosolic fraction) from each test tube was collected and kept in refrigerator at 4°C till use.

### Determination of glutathione content after treatment with cisplatin

Both the extracellular (plasma) and intracellular (Lysate) glutathione content estimation after treatment with different concentration of cisplatin were carried out using modified Ellman’s (DTNB) method (Ellman’s, 1959). To 1 ml (1000 µL) of plasma and/or cytosolic fraction taken in five separate test tubes, 1 ml (1000 µL) of 0.2, 0.4, 0.6, 0.8 and 1.0 mM isotonic solutions of cisplatin were added separately and shaken to obtain cisplatin plus plasma and/or cytosolic fraction stock mixtures. The final concentrations of cisplatin in these stock mixtures were 0.1 mM (100 µM), 0.2 mM (200 µM), 0.3 mM (300 µM), 0.4 mM (400 µM) and 0.5 mM (500 µM). Next, the test samples were prepared for "0" time readings by taking 0.2 ml (200 µL) of cisplatin plus plasma and/or cytosolic fraction stock mixture from each of the previously prepared test...
The intracellular (cytosolic fraction) GSH content was measured after exposure to different concentrations of cisplatin for increased period of time (0 to 5 h). Therefore, the change in GSH content was statistically significant (p < 0.05) as shown in Figures 6 to 10.

### Interaction of cisplatin with intracellular GSH

The intracellular (cytosolic fraction) GSH content was measured after exposure to different concentrations of cisplatin for varying period of time. Glutathione content in isolated cytosolic fraction of blood showed gradual decrease in GSH content as it was exposed to increasing concentrations of cisplatin as shown in Table 2. Similarly, GSH content was also found to be proportionally decreased as it is exposed to cisplatin for increased period of time (0 to 5 h).

**Table 1. Effect of different concentrations of cisplatin on the chemical status of glutathione (GSH) with time in plasma.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Conc. of cisplatin (µM)</th>
<th>Real abs/GSH conc. at 0 h</th>
<th>Real abs/GSH conc. at 1 h</th>
<th>Real abs/GSH conc. at 2 h</th>
<th>Real abs/GSH conc. at 3 h</th>
<th>Real abs/GSH conc. at 4 h</th>
<th>Real abs/GSH conc. at 5 h</th>
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<td>0.321</td>
<td>0.301</td>
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<td>0.232</td>
<td>0.225</td>
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<td>0.256</td>
<td>0.233</td>
<td>0.211</td>
<td>0.196</td>
<td>0.187</td>
</tr>
</tbody>
</table>

Abs.: Absorbance; Conc.: concentration. Absorbance of 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) blank solution was 0.064 at 412 nm. *Real Absorbance = Absorbance of mixture - Absorbance of DTNB blank solution.

**RESULTS**

**Interaction of cisplatin with plasma glutathione level**

Extracellular GSH content was measured after exposure to different concentrations of cisplatin for different period of time. Glutathione content in isolated plasma fraction of blood showed gradual decrease in GSH content as it was exposed to increasing concentrations of cisplatin as shown in Table 1. Similarly, GSH content was also found to be proportionally decreased as it was exposed to cisplatin for increased period of time (0 to 5 h). Therefore, the change in GSH content was statistically significant (p < 0.05) as shown in Figures 1 to 5.

**DISCUSSION**

Biological thiols are gaining increasing interest of researchers because of their emerging use as...
Table 2. Effect of different concentrations of cisplatin on the chemical status of glutathione (GSH) with time in cytosolic fraction after separation of blood.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Conc. of cisplatin (mM)</th>
<th>Real abs*/GSH conc. at 0 h</th>
<th>Real abs*/GSH conc. at 1 h</th>
<th>Real abs*/GSH conc. at 2 h</th>
<th>Real abs*/GSH conc. at 3 h</th>
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<th>Real abs*/GSH conc. at 5 h</th>
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<tr>
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<td>0.281 1.84</td>
</tr>
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<td>26.67</td>
<td>0.347 2.16</td>
<td>0.323 2.02</td>
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<td>0.235 1.51</td>
<td>0.196 1.35</td>
<td>0.187 1.27</td>
</tr>
<tr>
<td></td>
<td>Real abs./conc. for blank GSH</td>
<td>0.566 3.44</td>
<td>0.562 3.41</td>
<td>0.563 3.42</td>
<td>0.558 3.39</td>
<td>0.555 3.37</td>
<td>0.554 3.37</td>
</tr>
</tbody>
</table>

Abs.: Absorbance; Conc.: concentration. Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank solution was 0.064 at 412nm. *Real Absorbance = Absorbance of mixture - Absorbance of DTNB blank solution.

Figure 1. Effect of cisplatin (6.67 µM) with time on isolated Plasma GSH. ■ Control Plasma GSH; ● Cisplatin + GSH. Results are the mean ± SEM of 3 experiments of plasma GSH.

Biomarkers of disease status. Besides their vital role in antioxidant biochemistry, thiols have functions such as: synthesis and maintenance of proteins structure and activity, redox sensitive signal transduction and receptor modification, cell growth and proliferation, apoptosis, xenobiotic...
Figure 2. Effect of cisplatin (13.33 μM) with time on isolated Plasma GSH. ■ Control plasma GSH; ♦ Cisplatin + GSH. Results are the mean ± SEM of 3 experiments of plasma GSH.

Figure 3. Effect of cisplatin (20.00 μM) with time on isolated Plasma GSH. ■ Control plasma GSH; ♦ Cisplatin + GSH. Results are the mean ± SEM of 3 experiments of plasma GSH.

Glutathione metabolism, and immune regulation (Sen, 1998). Glutathione (GSH), an endogenous intracellular thiol containing tripeptide is an important thiol that is mainly the center of concern in cancer therapy (Arrick and Nathan, 1984). In the present study, our main interest was to ascertain the interaction of cisplatin with important bio-molecule, glutathione at ex. vivo level as a model of in vivo reaction to depict the picture of its acute toxicity. Glutathione-S-transferase is the focal point of the detoxification system that protect cells from oxidative and chemicals induced toxicity by promoting the conjugation between the thiol (SH) group of glutathione and the electrophilic moiety of toxic substrates, including cisplatin (Welters et al., 2001).

According to our findings, when glutathione, the most abundant thiol in the cell that is maintained in reduced form by NADPH-dependent glutathione reductase (Wang and Ballatori, 1998) in the plasma and cellular fraction of the blood was exposed to various concentrations of cisplatin, there was significant decrease in the concentration of reduced glutathione. This shows that cisplatin causes an increased deployment of reduced glutathione.
Our results confirmed the relationship between cisplatin-mediated toxicity and decreased GSH levels, which has been previously observed in Dalton's lymphoma cells. When glutathione-S-transferase activity was assayed in Dalton's lymphoma cells, it was found to be decreased by 60-80% after cisplatin treatment. However, the low activity of glutathione-S-transferase along with lower Glutathione concentration in Dalton's lymphoma cells suggests the possibility of a reduced conjugation of GSH with cisplatin because it is known that cisplatin-GSH conjugates can be formed directly or

(GSH) and converts this multifunctional molecule to its oxidized or disulfide form (GSSG). The concentration of GSH in the samples after treatment with varying concentration of cisplatin was determined using λmax as 412 nm according to a well known Elman's method (Ellman, 1959). The concentrations of cisplatin that were used during the experiments range from 6.67 - 33.34 µM. The pH was maintained at 7.6 by phosphate buffer, which is nearly equal to body pH and was proposed to be suitable for performing the in vitro experiments on thiols by Evans (1975).

Figure 4. Effect of cisplatin (26.67 µM) with time on isolated plasma GSH. ■ Control plasma GSH; ♦ Cisplatin + GSH. Results are the mean ± SEM of 3 experiments of plasma GSH.

Figure 5. Effect of cisplatin (33.33 µM) with time on isolated plasma GSH. ■ Control Plasma GSH; ♦ Cisplatin + GSH. Results are the mean ± SEM of 3 experiments of plasma GSH.
catalyzed by GST (Ishikawa and Osman, 1993). This may also suggest reduced elimination of the drug through export pumps and availability of more drugs in tumor cells causing cytotoxic effects. Glutathione-S-transferase is the focal point of the detoxification system that protects cells from oxidative and chemical-induced toxicity by promoting the conjugation between the thiol (SH) group of glutathione and the electrophilic moiety of toxic substrates, including cisplatin (Welters et al., 2001). Previous studies have shown that resistance to cisplatin develop due to: (1) reduction in the uptake of the drug; (2) increased detoxification and excretion by conjugation of cisplatin by thiols; and (3) changes in the capability of the cell to recognize and process cisplatin - DNA adducts which in turn triggers apoptosis (Fink et al., 1998). If any of the aforementioned processes fail to function properly, then it may lead to resistance to cisplatin.

During this study, time-dependent effect of different concentrations of cisplatin on the chemical status of glutathione was also observed and it was found that there was a gradual depletion of reduced glutathione as the time passed from 0 to 5 h, showing that cisplatin is responsible for depletion of glutathione. The decreased level of glutathione in the body then contributes to
nephrotoxicity, mutagenicity and defects in immune response. Indeed, when GSH levels were increased in the hosts, the nephrotoxic as well as mutagenic effects of cisplatin treatment in cancer were found to be decreased (Della Rovere et al., 2000). It is depicted from the data obtained herein that the cytotoxicity of cisplatin is due to cisplatin-induced biochemical changes in plasma and cellular fraction of blood, effecting buffering capacity of glutathione and metabolic processes within the cell.

**Conclusion**

The interaction of cisplatin with glutathione causes reduction in the level of glutathione in the body due to the formation of Pt-SG complex or conversion of reduced GSH to its disulfide form (GSSG), which in turn predisposes us to numerous pathological conditions. It is thus concluded that antioxidant supplementation would be beneficial to increase the concentration of GSH that is reduced due to anticancer therapy of cisplatin.

**ACKNOWLEDGEMENTS**

We are thankful to Prof. Dr. Gul Majeed Khan, Dean, Faculty of Pharmacy and Mr. Nusratullah Khan, Chairman, Department of Pharmaceutical Chemistry,
Gomal University D. I. Khan, for providing necessary work place and essential instruments/equipments. They were kind enough to provide all the chemicals required during the research work.

REFERENCES


Figure 10. Effect of cisplatin (33.34 µM) with time on isolated CF GSH. ■Control CF GSH; ● Cisplatin + GSH. Results are the mean ±SE of 3 experiments of plasma GSH.
Full Length Research Paper

The effect of adding hyoscine to vaginal misoprostol on shortening the time of abortion induction

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Induced abortion is the termination of pregnancy by medical or surgical methods before the fetus’ viability. Available evidences show that due to spasmolytic effects, use of Hyoscine with Misoprostol may reduce the pain during abortion induction. The aim of this study is to evaluate the effect of Misoprostol in combination with Hyoscine compared with Misoprostol alone in reducing the duration of abortion induction. In a clinical trial at the Department of Obstetrics and Gynecology, Qazvin University of Medical Sciences on 126 pregnant women with gestational age below 20 weeks elected for abortion, the effect of Misoprostol in combination with Hyoscine compared with Misoprostol alone was evaluated in reducing the duration of abortion induction. The mean duration of abortion induction in the group receiving Misoprostol suppositories and Hyoscine was significantly lower than in the group receiving Misoprostol suppositories alone. The need for analgesics in the group receiving Misoprostol suppositories and Hyoscine was significantly less than in the group receiving Misoprostol suppositories alone (p < 0.001). Finally, results of this study showed that adding 20 mg Hyoscine intravenous to vaginal Misoprostol is effective in significantly reducing the duration of the abortion induction, getting less pain killers, less vaginal bleeding and less decrease in hemoglobin changes.

Key words: Vaginal misoprostol, hyoscine, induction of abortion.

INTRODUCTION

Induced abortion is the termination of pregnancy by medical or surgical methods before the fetus’ viability (Cunningham et al., 2010). Induce abortion in pregnant women is done due to medical causes, social problems or fetal disorders. Currently, severe anatomical, metabolic or rational abnormalities are the most common indications for induced abortion (Cunningham et al., 2010). Failure of abortion induction in presence of unripe cervix may lead to surgical termination of pregnancy (Alfirevic et al., 2009); therefore, the use of prostaglandins was considered to be helpful for cervical ripening. E₁ and E₂ prostaglandins are usable for cervical maturation. Type E₁ (Misoprostol) is more clinical applicable (Cunningham et al., 2010).

Side effects of prostaglandins (nausea and vomiting, diarrhea and fever) are an obstructive element of their use (Cunningham et al., 2010). Available evidences show that due to spasmolytic effects, use of Hyoscine with Misoprostol may reduce the pain during abortion induction (Baracho and Kamat, 1982; Tehranian, 2010). Hyoscine with Misoprostol may also reduce duration of
abortion induction due to increased cervical dilatation (Baracho and Kamat, 1982; Tehranian, 2010). According to prior studies, due to its spasmolytic effect and effectiveness on cervical dilatation, Hyoscine was effective in reducing the active labor duration (Sirohiwal et al., 2005; Samuels et al., 2007). Use of Hyoscine with doses up to 30 mg, has no significant side effects (Corsen, 1983). The aim of this study was to evaluate the effect of Misoprostol in combination with Hyoscine, compared with Misoprostol alone in reducing the duration of abortion induction.

**MATERIALS AND METHODS**

In a clinical trial at the Department of Obstetrics and Gynecology, Qazvin University of Medical Sciences on 126 pregnant women with gestational age below 20 weeks elected for abortion, the effect of Misoprostol in combination with Hyoscine, compared with Misoprostol alone, was evaluated in reducing the duration of abortion induction. A total of 126 pregnant women with gestational age below 20 weeks elected for abortion due to maternal or fetal problems were selected according to the inclusion/exclusion criteria, and randomized into two groups. Inclusion criteria included null pregnancy, forgotten abortion, and fetal malformations, and exclusion criteria were severe anemia, coagulopathy, use of anticoagulant drugs, active hepatic disease, cardiovascular disease, uncontrolled convulsion, history of adrenal disease and having intrauterine device (IUD), and history of mother’s allergy to prostaglandin and Hyoscine.

After obtaining patients’ consent, they were randomly divided into two groups. The first group underwent vaginal Misoprostol regimen (400 mg/6 h) with 20 mg IV Hyoscine single dose, with the first dose of Misoprostol, and the second group got vaginal Misoprostol regimen (400 mg/6 h) with 1 cc IV normal saline. Patients were hospitalized and their vital signs were assessed every four hours. Patients’ symptoms including nausea, vomiting, fever, and vaginal bleeding was recorded. Vaginal examination was performed at the time of putting the first suppository, followed by vaginal bleeding.

**RESULTS**

In this study, there was no significant difference in demographic characteristics, including maternal age, gestational age, gravidity, parity and history of abortion between the two study groups, and the two groups were homogeneous. These parameters are shown in Table 1. Comparison duration of abortion induction and admitting duration between two groups are shown in Table 2. Side effects of treatment between two groups are shown in Table 3. Duration of abortion induction (p < 0.001) (Figure 1), the rate of vaginal bleeding (p < 0.001), and the rate of hemoglobin deficiency (p = 0.002) in the group receiving Misoprostol suppositories and Hyoscine was significantly lower than in the group receiving Misoprostol suppositories alone. The need for analgesics in the group receiving Misoprostol suppositories and Hyoscine was significantly less than in the group receiving Misoprostol suppositories alone (p < 0.001).

**DISCUSSION**

The results of this study show that in the group receiving
Table 2. Comparing duration of abortion induction and admitting duration between two groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of abortion induction (minute)</td>
<td>653.38±80.38</td>
<td>726.29±64.56</td>
</tr>
<tr>
<td>admitting duration (day)</td>
<td>103±0.17</td>
<td>1.02±0.12</td>
</tr>
</tbody>
</table>

Table 3. Side effects of treatment between two groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>11 (17.2%)</td>
<td>9 (14.3%)</td>
</tr>
<tr>
<td>Need to narcotic</td>
<td>8 (12.7%)</td>
<td>32 (50.8%)</td>
</tr>
<tr>
<td>Vaginal bleeding (Sanitary napkin count)</td>
<td>1 31 (48.4%)</td>
<td>2 (302%)</td>
</tr>
<tr>
<td></td>
<td>2 29 (45.3%)</td>
<td>53 (84.1%)</td>
</tr>
<tr>
<td></td>
<td>3 4 (6.3%)</td>
<td>8 (12.7%)</td>
</tr>
<tr>
<td>Hemoglobin change</td>
<td>0.4391±0.23612</td>
<td>0.5597±0.24858</td>
</tr>
</tbody>
</table>

Figure 1. Distribution of abortion induction time between two groups.

Misoprostol suppositories and Hyoscine, the mean duration of abortion induction was significantly shorter than in the group receiving Misoprostol suppositories alone (653.38 ± 80.38 versus 726.29 ± 64.56 min) (p ≤ 0.001). Hyoscine is a muscarinic antagonist drug that causes loss of cervical spasm (Bhattacharya and Joshi, 1985), and may help cervical dilatation affecting uterine cervical neural system (Baracho and Kamat, 1982).
Vagal stimulation leads to increased spasms in the lower uterine segment and the cervix, and Hyoscine, as a kind of parasympatholytic drug, helps cervical dilatation affecting the vagus (Baracho and Kamat, 1982). Thus, regarding the effects of Hyoscine on the cervix, it may reduce the duration of abortion induction in the group receiving Hyoscine.

Tewari et al. (2003) demonstrated that administration of 40 mg IV Hyoscine in two divided doses reduced the mean duration of labor by five hours and twelve minutes in the group receiving hyoscine compared with the control group. Battacharya et al. (1985) studied the effect of 20 mg IM Hyoscine in the active phase of labor in 10 prim gravid women and concluded that the mean duration of labor is reduced by three hours and 40 minutes. In a study by Samel et al. (1998) and Samuels et al. (2007), IV administration of 20 mg Hyoscine had also led to shorter duration of labor.

In our study, as well as the results of these studies, administration of Hyoscine led to reduced duration of induction abortion in patients under study. In terms of the need for analgesics, the intervention group than the control group had significantly less need for analgesics (12.7 versus 50.8%) (p ≤ 0.001). In the study by Tehranian (2010), pain score was lower in the intervention group than in the control group, but this difference was not statistically significant.

Aggarwal et al. (2008) demonstrated that IV Hyoscine relieves the pain by 36% during labor. Perhaps one of the reasons for differences among studies is the severity of the pain that patients can tolerate, and the pain threshold varies in different individuals. In our study, rate of nausea and vomiting in the intervention group was lower than in the control group, but this difference was not statistically significant. The rate of vaginal bleeding in terms of the number of sanitary pads used in our intervention group was significantly lower than in the control group (p ≤ 0.001) and no fever and diarrhea were observed in any patient. Mean hemoglobin changes before and 6 h after labor were 0.4391 ± 0.23 in the intervention group and 0.5597 ± 0.24 in the control group; that in the intervention group was significantly lower than in the control group (p = 0.002)

Conclusion

Results of this study showed that compared with vaginal Misoprostol, IV administration of 20 mg Hyoscine with 400 µg vaginal Misoprostol significantly reduces duration of abortion induction, the need for analgesics, vaginal bleeding and drop in blood hemoglobin levels. Therefore, further studies are recommended to be conducted with larger sample size to investigate this efficiency.

REFERENCES


**Full Length Research Paper**

**Effect of diabetan on blood glucose, glycosylated hemoglobin, lipid profile, liver and kidney function tests of diabetic patients: A clinical, double blind, randomized trial**

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Accepted 8 November, 2012

Considering the side effects of insulin and oral hypoglycemic agents, this study was performed with the aim of investigating the hypoglycemic effect of Diabetan (glycogol) tablet, which is a blend of *Salvia officinalis*, *Trigonella foenum* and ginseng. A double-blind clinical trial was carried out on type-2 diabetic patients who were referred to the Endocrine and Metabolism Clinic of Shahrekord University of Medical Sciences. Eighty type 2 diabetic patients who had not reached the ideal control of the disease were randomly divided into case and control groups. The case group received Diabetan and the control group received placebo tablets three times a day for three months. Glycosylated hemoglobin (HbA1c), lipid profile, liver and kidney function tests were carried out at the beginning and at the end of the trial. The fasting blood sugar (FBS) and 2 h postprandial (2hpp) glucose were also checked at the beginning and every 2 weeks, for three months and were compared in two mentioned groups. The 2hpp blood sugar and cholesterol levels decreased significantly in Diabetan treated patients as compared to the control group (*P*<0.05). There were no significant changes in glycosylated hemoglobin and FBS between the two groups. Results showed that Diabetan tablets might be beneficial in diabetic patients to reduce 2hpp and cholesterol. However, higher doses might be needed to decrease fasting blood glucose and glycosylated hemoglobin.

**Key words:** Glycogol tablet, *Salvia officinalis*, *Trigonella foenum*, ginseng.

**INTRODUCTION**

Diabetes mellitus is considered as the most common endocrine disease; its prevalence which is being increased in human population (American Diabetes Association, 1997). The metabolic aspect of diabetes is characterized by moderate to severe hyperglycemia and impaired metabolism of nutrients, including proteins, carbohydrates and lipids (Yanardag et al., 2002). The side effects of taking insulin and oral hypoglycemic agents have brought about a growing interest among this group of patients for using natural products having anti-diabetic activity (Holman and Turner, 1991). Herbs are rich sources of natural antioxidants, and are used in traditional medicine for the control and treatment of many diseases. The reducing effect of a large number of these plants on blood glucose has been confirmed in animal models and clinical studies (Kazemi et al., 2010; Asgary et al., 2011; Asgari et al., 2012). Studies on animals have shown that more than 400 plant species have hypoglycemic activity and several laboratories are isolating edible herbal hypoglycemic compounds. Among the herbal drugs whose effect on blood sugar reduction has been proved in several human and animal studies are *Salvia officinalis*, *Trigonella foenum* and Ginseng (Asgari et al., 2012; Nikravesh and Jalili, 2003; Vuksan et al., 2000). Antioxidant properties of or *S. officinalis* L (Sage) leaves are known (Asgari et al., 2012). Moreover, the hypoglycemic effect of the alcoholic extract of *T. foenum* seeds has been confirmed in laboratory animals.

*Corresponding author. E-mail: rafieian@yahoo.com.
(Puri et al., 2002; Roghani et al., 2005) and the hypoglycemic effect of its extract has been studied in a limited number of healthy volunteers (Abdel-Barry et al., 2000). In this study, the combination of *S. officinalis* (45 mg), *T. foenum* seeds (50 mg) and *Ginseng* (60 mg) was prepared as glycogol tablet and the effect of this compound on blood glucose, glycosylated hemoglobin (HbA1c), lipid profile, liver and kidney function tests were investigated.

**METHODOLOGY**

This study was a double-blind randomized clinical trial on type-2 diabetic patients referred to the Endocrine and Metabolism Clinic of Shahrekord University of MEDICAL Sciences. Eighty diabetic type-2 patients referred to the clinic who did not have any complications of diabetes (based on description of their medical situation, physical examination, and paraclinical findings) including retinopathy, nephropathy and cardiovascular diseases, and those who had not achieved ideal control of diabetes and were willing to participate in the study, were selected and divided into 2 groups of 40 patients.

Selection of subjects for the medical treatment or placebo control was carried out by convenience random method. The project was done with due consideration to the ethics and obtaining permission from the ethics committee of Shahrekord University of Medical Sciences and obtaining the written consent of participants. Glycogol tablets were given to the first group and the second group took a placebo similar to that of glycogol (3 times a day).

Patients continued receiving their anti-diabetic drugs and other oral medications. First, a questionnaire containing information on age, sex, weight, blood pressure, family history of diabetes and duration of the disease was completed. The treatment period was three months and at the beginning and the end of the study, the HbA1c and lipid profile as well as liver and kidney function tests were taken. At the beginning of the study and every 2 weeks, fasting blood sugar (FBS) and postprandial blood were checked. Moreover, indicators relating to medication tolerance and the drugs' side effects were evaluated.

Patients were instructed to follow their type of diet and daily activities during the course of the study. These factors were controlled at the two-week visits as well. Moreover, the symptoms of hypoglycemia and the tasks needed for its treatment were taught. After the end of the 3-month period, patients repeated the tests taken at their arrival into the study and the results were analyzed using statistical tests (paired and independent t-tests and repeated measures test).

**RESULTS**

Eighty patients were evaluated in this study and were divided into 2 groups; one received the drug and the other received placebo. Nineteen patients were men and 51 were women. Five patients of each group were excluded due to uncontrolled high blood sugar, need for insulin injection, hospitalization and lack of proper cooperation. On the other hand, two patients in the drug group showed mild gastrointestinal complications without stopping the drug use.

Table 1 illustrates the mean and standard deviation (SD) of variables including age, body mass index (BMI) and duration of the disease in drug and control groups, in which none of them has significant difference. HbA1c value were 7.94±0.64 and 7.79±0.60, respectively in drug and placebo groups at the beginning of the study (P>0.05) and were 7.43±0.75 and 7.51±0.70 (P>0.05), respectively, at the end of the study. Table 2 shows the comparison between the mean concentration of FBS and 2-h postprandial blood glucose (2hpp) at the beginning and every two weeks up to three months. The results show a significant decrease in 2hpp in the medication group as compared to the placebo group. The mean of total cholesterol did not reveal any significant difference at the beginning of the study; however, at the end of study, it was lower in medication group and also lower than its initial value (P<0.05). Table 3 compares the mean triglyceride, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) in the studied groups before and after intervention. Table 4 compares the mean indices of renal function (BUN and creatinine) and liver function (alanine aminotransferase and aspartate aminotransferase) in the medication and placebo groups before and after intervention that showed no significant difference.

**DISCUSSION**

This investigation was aimed to study the hypoglycemic effect of glycogol tablet, which is a blend of *S. officinalis*, *T. foenum* and ginseng. The effects of glycogol on lipid profile, liver and kidney function tests were also evaluated in diabetic patients.

In this study, although the fasting blood glucose in drug group was 25 mg/dl less than in control group; however, the difference was not significant (P>0.05). 2hpp had significant difference in the 12th week of the study (P<0.05). The lack of significant reduction in fasting blood glucose by medication may be attributed to the drug's ineffectiveness on gluconeogenesis and insulin secretion and significant reduction in 2hpp by medication in the twelfth week due to lower insulin resistance.

As mentioned, there was no significant change in glycosylated hemoglobin. Since HbA1c has a considerable correlation with postprandial glucose, the lack of significant result in HbA1c can be attributed to the mean postprandial blood glucose during the treatment.
Table 2. Comparison between the mean concentration of fasting blood sugar (FBS) and 2 h postprandial blood glucose (2 hpp) on arrival and every two weeks to three months.

<table>
<thead>
<tr>
<th>Blood factor (mg/dl)</th>
<th>Time (Week)</th>
<th>Medication (Mean±SD)</th>
<th>Placebo (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On arrival</td>
<td>2nd</td>
<td>4th</td>
</tr>
<tr>
<td>FBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>144.25±43.62</td>
<td>140.71±30.26</td>
<td>145.34±32.96</td>
</tr>
<tr>
<td>Placebo</td>
<td>142.88±34.66</td>
<td>138.57±29.80</td>
<td>137.02±32.48</td>
</tr>
<tr>
<td>BS2hpp (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td>221.97±58.43</td>
<td>194.22±50.96</td>
<td>196.20±51.44</td>
</tr>
<tr>
<td>Placebo</td>
<td>214.65±42.83</td>
<td>196.62±45.27</td>
<td>198.80±48.13</td>
</tr>
</tbody>
</table>

Comparison between the mean concentration of FBS and BS2hpp was not significant during different weeks, but *in 12th week BS2hpp in medication group was less than placebo group (P<0.05).

Table 3. The comparison between the mean triglyceride, total cholesterol, low density lipoprotein and high density lipoprotein in the studied groups before and after intervention.

<table>
<thead>
<tr>
<th>Blood factor</th>
<th>Statistical index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medication (Mean±SD)</td>
</tr>
<tr>
<td></td>
<td>Before intervention</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>175.14±91.80</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>192.68±35.83</td>
</tr>
<tr>
<td>Low density lipoprotein</td>
<td>110.37±28.67</td>
</tr>
<tr>
<td>High density lipoprotein</td>
<td>41.42±8.35</td>
</tr>
</tbody>
</table>

*P<0.05, in comparison with the initiation of the study and placebo group.

Table 4. The comparison between the mean indices of renal function (blood urea nitrogen and creatinine) and liver function (alanine aminotransferase and aspartate aminotransferase) in the medication and placebo groups before and after intervention.

<table>
<thead>
<tr>
<th>Blood factor</th>
<th>Statistical index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medication (Mean±SD)</td>
</tr>
<tr>
<td></td>
<td>Before intervention</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>17.91±6.56</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.88±0.18</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>22.02±8.17</td>
</tr>
</tbody>
</table>

None of the above cases were significant (P>0.05).

which has not been at the desirable level, but the reduction in 2hpp at the last week may indicate the fact that if the medication continues, there will be probability of reduction in HbA1c as well.

The results showed that the mean total cholesterol at the end of the study had significant difference in the medication group as compared to the placebo group. Mean triglyceride, LDL and HDL in the studied groups had no significant difference before and after the intervention (P>0.05). The reduction in total cholesterol in the medication group can be an indicator of beneficial effect of this drug on patients who have hyperlipidemia. In addition, glycochol tablets contain T. foenum and this herb, in turn contains large amount of tannin and saponin that are able to reduce the intestinal absorption of lipids and maybe part of the effect of glycochol on cholesterol that takes place in this way (Sauvare et al., 1991). Fortunately, the drug and placebo have had no adverse and unwanted effect on liver and kidneys that shows the drug’s safety.

Considering the mentioned issues, it is possible that not achieving a significant result in the reduction of fasting blood glucose and HbA1c has been due to patients‘ disloyalty to diets, insufficient and irregular use of the medication and the low level of effective substances in glycochol tablets. However, one should note
their unwanted side effects, while using high dosages of herbs (Khajehdehi, 2012; Gheissari et al., 2012; Ardalan et al., 2012). It is recommended that further researches should be conducted, regarding the mechanism of glycogol effect on blood glucose reduction in diabetic patients, as a considerable help for patients in order to reduce the use of chemical medications.

ACKNOWLEDGEMENTS

Goldaru Pharmaceutical Drug Company is appreciated for funding this research and preparing the glycogol as well as its placebo. This paper has been derived from a MD thesis. We also appreciate Mrs. Dehqharian for her kind cooperation in conducting and implementing the research.

REFERENCES


**Full Length Research Paper**

**Polymorphism in glutathione S-transferase P1 (GSTP1) on the effect of epirubicin or doxorubicin chemotherapy among breast cancer**

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To investigate the glutathione S-transferase P1 (GSTP1) expression with chemotherapy polymorphisms involved in the prognosis of breast cancer patients with epirubicin or doxorubicin chemotherapy among breast cancer. 219 primary breast cancer patients who were consecutively recruited in our study have been treated with anthracycline-based (epirubicin [E] or doxorubicin [A]) chemotherapy between March 2006 and March 2007. All the patients were followed up until January 2012. Genotyping was based upon duplex polymerase-chain-reaction with polymerase chain reaction with confronting two-pair primers (PCR-CTPP) method. The frequencies of GSTP polymorphisms were found have significant difference in progesterone receptor status (P<0.05). GSTP1 Val/Val genotype had significantly higher rates of response to chemotherapy when compared to GSTP1 Ile/Ile genotype, and the adjusted odds ratios (OR) (95% confidence interval, CI) of 2.19 (1.23 to 4.21). Additionally, the GSTP1 Val allele genotype had significantly higher rates of response to chemotherapy, with adjusted OR (95% CI) of 1.71 (1.12 to 2.76). Patients with glutathione S-transferase M1 (GSTM1) null genotype had a longer average survival time and significantly lower risk of death than those with non-null genotypes [Hazard ratio (HR) (95% CI) = 0.66 (0.31 to 0.93)]. Similarly, those carrying GSTP1 Val/Val genotype had 0.54-fold the risk of death of those with GSTP1 Ile/Ile [HR (95% CI) = 0.54(0.29 to 0.90)]. GSTP1 expression was found to be associated with response to chemotherapy treatment of breast cancer and Estrogen receptor (ER) status had some mechanism between GSTP1 polymorphism and response to chemotherapy.

**Key words:** Glutathione S-transferase P1 (GSTP1), breast cancer, epirubicin or doxorubicin chemotherapy.

**INTRODUCTION**

Breast cancer is by far the most frequent cancer among women with an estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers). In China, it is one of the most leading causes of death in Chinese women with an incidence of 14.2/10⁵ (IARC, 2008). Neoadjuvant chemotherapy (NAC) is used to enhance the operability of breast cancer patients with advanced tumors who is known to enhance the operability of patients with advanced tumors previously considered inoperable, as well as making breast-conserving surgery more feasible for patients for whom such surgery was previously not feasible due to large tumor size. Anthracycline-based chemotherapy regimens are preferred for downstaging breast cancer tumors (Bafaloukos, 2005).

Glutathione S-transferases (GST) are a multigene family of enzymes which catalyze the conjugation of glutathione to electrophilic xenobiotics to inactivate them, and thus prevent DNA damage and adduct formation (Watson et al., 1998). There were five classes of GST superfamily, and glutathione S-transferase P1 (GSTP1), glutathione S-transferase theta 1 (GSTT1) and glutathione S-transferase M1 (GSTM1) genes of them are

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studied extensively for the inter-individual variations of chemotherapeutic effect (Stoehlmacher et al., 2002, 2004). GSTP1 is reported to be associated with resistance to chemotherapy in vitro studies (Satta et al., 1992; Whelan et al., 1992). GSTP1 was found to be associated with resistance to 5-fluorouracil (5-FU), doxorubicin, mitomycin C and paclitaxel (Su et al., 2003). However, the results of the relationship between GSTP1 and breast cancer with chemotherapy are conflicting (Peters et al., 1993; Schmidt et al., 2003). Therefore, it is needed to explore the association between GSTP1 expression and response to chemotherapy.

In this study, a prospective study to investigate the association of GSTs expression with response to neo-adjuvant sequential epirubicin or doxorubicin chemotherapy among breast cancer patients was conducted.

MATERIALS AND METHODS

Study population

Primary breast cancer patients (219), who were consecutively recruited in this study have been treated with anthracycline-based (epirubicin [E] or doxorubicin [A]) chemotherapy. All the cases were histologically confirmed between March 2006 and March 2007, and the chemotherapy was indicated for breast patients with stage IIA to IIIb. All the patients were treated with NAC. The chemotherapy included anthracycline-based (epirubicin [E] or doxorubicin [A]) chemotherapy. Anthracycline-based chemotherapy consists of cyclophosphamide (C), the anthracycline agent (E or A), and/or 5-fluorouracil (F), (CEF and CAF regimens) combined with radiotherapy. All patients were followed up till March 2011.

Response to chemotherapy evaluation

The responses of treatment were evaluated on the basis of standard Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Patients with complete response (CR), partial response (PR), or stable disease remained in the protocol until progressive disease or unacceptable toxicity was documented. Common toxicities were assessed according to the National Cancer Institute Common Toxicity Criteria (NCICTC).

Examination of the GSTP1 polymorphisms

The DNA samples were obtained from stored blood samples using the Qiagen Blood Kit (Qiagen, Chatsworth, CA). The GSTP1 I105V polymorphism was examined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. 0.1 μg of genomic DNA, forward primer 5’-ACC CCA GGG CTC TAT GGG AA-3’ and reverse primer 5’-TGA GGG CAC AAG AAG CCC CT-3’, were used for PCR amplification. Initial denaturation was carried out at 95°C for 5 min. Cycling conditions were: primer annealing at 55°C for 30 s, polymerization at 72°C for 30 s, and strand separation at 94°C for 30 s. Thirty cycles were carried out. A final polymerization step of 72°C for 5 min was carried out to complete the elongation processes. PCR products, after being digested by BsmAI restriction enzyme (New England Biolabs, Beverly, MA, USA) at 37°C for 2 h, were separated on 2% Nusieve ethidium bromide-stained agarose gels to visualize the bands.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software version 11.0.1 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Association between the various parameters was assessed using Chi-squared test or Fisher’s exact test. The association of polymorphisms of GSTP1 with response to chemotherapy in breast cancer patients was calculated by odd ratio (OR) with a corresponding 95% confidence interval (CI). The overall survival curves were plotted using the Kaplan-Meier product limit method, and the statistical differences in survival among subgroups were compared by log-rank test. The relative risk [hazard ratio (HR)] and 95% CI were calculated with the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). A primary death from breast cancer was defined as a failure event, and the survival time was defined as the time between diagnosis and death. The cause of death was defined by specialists based on clinical documents and reports by patients’ family members. If a patient died from a cause other than breast cancer, her data was censored at the date of death. P-value less than 0.05 was considered statistically significant.

RESULTS

For clinical characteristics, there was no significant difference between patients with GSTP1 Ile/Ile genotype and those with Ile/Val or Val/Val genotype (Table 1). A total of 112 patients carried GSTP1 Ile/Ile genotype and 107 patients carried GSTP1 Ile/Val or Val/Val genotype. The frequencies of GSTP polymorphisms were found to be significantly different in progesterone receptor status (P<0.05). Among 219 patients, 125 patients showed response to chemotherapy. Among 125 responders, about 45.6% of them took GSTP1 Ile/Ile genotype, 24.8% showed GSTP1 Ile/Val genotype, and the remaining 30.4% were GSTP1 Val/Val genotype (Table 2). GSTP1 Val/Val genotype had significantly higher rates of response to chemotherapy when compared with GSTP1 Ile/Ile genotype, and the adjusted OR (95% CI) of 2.19 (1.23 to 4.21). Additionally, the GSTP1 Val allele genotype had significantly higher rates of response to chemotherapy, with adjusted OR (95% CI) of 1.71 (1.12 to 2.76). The average survival time was 36.6 months. Patients with GSTM1 null genotype had a longer average survival time and significantly lower risk of death than did those with non-null genotypes [HR (95% CI) = 0.66 (0.31 to 0.93)] (Table 3). Similarly, those carrying GSTP1 Val/Val genotype had 0.54-fold the risk of death of those with GSTP1 Ile/Ile [HR (95% CI) = 0.54(0.29 to 0.90)]. There was no significant association between GSTT1 gene polymorphisms and risk of death.

DISCUSSION

The aim of this study was to investigate whether GSTP1 expression was association with the anthracycline-based chemotherapy among breast cancer patients. This study found the GSTP1 expression to be associated with estrogen receptor (ER) status, and was association with
Table 1. Association of GSTP1 expression with clinic characteristics of breast cancer.

<table>
<thead>
<tr>
<th>GSTP1 expression</th>
<th>Total</th>
<th>Ile/Ile (wild-type) n=112</th>
<th>%</th>
<th>Ile/Val or Val/Val (mutant) n=107</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>81</td>
<td>43</td>
<td>38.39</td>
<td>38</td>
<td>35.51</td>
<td>0.66</td>
</tr>
<tr>
<td>≥50</td>
<td>138</td>
<td>69</td>
<td>61.61</td>
<td>69</td>
<td>64.49</td>
<td></td>
</tr>
<tr>
<td><strong>Menopausal status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>71</td>
<td>35</td>
<td>31.25</td>
<td>36</td>
<td>33.64</td>
<td>0.71</td>
</tr>
<tr>
<td>Post</td>
<td>148</td>
<td>77</td>
<td>68.75</td>
<td>71</td>
<td>66.36</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>26</td>
<td>23.21</td>
<td>24</td>
<td>24.43</td>
<td>0.58</td>
</tr>
<tr>
<td>II</td>
<td>91</td>
<td>43</td>
<td>38.39</td>
<td>48</td>
<td>44.86</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>76</td>
<td>42</td>
<td>37.50</td>
<td>34</td>
<td>31.78</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>24</td>
<td>14</td>
<td>12.50</td>
<td>10</td>
<td>9.35</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>146</td>
<td>77</td>
<td>68.75</td>
<td>69</td>
<td>64.49</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>22</td>
<td>11</td>
<td>9.82</td>
<td>11</td>
<td>10.28</td>
<td>0.61</td>
</tr>
<tr>
<td>T4</td>
<td>27</td>
<td>11</td>
<td>9.82</td>
<td>16</td>
<td>14.95</td>
<td></td>
</tr>
<tr>
<td><strong>Estrogen receptor (ER)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>109</td>
<td>53</td>
<td>47.32</td>
<td>84</td>
<td>52.34</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>-</td>
<td>110</td>
<td>59</td>
<td>52.68</td>
<td>23</td>
<td>47.66</td>
<td></td>
</tr>
<tr>
<td><strong>Progesterone receptor (PR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>122</td>
<td>42</td>
<td>37.50</td>
<td>80</td>
<td>74.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>-</td>
<td>97</td>
<td>70</td>
<td>62.50</td>
<td>27</td>
<td>25.23</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Distribution of GSTP1 in responders and non-responders to neoadjuvant chemotherapy for breast cancer.

<table>
<thead>
<tr>
<th>Genotype (GSTP1)</th>
<th>Responders (n=125)</th>
<th>%</th>
<th>Non-responders (n=94)</th>
<th>%</th>
<th>Odds ratio (95% CI)¹</th>
<th>Odds ratio (95% CI)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile/ Ile</td>
<td>57</td>
<td>45.6</td>
<td>51</td>
<td>54.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>31</td>
<td>24.8</td>
<td>20</td>
<td>21.28</td>
<td>1.39(0.67-2.9)</td>
<td>1.56(0.78-3.24)</td>
</tr>
<tr>
<td>Val/Val</td>
<td>38</td>
<td>29.6</td>
<td>22</td>
<td>24.46</td>
<td>1.55(0.78-3.22)</td>
<td>2.19(1.23-4.21)</td>
</tr>
<tr>
<td>Val allele</td>
<td>68</td>
<td>54.4</td>
<td>43</td>
<td>45.74</td>
<td>1.42(0.79-2.51)</td>
<td>1.71(1.12-2.76)</td>
</tr>
</tbody>
</table>

¹Non-adjusted; ²Adjusted for age, menopausal status, tumor grade, tumor size, ER, PR and therapeutic regimen.

Previous studies (Miyake et al., 2012). Previous evidences showed that the GSTP1 are involved in response to chemotherapy in various cancers (Ott et al., 2008; Mossallam et al., 2006; Funke et al., 2010; Nagle et al., 2007). However, few studies are conducted in Chinese breast cancer patients. Only several studies conducted in western countries investigation the association of GSTs with chemotherapy response and survival of breast cancer, but the results are conflicting (Oliveira et al., 2010; Mishra et al., 2011; Lourenço et al., 2010). A previous study showed that GSTP1 105 Val may be association with poor survival of breast cancer, and have lower response to chemotherapy treatment for this cancer (Oliveira et al., 2010). However, in another study in Asian population, no significant association was found in GSTP1 and responses to chemotherapy (Mishra et al., 2011), and another study conducted in Japan found a significant responses to chemotherapy patients with GSTP1 Val allele genotype (Ott et al., 2008). This study finds a significant association
between GSTP1 Val allele genotype and response to chemotherapy. These inconsistency results might be induced by differences in ethnicities, source of patients, or sample size. Therefore, further studies are needed to analyze the effect of polymorphism in GSTP1 on chemotherapy among breast cancer.

The relationship between GSTP1 expression and ER status was found, indicating that the GSTP1 plays a significant role in the suppression of anti-tumor activity of ER status. A previous study indicated the univariate and multivariate analysis of the pathological response to P-FEC showed that only GSTP1 expression was significantly associated with a lower pathological complete response (pCR) rate in ER-negative tumors, but not in ER-positive tumors (Miyake et al., 2012). It has been well established that the pCR rate of ER-positive tumors in response to chemotherapy is lower than that of ER-negative tumors (Kaufmann et al., 2007). In this study, GSTP1 expression was found to be associated with ER status, which indicates that ER had some mechanism between GSTP1 polymorphism and response to chemotherapy. The result is in line with previous ones.

Conclusively, GSTP1 expression was found to be associated with response to chemotherapy treatment of breast cancer, and ER status had some mechanism between GSTP1 polymorphism and response to chemotherapy. Further prospective studies incorporating larger numbers of patients are needed to validate these associations.

**REFERENCES**


Therapeutic antiepileptic drug monitoring pattern in a tertiary care hospital in Oman

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In epilepsy, therapeutic drug monitoring (TDM) could aid in individualizing dosage regimen and ascertaining compliance on anti-epileptic drugs (AEDs). The aim of this study was to survey the requests for TDM of AEDs to determine drugs involved, observed concentrations, reasons for requests and action undertaken. TDM requests for AEDs were surveyed at a university hospital in Oman from January 2006 to December 2009. A total of 151 patients with 354 TDM requests were collected. These requests were for valproic acid (46.9%), phenytoin (26.8%), carbamazepine (25.4%) and phenobarbital (0.8%). 50, 37 and 13% of all reported concentrations were below, within and above therapeutic range, respectively. For majority of the subjects (70%), there were no clear reasons for plasma concentrations to lie outside the therapeutic range. No change in the drug therapy/dosing was required subsequent to the TDM reports in 42.7% of the cases. Emergency department was the main unit requesting TDM (63.8%) and TDM was mostly indicated for an increase in the seizures frequency on the same day (62.7%). This study provides an overview of the specific requests for TDM of AEDs in routine clinical practice which might help in auditing and improving this service for optimal utilization.

Key words: Antiepileptic drugs, therapeutic drug monitoring, epilepsy, plasma.

INTRODUCTION

Therapeutic drug monitoring (TDM) is a useful tool in individualizing drug therapy, enhancing drug’s efficacy and improving patient’s safety (Kang and Lee, 2009). Epilepsy is one of the most common disorders in which TDM has been utilized for optimizing pharmacotherapy (Eadie, 1976; Kutt and Penry, 1974). This is due to the pharmacokinetic characteristics of the most commonly used drugs such as carbamazepine, phenytoin, phenobarbital and valproic acid in the developing countries. These antiepileptic drugs (AEDs) have narrow therapeutic indices, inter-individual pharmacokinetic variations, multiple drug interactions, pharmacokinetic interactions and a good correlation between plasma concentrations and clinical efficacy and safety (Johannessen and Tomson, 2006; Patsalos and Perucca, 2003, Patsalos et al., 2008; Toledano and Gil-Nagel, 2008). TDM could also help in ascertaining patient’s compliance with AED. In contrary to older AEDs, the newer AEDs such as levetiracetam, topiramate and tigabine have less drug interaction profile, larger therapeutic indices and more predictable pharmacokinetic profiles (Hachad et al., 2002; Zaccara et al., 2006). Moreover, there is no clear correlation between TDM and clinical efficacy or adverse effects of the newer AEDs. Thus, TDM has little, if any, clinical role in the manage-ment of epileptic patients on newer AEDs (Krasowski, 2010; Neels et al., 2004; Striano et al., 2008).
At Sultan Qaboos University Hospital (SQUH), a university teaching hospital in Oman, monitoring of the free plasma concentrations is available for carbamazepine, valproic acid and phenytoin and total serum concentrations for phenobarbital. The aim of this survey of requests for TDM is to identify the drugs involved, the plasma concentrations observed, the reasons for the requests and clinical actions taken towards the results.

**METHODOLOGY**

This study was part of a utilization study of AEDs in adult (>18 years) epileptic patients undertaken over a period of 4 years from January 2006 to December 2009 at SQUH. All the TDM requests for AEDs were collected retrospectively from the electronic patients’ records where the requests were punched in. Plasma concentrations were measured by fluorescence polarization immunoassay using semiautomatic analyzer (Roche Diagnostic Systems, USA). A standard TDM data sheet included information on epilepsy type and cause(s), co-morbidities, list of prescribed AEDs, frequency of seizures and the indications for TDM, apart from demographic characteristics.

This study was approved by the SQU Medical Research and Ethics Committee. For categorical variables, frequencies and percentages were reported. For continuous variables, means and standard deviation were used to summarize the data. Statistical analyses were performed using Statistical Package of Social Sciences version 15 (SPSS, Inc., Chicago, IL, USA).

**RESULTS**

A total of 151 patients with 354 TDM requests were collected (2.32 requests per patient) over the 4-year study period. The mean age of the patients was 34.6 ± 14.3 years (range 19 to 93 years) with a male to female ratio of 1:3.1.

Drugs that were prescribed along with the monitored AEDs were levetiracetam (19.8%), clonazepam (15.3%), topiramate (14.4%) and lamotrigine (12.4%). Majority of the patients (62.7%) have had a seizure on the same day of the TDM request. However, information about the pattern, type or frequency of seizures could not be determined reliably in some of the cases (22.3%). Emergency department was the main unit for requesting TDMs (63.8%) and these were mostly indicated for an increase in seizures frequency (69.8%) and suspected side effects (7.9%).

Table 1 summarizes the frequency of seizures, TDM indications, the locations for ordering TDM requests and possible reasons for sub-therapeutic and above therapeutic concentrations, while Table 2 lists the clinical actions taken towards the concentrations outlying the therapeutic range for the monitored AEDs. Majority of the TDM requests were for valproic acid (46.9%) followed by phenytoin (26.8%), carbamazepine (25.4%) and phenobarbital (0.8%).

The reported concentrations that were lying below the therapeutic range, within the therapeutic range and above the therapeutic range were 50, 37 and 13%, respectively. No clear clinical reasons were identified in majority of the cases (70%) in whom the plasma concentration was outside the therapeutic ranges. Furthermore, there were no clinical actions taken towards these concentrations in many of these cases (42.7%). Poor compliance with the treatment regimen was difficult to be evaluated and it was only apparent in 24.2% of the requests.

**DISCUSSION**

In this study, 354 TDM requests for 151 patients were surveyed retrospectively at a university hospital in Oman. The most requested plasma concentrations were for valproic acid (46.9%) followed by phenytoin (26.8%), carbamazepine (25.4%) and phenobarbital (0.8%). Majority of these requests were ordered from the emergency department (63.8%) for patients presenting with seizures on the same day of the request. Half of all the requests (50%) were below the therapeutic range, while the other was either within (37%) or above (13%) the therapeutic range. A seizure due to poor compliance to medication was apparent in only 24.2% of the requests.

Despite decades of use of TDM of AEDs in the management of epilepsy, the correlation between the TDM and clinical efficacy of AEDs is not fully clear (Camfield and Camfield, 2006; Chan and Beran, 2008; Dreyfus, 2010; Glauser and Pippenger, 2000; Jannuzzi et al., 2000; Johannessen and Landmark, 2008). TDM have been used with almost all old AEDs, but such use has declined with time. This is partly due to the introduction of newer AEDs (Schmidt, 2009). In developing countries, TDM of older AEDs is requested in the setting of suspected problems with drug compliance, adverse effects or overdose (Jose et al., 2006; Palmini, 2000; Radhakrishnan, 2009). The same indications were observed in this study. Thus, the main indication for TDM requests was an increase in seizure frequency (69.8%) possibly due to suspected insufficient plasma concentrations of AEDs. Most of these seizure occurred at the same day of the requests (62.7%). Poor compliance was apparent in only 24.2% of the subjects. However, it might be attributed to the reported sub-therapeutic concentrations in most of the subjects (50%), though it could not be ascertained. This could also be supported from the finding that no actions were taken towards the sub-therapeutic reported concentrations in most of the subjects (42.7%). Furthermore, for the three drugs: carbamazepine, phenytoin and valproic acid, most of the subjects were discharged with the same dosage regimen they were on before experiencing seizures. This might indicate that TDM played little, if any, role in the clinical management of the patients. Similar conclusions have been reached in previous studies (Jannuzzi et al.,...
Table 1. Frequency of seizures, TDM indications, the locations of TDM requests and reasons for sub-therapeutic and above therapeutic range concentrations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency of seizures/month</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>29 (8.2)</td>
</tr>
<tr>
<td>4-6</td>
<td>19 (5.4)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>20 (5.6)</td>
</tr>
<tr>
<td>Information not available</td>
<td>247 (69.8)</td>
</tr>
<tr>
<td>Others</td>
<td>39 (11.0)</td>
</tr>
<tr>
<td><strong>Time of last episode of seizure(s) to TDM request</strong></td>
<td></td>
</tr>
<tr>
<td>Same day</td>
<td>222 (62.7)</td>
</tr>
<tr>
<td>Same week</td>
<td>35 (9.9)</td>
</tr>
<tr>
<td>Within 4 weeks</td>
<td>10 (2.8)</td>
</tr>
<tr>
<td>1-3 months</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>&gt; 3 months</td>
<td>5 (1.4)</td>
</tr>
<tr>
<td>Information not available</td>
<td>79 (22.3)</td>
</tr>
<tr>
<td><strong>Indications for TDM request</strong></td>
<td></td>
</tr>
<tr>
<td>Side effects</td>
<td>28 (7.9)</td>
</tr>
<tr>
<td>Increase seizures frequency</td>
<td>247 (69.8)</td>
</tr>
<tr>
<td>Routine</td>
<td>22 (6.2)</td>
</tr>
<tr>
<td>Upward titration of dose</td>
<td>21 (5.9)</td>
</tr>
<tr>
<td>Others</td>
<td>36 (10.2)</td>
</tr>
<tr>
<td><strong>Locations of the TDM request</strong></td>
<td></td>
</tr>
<tr>
<td>Emergency department</td>
<td>226 (63.8)</td>
</tr>
<tr>
<td>Outpatient department</td>
<td></td>
</tr>
<tr>
<td>Neurology</td>
<td>15 (4.2)</td>
</tr>
<tr>
<td>Psychiatry</td>
<td>15 (4.2)</td>
</tr>
<tr>
<td>Others</td>
<td>5 (1.4)</td>
</tr>
<tr>
<td>Inpatients wards Medical</td>
<td>62 (17.5)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>7 (2.0)</td>
</tr>
<tr>
<td>ICU</td>
<td>11 (3.1)</td>
</tr>
<tr>
<td>Others</td>
<td>13 (3.7)</td>
</tr>
<tr>
<td><strong>Reasons for therapeutic range outliers (n = 223)</strong></td>
<td></td>
</tr>
<tr>
<td>Toxic concentrations (n = 46)</td>
<td></td>
</tr>
<tr>
<td>Errors in taking the drugs</td>
<td>3 (6.5)</td>
</tr>
<tr>
<td>Interaction with other drugs</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Unclear reasons</td>
<td>42 (9.3)</td>
</tr>
<tr>
<td>Subtherapeutic concentrations (n = 177)</td>
<td></td>
</tr>
<tr>
<td>Poor compliance</td>
<td>54 (30.5)</td>
</tr>
<tr>
<td>Out of medication</td>
<td>8 (4.5)</td>
</tr>
<tr>
<td>Interaction with other drugs</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Unclear reasons</td>
<td>114 (64.4)</td>
</tr>
</tbody>
</table>

2000; Minshall et al., 2011; Sharma et al., 2009; Walters et al., 2004). Other subjects required the addition of another drug (15.0%), an increase or a decrease in the dosage regimen (17.5%) or completely stopping the drug (5.1%). This might be an indication to ineffectiveness of the drug, insufficient plasma concentrations or intolerable
side effects. In general, due to significant fluctuations in the plasma concentrations of sodium valproate, determination of valproate level is being discouraged (Shorvon, 2010). Since the timing of the blood collection with regard to the last dose of AEDs was not available, it was difficult to determine whether the plasma level reflected trough or peak concentration.

The concept of “therapeutic range” for AEDs has always been debated (Jannuzzi et al., 2000; Johannessen and Landmark, 2008; Patsalos et al., 2008; Tomson et al., 2007). While some patients might be well controlled with plasma concentrations well below the therapeutic range, others might require a higher than therapeutic range plasma concentrations without demonstrating unacceptable adverse effects. To overcome the limitations that aroused with the “therapeutic range”, the concept “individual therapeutic concentration” emerged and appeared to be well accepted (Jannuzzi et al., 2000; Johannessen and Landmark, 2008; Patsalos et al., 2008; Tomson et al., 2007). Thus, TDM of AEDs has become more rewarding in selected patients with specific clinical conditions and for specific AEDs. Furthermore, pharmacogenomics also play a role in the inter-individual differences in AEDs concentrations (Cavalleri et al., 2011; Pandolfo, 2011). A prospective study incorporating pharmacogenomics would aid in individualizing therapy in certain patients in this era of personalized medicine.

Owing to the nature of the clinical presentations of seizures, majority of these requests were ordered from the emergency department. Epilepsy at SQUH is primarily managed by neurology unit, despite the fact that only 4.2% of all requests originated from this unit. This might reflect an awareness and adherence to the current guidelines in adopting selective request for TDM of AEDs (Minshall et al., 2011; Patsalos et al., 2008).

Several questions emerged while interpreting the collected data and could not be well resolved. Potential concerns were: was the reported concentration trough or peak? Was the sampling time appropriate for these concentrations? Was the reported values steady state concentrations? What was the nature of drug interactions?

**Conclusion**

This study provides an overview of the specific requests for TDM of AEDs in routine clinical practice and this could be the first step towards subsequent auditing of this laboratory service for optimal utilization. The results of this study suggest that the TDM of older AEDs can still be of some value in the management of patients with epilepsy mainly if indicated for therapeutic noncompliance, exploring sub-therapeutic concentrations and toxicity which might aid in subsequent clinical decision.

**REFERENCES**

**UPCOMING CONFERENCES**

International Conference on Pharmacy and Pharmacology, Bangkok, Thailand, 24 Dec 2013


1st Annual International Conference on Pharmacology and Pharmaceutical Sciences (PHARMA 2013)

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