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Research Article

Renal Protective effects of Melatonin in rat treat by Gemcitabine

Abstract

Nephrotoxic side effects of gemcitabine have been reported in patients receiving this drug medication. Melatonin is a potent free radical scavenger and antioxidant and has protective effects against ischemic damage. In the present study, we examined the relationship between the renal protective effects of melatonin in tubular damage induced by gemcitabine. This study was conducted simultaneously as a case of 48 male wistar rat, weighing approximately 20 ± 250 g and aged 8 weeks were used. The rats were randomly divided into 3 groups.

Group 1; has found the amount of normal saline. The treatment group was divided into two sub-groups, groups, (T1) the melatonin doses 10 (mg/kg), as well as gemcitabine dose 50 (mg/kg) intraperitonealy (IP) received. (T2) the melatonin doses 20 (mg/kg),), as well as gemcitabine dose 50 (mg/kg) intraperitonealy (IP) received. The experimental group was divided into 3 sub-groups, (T3, T4, and T5). (T3); received gemcitabine with dose of 50 (mg/kg) intraperitonealy (IP). Group (T4) melatonin doses 10 (mg/kg) and (T5); melatonin doses 20 (mg/kg) and intraperitonealy (IP) received. Each group consisted of eight rat. In the group receiving only gemcitabine (T3), renal lesions were observed as tubular degeneration and necrosis. The combination of gemcitabine and melatonin reduced the renal injury. The lesions in group T5 were remarkably reduced. The results of this study shows that melatonin was effective in reducing nephrotoxicity effects of gemcitabine.

Introduction

Due to the increasing numbers of cancer patients and breaking this out, medical science presents new drugs to market every day, but in spite of having high performance, most treatment of these drugs have side effects and are toxic to other cells and tissues. Among the most effective anti-cancer drugs which treat a variety of malignant tumors is gemcitabine. Previous studies on the effects of toxic side effects in other tissues have been observed during treatment [1].

Gemcitabine mechanism based on the specification phase of the cell or cells which were mainly made DNA (Phase - s) are destroying it. [2] Melatonin is a hormone that is secreted by the brain's pineal gland. Melatonin has a hormone neuronal and works as well as safety regulator and temperature. Moreover, melatonin effects on proliferation and cell differentiation too. Also, studies have shown that melatonin acts as an antioxidant and protects against cancer [3,4]. Melatonin participates in many important physiological functions, including anti-inflammation [5], and immunoregulation [6], as well as acting as a broad spectrum antioxidant [7]. In addition, melatonin protects against liver injury induced by endotoxin shock [7], ischemia/reperfusion [8], and protective effect of melatonin against UV radiation (Scheuer et al. 2014) in rats through its antioxidant action. The antioxidant property of melatonin in the treatment of cancer patients, taking chemotherapy drugs, are important. Melatonin can be taken to neutralize free radicals, including hydroxyl radicals, proxy and anions proxy nitrate [9]. Taking melatonin in rat cells against oxidative damage can protect various ways to reduce the oxidant stress [10]. The purpose of this study was to investigate of protective effect of melatonin on nephrotoxicity induced by gemcitabine in rat.

Materials and Methods

Animals

Adult male Wistar rats weighing 200-230 g were used in the study. The animals were maintained in an air conditioned animal house at a temperature of 22±2 °C, relative humidity of 57±2% and photo-cycle of 12:12 h light and dark. The animals were provided with standardized pelleted feed and drinking water ad libitum. All the experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee. The animals were observed daily for any signs of toxicity. Body weight was recorded at regular intervals throughout the experimental period.

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After acclimatization a period of one week, the animals were randomly divided into three groups and each group consisted of 8 rat.

The treatment group was divided into two sub-groups, groups, (T1) the melatonin doses 10 (mg/kg), as well as gemcitabine dose 50 (mg/kg) intraperitonealy (IP) received. (T2) the melatonin doses 20 (mg/kg),), as well as gemcitabine dose 50 (mg/kg) intraperitonealy (IP) received. The experimental group was divided into 3 sub-groups, (T3, T4, and T5). (T3); received gemcitabine with dose of 50 (mg/kg) intraperitonealy (IP). Group (T4) melatonin doses 10 (mg/kg) and (T5); melatonin doses 20 (mg/kg) and intraperitonealy (IP) received. The selection of melatonin dosage was based on previous studies [11].

Melatonin

The Amount of 500 mg melatonin dissolve in propylene glycol (PG) and dilute with double distilled water to reach the PG final concentration of 10% (v/v) and melatonin to 10 mg/ml. sodium chloride was added in order to prepare isotonic solution. The final solution was filtered through 0.22 µm membrane (Merck Millipore, USA). The container was preserve from light and kept in 4°C. the lethal dose of PG in rat is 20.9g/Kg[1] and the injection dose of PG in our examination for injection is very lower than it [12].

Gemcitabine

To prepare the injectable form gemcitabine, Jmzar 250 mg vials was used. Based on the structure and resources with sodium chloride 0.9%, the only proven solution is to dilute gemcitabine sterile powder.

Histopathology

After 14 days, rats were anesthetized and euthanized. A fresh piece of the kidney from each rat, previously trimmed to approximately 2 mm thickness, was rapidly fixed in 10% neutral formalin. The fixed tissues were then embedded in paraffin, sectioned (5 µm) with a rotary microtome and stained with histological dye, Hematoxylin and Eozine (H&E). The typical histopathological lesions, such as inflammation, tubular necrosis, tubular degeneration were evaluated. Histopathology scoring was done according to the below pattern [13]:

Tubular degeneration (each of 100 tubules): 0= No degeneration, 1 = <25%, 2 = 25-50%, 3 = 50-75%, and 4 =.> 75%.

Tubular necrosis (each of 100 tubules): 0= No necrosis, 1 = <25%, 2 = 25-50%, 3 = 50-75%, and 4 =.> 75%.

Inflammation: 0 = no inflammation, 1 = focal inflammation in less than 25% of the tissue, 2 = focal inflammation in 25-50% of the tissue, 3 = extensive, but focal inflammation, and 4 = global inflammation.

Statistical analysis

Statistical analyses were carried out using SPSS 10 program for Windows (SPSS, Chicago, Ill). Data were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance followed by Turkey post hoc test. The criterion for statistical significance was $p<0.05$.

Results

Histopathology show that no tubular degeneration, necrosis and inflammation in the only melatonin group (T1 and T2) rats. Sever tubular degeneration and moderate necrosis with infiltration of mononuclear inflammatory cells in renal interestitum were found in gemcitabin rats (T3). In the other hand, administration gemcitabine with melatonin significantly decreased tubular degeneration, necrosis and inflammation (Figures 1-3, Table 1).

Discussion

The study considers protective effect of melatonin on kidney tissue of adult male rats treated by gemcitabine. Dose of chemotherapy drugs, which depend on the patient›s weight and other factors in their treatment, varies according to the severity of the disease and the treatment is different. In this study, we have used the lowest dose that even the lowest dose, gemcitabine cause significant side effects and complications such as kidney was full of blood and inflammation, necrosis, inflammatory cells which clearly perceived. Studies have shown that the level of risk in using gemcitabine increases while the oncologists increase the efficiency of chemotherapy and use gemcitabine with other drugs [14]. Tissue and cellular

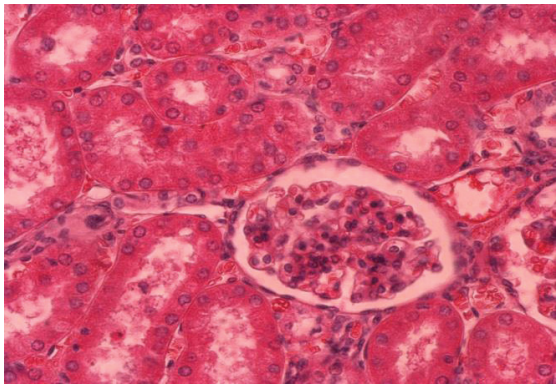

Figure 1: Renal tissue in melatonin group (T2) showed normal renal tissue (H & E, ×640).

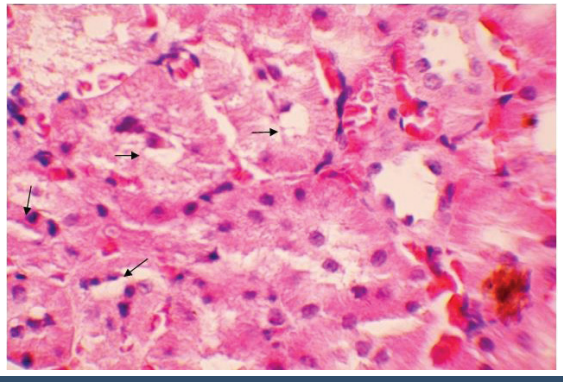

Figure 2: Renal tissue in gemcitabine group (T3) showed degeneration and necrosis of renal tubular epithelial cells (arrow); (H & E, ×640).

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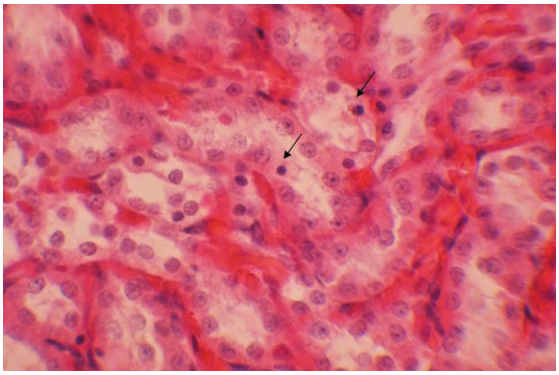

Figure 3: Renal tissue in treatment group (T5) showed mild degeneration and necrosis in tubular epithelial cells (arrow); (H & E, ×640).

Table 1: Histopathologic lesions in experimental group (Mean±SD).

Lesions / Group	Tubular degeneration	Tubular Necrosis	Inflammation
T1	0±0	0±0	0±0
T2	0±0	0±0	0±0
T3	3±0.05*	2±0.02*	2±0.007*
T4	2±0.087	2±0.003	2±0.04
T5	1±0.054*	1±0.037*	1±0.06*

changes observed toxic side effects of this drug, and it reflects the nature of the alkylating gemcitabine [15].

Previous studies have demonstrated that the isolated DNA gemcitabine causes lymphatic cancer cell apoptosis [16]. In this study, these were found in the gemcitabin group (T3) which gemcitabine affected and showed that all these findings are consistent with the results of the present study as nephrotoxicity effects. Melatonin is a hormone that is secreted by the brain's pineal gland in the regulation of certain physiological phenomena and other hormones in the body and plays an important role. Melatonin hormone neuronal function, as well as safety regulator and temperature [3,4]. According to studies and reports which have been published recently, melatonin mechanism remained unknown. However, the beneficial effect of the therapy has been well observed and reported. Also, studies have shown that melatonin can protect against cancer as an antioxidant agent used [3,4]. A study performed on the effects of melatonin (5 methoxy - N - acetyl Trytpamyn) in cancer and has proven its anti-cancer effects [17,18]. The protective effect of hormone in treatment groups was used in this study (T4, T5). Renal protective effect of melatonin in experimental group was determined in this study. They were same with the results of previous studies of the relationship between dose groups inversely which clearly decreased the side effects.

Conclusions

In general, the results obtained in this study have shown that most lesions were observed in gemcitabine administration, respectively. The injuries in treatment groups (T4, T5) that received different doses of melatonin with gemcitabin, have decreased remarkably. Compared with (T3), this decreasing reflects on protective effect of melatonin on the basis of the results obtained with the lower dose of melatonin linked to injuries.

Only melatonin group (T1, T2) received different doses of melatonin show that no significant renal lesions. No toxicity of high doses of melatonin has been found.

The study suggests that melatonin may have a protective effect on the renal tissue after prescription of gemcitabine, due to the diagrams and examined microscopically. This protective effect was most observed at the dose of 20 mg/ kg melatonin [18].

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