Vasoprotection by melatonin and quercetin in rats treated with cisplatin

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Received 23 December 2009; revised 5 August 2010

Cisplatin-based chemotherapy has a variety of vascular side effects. The aim of the present study was to evaluate the beneficial effect of melatonin and cisplatin on the alterations in vascular reactivity and structure of cisplatin-treated rats. Phenylephrine (PHE) and KCl-caused concentration-dependent contractions of rat aorta. Pretreatment with cisplatin increased the sensitivity but not the max response to PHE and KCl. In rats treated with melatonin or quercetin before cisplatin, the EC_{50} values, but not the maximal response to both agents were significantly higher than cisplatin-treated group. Compared to the control group, cisplatin-treatment significantly reduced the luminal area of the aorta. In melatonin and quercetin-treated aortas the luminal area values were significantly higher than cisplatin-treated group. The results demonstrate for the first time that melatonin and quercetin treatment may protect the aorta in cisplatin-based chemotherapy.

Keywords: Aorta, Cisplatin, Melatonin, Quercetin, Vasoprotection

Cisplatin (Cis-diaminedichloroplatinum II) shows high anti-tumor activating effect against several types of cancer and has been widely used as an agent for cancer chemotherapy. Cisplatin-based chemotherapy has been reported to cause a variety of vascular side effects. There are many reports, both of single case and of series of cases, of cerebrovascular and cardiovascular arterial occlusion in patients treated with cisplatin-based chemotherapy. The wide spectrum of arterial and venous thromboembolic events are well-known complications of cisplatin-based chemotherapy. Further, incubation of cells with antineoplastic drugs such as cisplatin, has been found to induce free radical formation in vitro. Melatonin may scavenge hydroxyl radical directly and thereby prevent renal tissue damage caused by hydroxyl radical produced by cisplatin treatment.

Melatonin, a hormone mainly produced in the pineal gland, has been known for decades to regulate circadian rhythms. This hormone may also participate in the regulation of cardiovascular functions. In addition, melatonin is a potent scavenger of the hydroxyl radical and peroxynitrate, suggesting that it may be useful in treating oxygen radical pathophysiology. Sener et al. and Hara et al. have reported that in animal experiments, melatonin confers protection against the oxidative damage associated with cisplatin.

Quercetin, a member of the flavonoids family, is ubiquitously present in food, including vegetables, fruits, tea, and red wine. Several biological properties of quercetin have been reported to be beneficial to human health, including protection against various diseases, such as osteoporosis, certain forms of cancer, pulmonary and cardiovascular diseases, and aging. Quercetin has been shown to possess potent vasodilator and anti-oxidant activities.

To our knowledge, no studies have investigated the protective effects of melatonin and quercetin against cisplatin-induced alterations of aorta. Therefore, the effects of melatonin and quercetin on the cisplatin-induced aortic responses and the histopathological changes during the treatments have been investigated. This goal is achieved by studying the effect of cisplatin on the responses of isolated rat aorta rings to phenylephrine (PHE) and potassium chloride (KCl).

Materials and Methods

Animals—Female Sprague Dawley rats weighing 180-220 g were divided randomly into four groups of 8 animals each. The experimental procedures were approved by the Animal Ethical Committee of the
university. The first group (control) received physiological saline solution, ip for 8 days. The second group (cisplatin-treated) was injected with cisplatin (10 mg/kg, ip) in physiological saline solution (0.5 ml), for 8 days. The third group (melatonin+cisplatin-treated) received cisplatin (10 mg/kg, ip) and melatonin (4 mg/kg, ip) in physiological saline solution (0.5 ml), for 8 consecutive days, and the fourth group (cisplatin+quercetin-treated) received cisplatin (10 mg/kg, ip) and quercetin (50 mg/kg, ip) in physiological saline solution (0.5 ml) for 8 consecutive days. The last two groups began to receive antioxidants one day prior to cisplatin injection. After 14 days of cisplatin treatment, rats were anesthetised with sodium thiopenthal injection. After 14 days of cisplatin treatment, rats were anesthetised with sodium thiopenthal (40 mg/kg, ip), the abdominal aorta was dissected and placed in a Petri dish containing Krebs-Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, MgSO4 1.50, KH2PO4 1.20, CaCl2 2.50, NaHCO3 25, Glucose 11), the connective tissues were cut away.

Drugs—The following compounds were used: phenylephrine chloride (Sigma, St. Louis, MO, USA), cisplatin (Sigma, St. Louis, MO, USA), melatonin (Merck, Germany), quercetin (Aldrich, Germany). Phenylephrine chloride was dissolved in distilled water. Cisplatin was dissolved in physiological saline, melatonin and quercetin were dissolved in absolute ethanol and further dilutions were made in saline (the final concentration of ethanol was 1%).

Experimental design—The preparations were mounted in 25 ml organ baths containing KHS maintained at 37°C and aerated with 95% O2 and 5% CO2. 0.5 g tension was applied to tissues and tissues were allowed to equilibrate for 1 h. The tissue responses were recorded isometrically by a force-displacement transducer (Grass FT04, Grass Instruments Co, W. Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D, Grass Instrument Co). First, cumulative concentration-response curves were determined for potassium chloride (KCl, 5-100 mM). After the first concentration-response curve was completed, preparations were washed and allowed to restore resting tension. Then the tissues were contracted with cumulative addition of phenylephrine (PHE, 10^-9 - 10^-4 M).

Histological morphometric analysis of the abdominal aorta—After formaldehyde fixation, the entire abdominal aorta was sectioned at 5 segments of 4 mm each. Tissues were embedded in parafin, cut into 4-6 µm thick sections and stained by Hematoxylin and Eosin. In total, five artery sections were analyses per animal, consequently, 35 sections were studied for group. Measurements of the abdominal aorta cross-sectional area, were made in a single-blind fashion by pathologist. Morphometric measurements on all five segments of abdominal aorta were performed by using the Image Analysis System (BAB Bs200ProP Image Processing and Analysis System, Turkey, Ankara). The luminal area was calculated from the perimeter of the luminal border. The luminal area for each abdominal aorta was obtained by averaging these measurements. The mean ± SD value for a particular vessel, after averaging five consecutive abdominal aorta segment values for cross-sectional area were obtained.

Statistical analysis—Concentrations of the contractile agents causing 50% of the maximal response (EC50) were calculated from each individual concentration-response curve and its 95% confidence interval were obtained for each group of experiments. Maximal responses and EC50 values for curves obtained from control and cisplatin-treated or cisplatin-treated and cisplatin-antioxidant (between two groups) were compared by using Student’s t test. Statistical significance was set at P<0.05. The histopathological results are expressed as the mean ± SD. All of the numerical data were analyzed first using the Kruskal-Wallis test to test whether there was a difference between groups and then the Mann-Whitney U-test was performed to analyze two groups consecutively.

Results

Contractile response to phenylephrine—Phenylephrine (PHE, 10^-9-10^-4 M) produced concentration-dependent contractions of rat aorta (Fig. 1a). Treatment with cisplatin significantly enhanced the sensitivity of the preparations towards PHE, as compared with the control group. Treatment of the rats with melatonin or quercetin before cisplatin showed a significant decrease in the sensitivity, but not max response, to PHE compared to ciplatin alone. EC50 and Emax values of PHE, are given in Tables 1 and 2 respectively.

Contractile response to KCl—KCl-induced concentration-dependent contractions in all groups (Fig. 1b). Treatment with cisplatin significantly enhanced the sensitivity of the preparations towards
KCl, as compared with the control group. Compared to the cisplatin-treatment, treatment of the rats with melatonin or quercetin before cisplatin both significantly reduced the sensitivity, but not max responses, to KCl.

**Histopathologic examination**—The mean values for the measurements of aortic luminal area were analysed statistically; confidence interval was assessed at 95%. The average luminal areas of rat aorta were found to be 1194049.43±64164 µm² in control group (n=7), 1008250.43±34952 µm² in cisplatin-treated group (n=7), 1101059.86±32575 µm² in melatonin plus cisplatin group (n=7) and 1154531±92442 µm² in quercetin plus cisplatin group (n=7) (Fig. 2). The luminal area of the cisplatin-treated group decreased by nearly 15.56% compared to control group (P<0.05). The antioxidants significantly (P<0.05) reversed the vasospasm induced by cisplatin. The luminal area in the melatonin plus cisplatin group and the quercetin plus cisplatin group was also increased by nearly 9.20 and 14.50%, respectively compared to the cisplatin alone group (P<0.05). There was no statistical differences between the melatonin and quercetin (P>0.05).

**Discussion**

In the present work, the effects of cisplatin treatment on PHE- and KCl-induced contractions of rat aorta, with special attention to the influence of antioxidants melatonin and quercetin in these effects were studied. To our knowledge, this is the first study to show the effects of cisplatin treatment on the contractile responses of rat aorta and the role of melatonin and quercetin on contractions of cisplatin-treated rat aorta.

Although cisplatin can cause nephro-, neuro-, and other toxicities as cardiovascular arterial occlusion³-⁵, and despite the availability of some newer and less toxic platinum drugs, cisplatin remains a major antineoplastic drug for the treatment of solid tumours. Therefore, strategies of ameliorating the toxicity of
cisplatin are of clinical interest. Further, incubation of cells with antineoplastic drugs such as cisplatin, has been found to induce free radical formation in vitro. It has been shown by many workers that dietary antioxidants may detoxify reactive oxygen species and may also enhance the anticancer effects of chemotherapy, and reduce some of the side effects.

The mammalian pineal secretory product melatonin, is a highly evolutionarily conserved molecule that is present in organisms ranging from algae to humans; it is likely that melatonin and structurally related compounds may be present in all organisms. Melatonin participates in the control of many important physiological functions including seasonal reproduction, the immune system and circadian rhythms. This hormone may also participate in the regulation of cardiovascular functions. In addition, melatonin is a potent scavenger of the hydroxyl radical and peroxynitrate, suggesting that it may be useful in treating oxygen radical pathophysiology. Oxidative stress caused by reactive oxygen species (ROS) induces apoptosis, which is characteristically identified by cell shrinkage and DNA fragmentation. Cisplatin also induces apoptosis. In animal experiments melatonin confers protection against the oxidative damage associated with cisplatin.

Quercetin, is a flavonol that is commonly consumed in the diet. Epidemiological studies indicating that high dietary intake of flavonols reduces the risk of mortality due to coronary heart disease have provoked interest in the mechanism of this cardioprotective effect. In the field of cardiovascular diseases, it has been shown that quercetin together with the improvement of endothelial function, has direct vasodilator effects, and are good candidates to explain the blood pressure reduction and vascular protective effects of quercetin in animal models of hypertension and possibly in human cardiovascular diseases. Furthermore, restoration of endothelial dysfunction by quercetin has also been observed in diabetic rats. The investigators reported that quercetin and its methylated metabolite isorhamnetin...
have reported that quercetin exhibited endothelium-independent vasodilator effects in vitro. The present results indicate that PHE- and KCl-induced reproducible contractions in rat aorta. Compared with the control responses, in preparations obtained from cisplatin-pretreated rats, the sensitivity to both PHE and KCl were increased. The mechanism under the increased sensitivity to aorta in cisplatin treated rats is not understood. In the present study, treatment with melatonin or quercetin plus cisplatin significantly decreased the sensitivity to both PHE and KCl compared to cisplatin alone. Both melatonin or quercetin-treatment before cisplatin increased the luminal areas of rat aortas compared to the cisplatin-treated group. Reyes-Toso et al. reported that melatonin decreased aortic ring contractility response to phenylephrine. Prevailing evidence from both human and animal studies indicate that dietary antioxidants can prevent the development of the vascular complications. The present results are in agreement with those reported by Lee et al. who have reported that quercetin exhibited in vivo hepatoprotective and anti-fibrogenic effects against dimethylnitrosamine-induced liver injury in rats. Further, Huang et al. reported that quercetin inhibited neointima hyperplasia after balloon injury in a rat model. Some investigators also concluded that quercetin may possess both antiatherogenic and anti-restenosis properties. To the best of our knowledge, no previous data on the effects of melatonin and quercetin of cisplatin-treated rat aorta are available. The present studies demonstrated that melatonin and quercetin, both decreased the aortic alteration induced by cisplatin treatment in rat. Similarly, Yoshida et al. reported that melatonin is an effective scavenger of hydroxyl radical and indicated that melatonin may play a protective role against renal damage induced by hydroxyl radical produced upon cisplatin treatment.

In conclusion, the results of the present study suggest that the pretreatment with cisplatin increased the sensitivity to PHE and KCl to aorta. Melatonin or quercetin treatment decreased the sensitivity to both contractile agents. Compared to the control group, cisplatin-treatment significantly reduced (15.56%) the luminal area of the aorta. In melatonin and quercetin-treated aortas the luminal area values were significantly higher (9.20 and 14.50%) than cisplatin alone-treated group. This study demonstrates for the first time that melatonin and quercetin treatment may prevent the aortic alteration that is produced by cisplatin treatment. These results may be helpful to take advantage of the therapeutic potential of melatonin and quercetin in the prevention and treatment of structural alteration of aorta after cisplatin-treatment.

References
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