Effects of Piracetam Supplementation on Cochlear Damage Occuring in Guinea Pigs Exposed to Irradiation

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In this study we aimed to determine the role of piracetam (PIR) in preventing radiation induced cochlear damage after total-cranium irradiation (radiotherapy; RT). Male albino guinea pigs used in the study were randomly divided into three groups. Group 1 (Control group) (n=11) received neither PIR nor irradiation, but received saline solution intraperitoneal (i.p.) and received sham irradiation. Group 2 (RT group) (n=32) was exposed to total cranium irradiation of 33 Gy in 5 fractions of 6.6 Gy/d for five successive days, with a calculated (α/β=3.5) biological effective dose of fractionated irradiation equal to 60 Gy conventional fractionation, then received saline solution for five successive days i.p. Group 3 (PIR+RT group) (n=33) received total cranium irradiation, plus 350 mg/kg per day PIR for five successive days i.p. After the last dose of RT, the guinea pigs were all sacrificed at the 4th, 24th and 96th hours, respectively. Their cochleas were enucleated for histopathologic examination. It was observed that total cranium irradiation (RT group) promoted degeneration in stria vascularis (SV), spiral ganglion cells (SG), outer hair cells (OHC) and inner hair cell (IHC) of cochleas at these times (p<0.05). While in the PIR+RT group, there was no statistically significant difference on radiation-induced cochlear degeneration in SV and OHC at 4th (p>0.05) and IHC at 4th, 24th hours (p>0.05), there was a significant difference on radiation-induced cochlear degeneration in SV and OHC at 24th and 96th hours (p<0.05), IHC at 96th hour (p>0.05) and SG at 4th, 24th and 96th hours (p>0.05). There was no any cochlear degeneration in the control group. Piracetam might reduce radiation-induced cochlear damage in the guinea pig. These results are pioneer to studies that will be performed with PIR for radiation toxicity protection.

Key words piracetam; cochlear damage; gamma-irradiation

Since the discovery of X-rays, radiotherapy (RT) has been used widely in both primary and adjuvant treatment of cancer diseases, but some side effects of RT have occurred in addition to these curative effects. One of these side effects is oto-toxicity. Hearing loss may be severe and uncomfortable enough to disrupt the life quality of some patients.1—4) In order to minimize or prevent otologic side effects induced by RT and various other drugs, studies have continued to the present day. These include pantothenic acid, coenzyme A, D-methionine, histidine, brain derived neurotrophic factor with D-methionine, L-methionine, diethylthiocarbamate, 4-methylthiobenzoic acid, ebselen, alpha lipoic acid, alpha tocopherol, vitamins B, A, C, E and K, zinc–copper-superoxide dismutase, nicotinamide, amino acids, choline, ATP, gluturonic acid, chondroitin sulfate, corticoids, sulfhydryl compounds (i.e. pantothenic acid, glutathione, sodium thiosulfate), carnitine and piracetam.5—15)

Piracetam (PIR) is a drug, with a fairly wide effect spectrum. Also, it has been used in the treatment of epilepsy, cerebro-vascular occlusion, motor aphasy, amnesia and sudden hearing loss, and good results have been obtained from the treatment.16—19) Piracetam (2-oxo-1-pyrrolidine-acetamine) is a low molecular weight derivative of gamma-aminobutyric acid. Different but complementary effects have been recognized, such as effects on cognitive function, platelet anti-aggregant, rheological and antioxidant mechanisms.20—22) Nootropic (cognitive) effects were the first established. These relate both to the ability to cross the blood–brain barrier and local vasomotor and metabolic effects, such as increase in oxygen and glucose utilisation, decrease in the lactate/pyruvate ratio and restoration of regional metabolism via the ATP pathway.21,22) Platelet anti-aggregant effects might be secondary to a direct inhibitory effect of piracetam on tromboxane A2 or tromboxane A2 synthetase in the chain of synthesis of platelet prostaglandins. The rheological effects of piracetam, especially those related to red cells, are probably related partly to cell membrane changes, such as lipid structure, prostaglandin synthesis, and degree of phosphorylation of spectrin, especially in erythrocytes but also in white cells and platelets, and partly to qualitative changes in some plasma protein fractions such as fibrinogen, macro-globulins and von Willebrand’s factor.20)

The purpose of this study was to examine the protective effects of parenteral piracetam supplementation on cochlear damage occurring in guinea pigs exposed to total cranium irradiation.

MATERIALS AND METHODS

Animals, Drugs and Irradiation All procedures including albino guinea pigs were carried out adhering to principles of the Use of Animals in Research. The guinea pigs were quarantined for at least 1 d before irradiation, housed in cages in a windowless laboratory room with automatic temperature (22±1 °C) and lighting controls (12 h light/12 h dark), and fed standard laboratory chow and water ad libitum.

Male albino guinea pigs (450—600 g body weight) (n=76) were randomly divided into three groups. Group 1 (Control group) (n=11) received neither PIR nor irradiation, but received saline solution (3.5 ml/kg) i.p. and received sham irradiation for five successive days. Group 2 (RT group)
(n=32) was exposed to total cranium irradiation of 33 Gy in 5 fractions of 6.6 Gy/d for five successive days, with a calculated (α/β=3.5) biological effective dose of fractionated irradiation equal to 60 Gy conventional fractionation, plus receiving saline solution (3.5 ml/kg) i.p. for five successive days as a placebo. Group 3 (PIR+RT group) (n=33) received total cranium irradiation, plus 350 mg/kg per day PIR (UCB Pharma, Istanbul, Turkey) for five successive days i.p. In our study, 350 mg/kg/d dose of piracetam (i.e. 3.5 ml/kg) was used 1 h before the total cranium irradiation.

Prior to total cranium radiotherapy, the guinea pigs were anesthetized with 50 mg/kg ketamin HCl (Pfizer, Istanbul) and placed on a plexiglas tray in a prone position. The guinea pigs in the RT and PIR+RT groups were irradiated using a cobalt-60 teletherapy unit (Picker, C-9, Maryland, NY, U.S.A.) from a source-to-surface distance of 80 cm, by 7.5×7.5 cm anterior fields with 33 Gy to the total cranium in 5 fractions of 6.6 Gy/d for five successive days; the calculated (α/β=3.5) biological effective dose of fractionated irradiation was equal to 60 Gy conventional fractionation. The dose was calculated for the central axis at a depth of 1.5 cm. The dose rate was 0.65 Gy/min.

After the last dose of irradiation and/or drug, the guinea pigs were anesthetized by i.p. administration of 100 mg/kg of ketamine hydrochloride (Pfizer) and 3 mg/kg of diazepam (Deva, Istanbul), and were sacrificed with overdoses (100 mg/kg) of pentobarbital (Abbott, Istanbul) this was administered to all of the control group (n=11), 12 and 11 animals at the 4th hour from 2 and 3 groups, 10 and 12 animals at the 24th hour, and 10 and 10 animals at the 96th hour, respectively. Their right and left temporal bones were removed within 5 min of sacrifice and it was reached to the tympanic bulla after removal of the lateral wall of the mastoid like process of these temporal bones. Their left cochleas were enucleated for histopathologic examination, and their right temporal bones were used for another study.

**Histopathological Evaluation** The left tympanic bullas were fixed in 10% neutral buffered formaldehyde solution and kept in this solution at +4 °C for 24 h. For decalcification, specimens were stored in 10% ethylenediaminetetraacetic acid solution at 4 °C for 20 d. All specimens were dehydrated, embedded in paraffin and serially cut into 5 µm slices. Sections were stained with haematoxylin and eosin for light microscopic examination by two independent pathologists. Changes in the organ of Corti (hydropic and vacuolar degeneration and loss of outer hair cells (OHC) and inner hair cell (IHC)); in spiral ganglion (SG) (cytoplasmic and nuclear condensation, nucleus and neuron loss); and in the stria vascularis (SV) (edema, vacuolization and loss of cells) were noted. While changes in SV were scored as absent (0), mild (1), moderate (2) or severe (3), the percentage of degenerated cells in SG and the number of degenerated cells in the organ of Corti were evaluated.

**Statistical Analysis** After necessary data had been collected, statistical analyses were made using the SPSS 11.0 packet programme (Statistical Package for Social Science; Windows version 11.0). The results were given as means± standard deviation. Statistical significance of differences between the groups was tested with the One-way ANOVA test. p<0.05 was accepted as statistically significant.

**RESULTS**

There was no cochlear degeneration in the control group (Table 1, Figs. 1, 4, 7). When the RT and PIR+RT groups
were compared with control group, cochlear damage in the guinea pigs at 4th, 24th and 96th hours was significant ($p<0.05$). We found that the total cranium irradiation (RT group) promoted degeneration in the SV (severe hydropic and vacuolar degeneration) (Fig. 2), OHC and IHC (hydropic, vacuolar degeneration, and loss of cells) (Fig. 5) and

Fig. 4. Organ of Corti (Outer Hair Cells and Inner Hair Cell) by Light Microscopy in the Cochleas of the Control Group (H&E×400)

Fig. 7. Spiral Ganglion by Light Microscopy in the Cochleas of the Control Group (H&E×200)

Fig. 5. Changes in Organ of Corti (Severe Degeneration, and Loss of Cell in Outer Hair Cells and Inner Hair Cell) by Light Microscopy in the Cochleas of the Radiotherapy (RT) Group (H&E×200)

Fig. 8. Changes in Spiral Ganglion (Inconspicuous Cytoplasmic and Nuclear Condensation, Nucleus and Neuron Loss) by Light Microscopy in the Cochleas of the Radiotherapy (RT) Group (H&E×400)

Fig. 6. Changes in Organ of Corti (Mild Degeneration in Outer Hair Cells and Inner Hair Cell) by Light Microscopy in the Cochleas of the Radiotherapy Plus Piracetam (PIR+RT) Group (H&E×200)

Fig. 9. Changes in Spiral ganglion (Mild Cytoplasmic and Nuclear Condensation, and Loss of a Few Nuclei and Neuron) by Light Microscopy in the Cochleas of the Radiotherapy Plus Piracetam (PIR+RT) Group (H&E×400)
SG (inconspicuous cytoplasmic and nuclear condensation, nucleus and neuron loss) (Fig. 8) of cochleas at 4th, 24th and 96th hours ($p<0.05$) (Table 1). PIR supplementation (PIR+RT group) decreased damage in SV (mild hydropic and vacuolar degeneration) at 24th and 96th hours (Fig. 3), OHC (hydropic degeneration) at 24th and 96th hours and IHC (hydropic and nuclear degeneration) (Fig. 6) and SG (cytoplasmic and nuclear condensation, and loss of a few nucleus and neuron) at 4th, 24th and 96th hours (Fig. 9), at 96th hour ($p<0.05$) the radiation-induced cochlear degeneration. But, none of these changes was not observed in SV and OHC at 4th, 24th and 96th hours ($p>0.05$) (Tables 1, 2).

DISCUSSION

The goal of radiation treatment is to deliver completely measured doses of ionizing radiation to a defined tumor volume with the minimum acceptable injurious effects of ionizing radiation to surrounding healthy tissue by eliminating tumor cells, providing a high quality of life and prolongation of survival at reasonable cost to cancer patients.$^{24}$ Cochlear toxicity may occur, however, during radiation therapy of head and neck cancer.$^{13}$

In the literature we found that cochlear damage increased in proportion to RT dose. Kim and Shin$^{25}$ applied a single dose of 2, 6, 10, or 15 Gy to guinea pigs, and evaluated cochleas histopathologically. They found that OHC damage appeared with irradiation of more than 10 Gy, and IHC damage with irradiation of more than 15 Gy. They also found that the morphologic changes in SV were intercellular and perivascular fluid accumulation that have appeared to be a reversible process. Young et al.$^{26}$ carried out experimental studies in which they gave 2 Gy-dose ionized radiation to guinea pigs for 30 d, then evaluated the inner ear structure microscopically. They detected degeneration in support cells and in IHC of the Corti organ, in vessel endotheliocytes, reduction in the number of capillaries, atrophy and degeneration in SV. In the present study at the applied high RT dose of 60 Gy, we demonstrated that irradiation caused damage in all...
the investigated parameters of SV, OHC, IHC and SG.

Oxidative stress has an important place in ototoxicity pathophysiology.\(^{27}\) Previous studies have suggested that cisplatin or aminoglycoside ototoxicity was related to an increase in lipid peroxidation and a decrease in endogenous antioxidant enzyme status.\(^{28,29}\) In cisplatin and aminoglycoside-induced ototoxicity, it has been reported that L-methionine, alpha lipoic acid, vitamin E, vitamin B, pantothenic acid, co-enzyme A, and piracetam were used as protective agents because of their antioxidant properties.\(^{8—15}\)

Exposure of cells to ionizing radiation cause oxidative stress because it occurs through which a variety of reactive oxygen species, such as the superoxide radical (\(\text{O}_2^-\)), the hydrogen peroxide (\(\text{H}_2\text{O}_2\)) and the hydroxyl radical (\(\text{OH}^-\)) that are associated with radiation-induced cytotoxicity.\(^{30}\) Although all respiring cells are equipped with protective enzymes such as SOD and CAT or GSH-Px, increased oxidative stress in cells stemming from ionizing radiation may overwhelm the protective systems, leading to cell injury.\(^{31}\) Consequently, piracetam is a drug that has antioxidant,\(^{22,32,33}\) platelet antiaggregant, rheological,\(^{20—22}\) anti-inflammatory,\(^{34}\) anti-apoptotic, cytoprotective\(^{35}\) and immunomodulatory effects.\(^{36,37}\) Minkova et al.\(^{38}\) reported that 200 mg/kg piracetam combined with vitamin E 20 mg/kg and anthocyan 50 mg/kg has a radioprotective effect. They found that the combination significantly increased survival rate (50%) in mice, blood-forming organs underwent less radiation damage, and recovery processes in them were stimulated. In a recent study, we demonstrated that piracetam prevented ototoxicity by interfering with the neuroprotective mechanism in the guinea pigs in vivo. We also determined that piracetam improved the ototoxic effects of combined cisplatin–gentamicin upon auditory pathways from cochlea to middle brain. Thereby, we deduced that, because of the protective effects of piracetam, it was able to provide more effective treatment of otorhinolaryngologic, neurologic, infectious and oncologic diseases by reducing combined cisplatin–gentamicin ototoxicity.\(^{15}\) In terms of the above expressed information, one can allege that piracetam might play a role by increasing oxygenation in the tumor cells with its rheological effects and decreasing apoptosis in surrounding healthy cells with its anti-apoptotic effects in the prevention of radiation-induced oxidative damage. Animal studies showed that rats treated chronically with 100 to 1000 mg/kg orally for 6 months did not show any toxic effect; Nor were any teratogenic effects found.\(^{39}\) Koskineni et al.\(^{40}\) reported that a dose of 24 g/d for two weeks is highly beneficial, more effective than lower doses as an effective and safe medication in patients. Thus, a piracetam dose of 350 mg/kg/d in our study was similar to 24 g/d used in a 70 kg of human. The plasma elimination half-life was short in all the species studied, ranging from a mean of 1.8—2.0 and 4.5 h following administration in rats and man, respectively.\(^{41,42}\) Piracetam was used for 1 h to prevent barbiturate intoxication in rabbit.\(^{13}\) We also used it 1 h before the total cranium irradiation. These are some of other reasons why we used piracetam as a potent radioprotector agent in the present study.

We found in this study that fractionated total cranium irradiation of 60 Gy (RT group) promoted damage in SV, SG, OHC and IHC of cochleas, and piracetam supplementation (PIR + RT group) decreased the radiation-induced cochlear damage.

In conclusion, piracetam seems to have beneficial effects in the reduction of radiation-induced ototoxicity. These are pioneer results for studies that will be performed with PIR to protect from radiation toxicity. It would be worthwhile studying the effects of PIR supplements in radiation-treated cancer patients, in the hope of reducing radiation-induced toxicity.

**REFERENCES**