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THE INVESTIGATION OF POSSIBLE BENEFICIAL EFFECTS OF GABAPENTIN ON ADVERSE CARDIAC EFFECTS OF HIGH DOSE ZOLEDRONIC ACID: AN EXPERIMENTAL STUDY

ANIL GULCU[1] AND OZGUR AKKAYA[2]

[1]Medical School of Alaaddin Keykubat University
[2]Alanya Training and Research Hospital

Abstract: Zoledronic acid (ZA) is prescribed for different kinds of clinic conditions to suppress osteoclastic activity and protect bone mineral density. However, some reports claim that ZA treatment can lead to adverse cardiac events. Gabapentin (GP), a widely prescribed agent for neuropathic pain, can ameliorate some other medical agents' cardiac side effects, through its anti-inflammatory and antioxidant properties. This study aimed to experimentally investigate the combined effect of ZA and GP on the myocardium. Four different groups were created with rats, as follows: I. Control (n:3): investigating the normal myocardial tissue in rat genus. II. Sham (n:4): weekly intraperitoneal (i.p.) saline injection and daily 30 mg/kg GP (oral gavage) for obtaining possible cardiac side effects of i.p. injection. III. ZA (n: 7): weekly 100 mg/kg i.p. ZA infusion for four weeks. IV. ZA+ GP (n : 7): weekly 100 mg/kg ZA (i.p.) and daily 30 mg/kg GP (oral gavage) utilized for four weeks. Histopathological examinations were made to obtain cardiac tissues at the end of the four weeks. A severe myocardial injury was detected in the ZA group. Regarding injury grade, high dose ZA leads to significant myocardial edema and cellular damage when compared with control subjects ($p = 0.000$). On the other hand, reduced damage was detected in ZA + GP Group when compare with ZA alone treated group ($p = 0.030$). Our results suggest that the possible cardiac side effects of ZA can be prevented or reduced by GP. However, more comprehensive studies are needed to elucidate this potential effect.

Keywords: zoledronic acid, cardiac side effects, gabapentin, combination treatment

Abbreviations: ZA: Zoledronic acid; GP: Gabapentin; i.p.: intraperitoneal; FDA: Food and Drug Administration; H&E: hematoxylin and eosin; ASBMR: American Society for Bone and Mineral Research

There have been various reports of cardiac side effects of bisphosphonate-derived drugs. Studies have focused on rhythm disorders and their careful use has been recommended in patients with cardiac problems (1, 2). Zoledronic acid (ZA) is a nitrogen-containing bisphosphonate derivative used to support bone tissue, particularly in cases of increased osteoclastic activity such as osteoporosis. It is also used routinely in similar diseases that cause disorders in bone metabolism (3, 4). In addition to its positive effects such as an increase in muscle mass in patients with osteoporosis, it has been reported that it can induce apoptosis in cancer cells (5). There is, however, no definite consensus about the optimum dose of this agent used in different clinical situations and moreover, although the exact mechanism is not known, cardiac side effects have also been reported. The most-reported cardiac complaints in patients, especially during use, are palpitations and atrial fibrillation (6).

Gabapentin (GP) is in fact a drug in the anticonvulsant group approved by the FDA, with highly successful results in neuropathic pain (7). Additionally, the anticonvulsants, analgesics, and anxiolytics effects detected in its application have led to its use with increasingly common indications. As a result of these effects, its use has become widespread in the treatment of restless leg, various psychiatric disorders, and substance abuse disorders (7, 8). In addition, recent reports have emphasized potential cytoprotective effects, stating that it reduces oxidative stress and apoptosis (9).

In this study, we aimed to experimentally investigate the cardiac effects of high-dose ZA and evaluate the possible beneficial effects of concomitant GP treatment.

* Corresponding author: e-mail: oakkaya369@gmail.com

MATERIAL AND METHOD

Study design

This randomized animal study was designed as a single-blinded controlled experiment so that the tissue histopathological analysis was made without group names. All steps of the experiment were conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals. Thereafter, the ethical approval was obtained from the Local Ethics Committee of Kobay Animal Laboratories

Study subjects

Twenty-one male Albino-Wistar rats (aged 10 to 11 weeks) weighing 180 ± 20 g (mean ± standard deviation), were included in this experimental study. All animals were stored in temperature (22 ± 2°C) and humidity (50 ± 5%) controlled cages during the study, under a standard animal diet.

Protocols

The rats were divided into four groups as follows: I. Control group (n: 3): rats were observed for 4 weeks without any application to obtain normal histological findings of the rat genus. II. Sham group (n: 4): rats were observed for a period of 4 weeks. Additionally, weekly intraperitoneal saline was administered together with daily 30 mg/kg Gabapentin (GP) application via oral gavage for creating a sham group. III. Zoledronic acid (ZA) group (n: 7): weekly 100 mg/kg intraperitoneal (i.p.) ZA was applied to this group, in addition to the standard diet for 4 weeks, to investigate the effects of the drug on myocardial tissue (10). IV. ZA + Gabapentin (GP) group (n: 7): weekly 100 mg/kg ZA (i.p.) and daily 30 mg/kg GP (via oral gavage) were utilized in this group, in addition to a standard diet for 4 weeks, to investigate the effects of drugs on myocardial tissue (the dose was determined according to previous experimental reports (11).

All rats were sacrificed, and hearts were excised in all groups after the 4 weeks observation period. The myocardial tissues were examined histologically.

Histopathological examination

The heart tissues were fixed in 10% formalin after excision and embedded in paraffin blocks. The blocks were then sliced 3 μm thick with a rotary microtome for light microscopy evaluation and prior to the examination, each slice was stained with hematoxylin and eosin (H&E). Finally, tissues were examined under an inverted fluorescence Nikon ECLIPSE TS-100F (Nikon Instruments Inc., Tokyo, Japan) microscope.

The myocardial tissue damage was classified into four main categories, as described previously (12):

Grade 0: Normal tissue findings

Grade I: Mild injury with weak cellular (myocardial) swelling: interstitial edema and fibrosis, leukocyte accumulation in capillaries, few contraction bands in a small field.

Grade II: Moderate myocardial injury with marked interstitial and myocardial edema, fibrosis, leukocyte accumulation in capillaries, and increased contraction bands in some fields.

Grade III: Severe injury with widespread contraction bands, severe cellular irregularity, and severe edema

Statistical analysis

The statistical evaluations were made using a statistical software program (SPSS 15.0; SPSS Inc., Chicago, IL, USA). The myocardial injury degrees were calculated numerically and expressed as mean and standard deviation. The myocardial injuries in groups were compared using the Mann-Whitney U test. The injury grades were compared using the one-way analysis of variance and significant differences were analyzed using Tukey's honest post hoc test. The p-value ≤ 0.05 was considered statistically significant.

RESULTS

Histopathologically, Grade 0 normal tissue morphology was obtained from both control and sham groups (Image 1A). In the ZA group, diffuse contraction bands, cellular irregularity, and advanced edema were detected, and most of the tissues were consistent with Grade III myocardial damage (Image 1B). In the ZA + GP group, partial edema, more regular cell morphology, and fewer contraction bands were consistent with Grade I - Grade II myocardial damage (Image 1C). The accumulation of histopathological grades in each group was presented in Table 1.

The post-hoc test revealed that a high dose of ZA leads to significant myocardial edema and cellular damage when compared with control subjects (p=0.000). On the other hand, reduced damage was detected in the ZA + GP Group when compare with the solely ZA-treated group (p = 0.030). The comparison of injury grades and ANOVA test results are reported in Table 2.

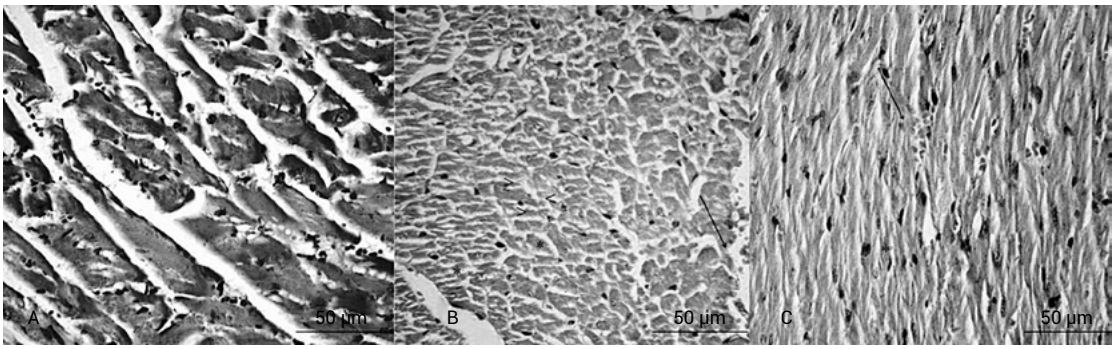

Image 1. A. Normal myocardial morphology of rat genus (Grade 0); B. The severe myocardial injury (Grade III) in the Zoledronic acid group with cellular irregularity (>: nuclear distribution is disturbed), cellular swelling (*), hemorrhage (black arrow), and edema; C. Mild, moderate myocardial injury (Grade I-II) in ZA+GP group with more regular cell morphology (*), fewer contraction bands, less cellular swelling (black arrow) and decomposition. [Hematoxylin and eosin (H&E), Bar=100 µm]

Table 1. The accumulation of myocardial injury in groups.

	Control Group n : 3	Sham Group n : 4	Zoledronic Acid Group n : 7	Zoledronic Acid + Gabapentin Group n : 7
Grade 0 (n)	3	4	-	-
Grade I (n)	-	-	-	3
Grade II (n)	-	-	2	3
Grade III (n)	-	-	5	1
TOTAL	3	4	7	7

Table 2. Comparison of injury grades and post hoc test analyze

Experimental Subject	Control & Sham (C&S) Group	Zoledronic Acid Group (Group 1)	Zoledronic Acid + Gabapentin Group (Group 2)			Groups	p
1	+	+++	++		C&S	Group 1	0.000
2	+	++++	++			Group 2	0.000
3	+	+++	+++		Group1	Group 2	0.030
4	+	++++	++	Tukey HSD			
5	+	++++	+++				
6	+	++++	++++				
7	+	++++	+++				
Mean±SD	1.0 ± 0.0	3.7 ± 0.5	2.7 ± 0.8				
p ANOVA		0.000					

+: Grade 0 injury; ++: Grade 1 injury; +++: Grade 2 injury; ++++: Grade 3 injury; SD: Standard deviation; $p < 0.05$ is considered significant; ANOVA: One-way analysis of variance; HSD: Honest significant difference.

DISCUSSION

According to our findings, a high dose of ZA leads to myocardial injury, though cardiac side effects may be related to this impact on the myocardium. Our results supported that concomitant GP treatment with ZA may reduce cardiac adverse effects and to our knowledge, this is the first study to investigate the additive effects of GP with ZA on the myocardium.

The cardiac side effects of bisphosphonate-derived drugs are mentioned in many clinical reports (13, 14). In particular, cardiac arrhythmia and palpitation are the main reported clinical conditions in bisphosphonate-derived drug usage (13). For instance, in the 2017 meeting of the American Society for Bone and Mineral Research (ASBMR), it was reported that the incidence of heart failure increased in patients receiving ZA therapy (6). According to the results of the double-blind, placebo-controlled

three-year HORIZON Pivotal Fracture Trial, which has provided some of the most important data on this subject, ZA significantly reduces the fracture risk in patients with osteoporosis, but it has shown a significant side-effect potential compared to placebo, and atrial fibrillation has been reported as the most important and critical side effect (14). Conversely, the cardiac adverse effect of ZA remains controversial. Some clinical data in cancer patients treated with ZA suggests that this agent does not lead to electrocardiographic changes in the acute period (15). In another report, although increased cardiac ectopy was detected in some patients, it was declared that there is no atrial fibrillation detected in osteoporosis patients, during or early after ZA therapy (16). Moreover, a study based on Danish and Swedish health registries indicated that ZA infusion has a higher risk of heart failure, fractures, and death when compared with oral bisphosphonate usage (17). However, they added that it is difficult to determine if the increment of cardiovascular risk is related to a true drug effect, or higher baseline risk in patients who were treated with ZA (17). Interestingly, acute myopericarditis was reported in a case after intravenous ZA administration (18). In a cohort study, it was indicated that ZA usage has led to hypocalcemia. In the same study, this situation is blamed as responsible for the aggravation of heart failure and also claimed that prolonged QT distance can be induced with severe hypocalcemia (19). In another study, it was shown that the application of ZA in risky patients may cause severe atrioventricular blocks and supraventricular arrhythmias, which may also be due to electrolyte deficits. It was mentioned that this may occur as a result of electrolyte imbalance in the myocardium caused by ZA, and it was concluded that this effect may result in functional problems in the heart (20). Based on these reports, we investigated the myocardial effects of high dose ZA experimentally and according to our findings, ZA might have myocardial injury potential.

Unlike ZA, GP, which has been reported to cause bone loss in long-term use by reducing bone mineral density, is an anticonvulsant agent used in neuropathic pain (21). Bone mass monitoring is recommended for the long-term use of this agent, which has different clinical uses as a result of its wide range of effects as well as various pain derivatives and anticonvulsive effects (21). In opposition to these results, another study reported that there were no significant alterations in bone mineral density or biomechanical bone strength with GP treatment and therefore, GP was proposed as an antiepileptic drug with lower risks to bone health (22). It is known that GP is quite effective in muscle, skeletal, and organ origin pain treatment. It has been shown in rats that these effects are mediated by arachidonate, nitrergic and serotonergic systems and are regulated by nitric oxide synthetase and prostaglandin release (23). However, its effects on cardiac tissue, where muscle tissue is concentrated, are still not clearly clarified. In a study conducted on rats, the hemodynamic effects of GP were investigated with different application techniques (intrathecal, intracerebroventricular, and intraperitoneal route). Post-administration blood pressure and heart rate values were recorded in this study, although GP increased blood pressure via the intracerebroventricular route, it did not change blood pressure or heart rate in the study when administered intrathecally and intraperitoneally. Based on these findings, it has been suggested that it is safe to use these routes (24). Furthermore, some studies have suggested that GP has cytoprotective effects (9). In preclinical studies, the neuroprotective effects of GP have been demonstrated in the ischemic brain, and it has been emphasized that this neuroprotective effect may be related to conditions such as Hsp7 expression (25). On the other hand, it has been reported that GP may exert neuroprotective effects by inhibiting oxidative stress-induced neuronal autophagy through the regulation of PI3K/Akt/mTOR signaling pathways (26). In a more recent preclinical study, myocardial protective effects of GP were demonstrated in doxorubicin-induced myocardial toxicity. It has been stated that this cardioprotective effect may be related to the modulation of the inflammatory/apoptotic signaling pathway (27). While there are publications showing that ZA increases the inflammatory response stimulated by the lipopolysaccharide pathway, studies have also reported that GP reduces the inflammatory response stimulated by lipopolysaccharide (28,29). In our study, it was determined that myocardial injury was reduced with the addition of GP to high-dose ZA treatment. Notably, all the opposite effects listed above may in fact enable these two agents to tolerate each other's negative effects.

CONCLUSION

In line with our results, it can be said that repeated high-dose ZA treatments have toxic effects on the myocardium and that these decrease with the addition of GP to the treatment. However, we believe that it would be beneficial to reveal these results and potential mechanisms of action with more comprehensive studies.

Limitations

The main and primary limitation of this study is that it was a preclinical animal experiment, thus

the results are representative and need to be confirmed in human subjects. The second limitation is that the possible mechanisms could not be investigated in the study and this issue may be further clarified by revealing possible cellular mechanisms, in larger planned studies. The third is the use of a limited animal group in the study: as a pilot study, the number of animals allowed by the ethical committee was duly restricted. The last limitation is related to our laboratory facility. In our laboratory, we did not have a device suiTable for echocardiographic examination of rats, and echocardiographic changes could not be recorded during the study. Therefore, the obtained histopathological results could not be compared with echocardiographic evaluations.

Conflict of interest

The author declares that there is no conflict of interest.

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REFERENCES

1. Kennel K.A., Drake M.T.: Mayo Clin. Proc. 84, 632 (2009).
2. Kausar S.A.: BMJ 351, h5870 (2015).
3. Pan B., To L.B., Farrugia A.N., Findlay D.M., Green J., et al.: Bone 34, 112 (2004).
4. Giannasi C., Niada S., Farronato D., Lombardi G., Manfredi B., et al.: Int. J. Med. Sci. 16, 23 (2019).
5. Huang C.F., Shiao M.S., Mao T.Y.: Drug Des. Devel. Ther. 15, 3711 (2021).
6. Abrahamsen B., Rubin K.H., Möller S., Choudhury A., Eriksen E.F., Andersen M.: in American Society for Bone and Mineral Research (ASBMR) 2017. Annual Meeting, Abstract MO0652, September 8-11, Denver 2017.
7. Wallach J.D., Ross J.S.: JAMA 319, 776 (2018).
8. Hyatt J.D., Daniel D., Tran Q.V., Millares M., Kubota D.R.: Am. Fam. Physician 84, 480 (2011).
9. Ola M.S., Alhomida A.S., LaNoue K.F.: Neurotox. Res. 36, 81 (2019).
10. Aydin I., Sehitoglu I., Ozer E., Kalkan Y., Tumkaya L., et al.: Eur. Rev. Med. Pharmacol. Sci. 25, 3567 (2021).
11. Samra Y.A., Amin M.N., Said E.: Int. Immunopharmacol. 90, 107125 (2021).
12. Caliskan A., Yavuz C., Karahan O., Yazici S., Guclu O., et al.: Perfusion. 29, 260 (2014).
13. Pazianas M., Compston J., Huang C.L. Atrial fibrillation and bisphosphonate therapy. J. Bone Miner. Res. 25, 2 (2010).
14. Black D.M., Delmas P.D., Eastell R., Reid I.R., Boonen S., et al.: N. Engl. J. Med. 356, 1809 (2007).
15. Demirtas D., Bilir C., Demirtas A.O., Engin H.: Clin. Cases Miner. Bone Metab. 14, 35 (2017).
16. İlgezdi Z.D., Aktaş İ., Doğan Metin F., Kepez A., Ünlü Özkan F., et al.: Anatol. J. Cardiol. 15, 320 (2015).
17. Rubin K.H., Möller S., Choudhury A., Zorina O., Kalsekar S., et al.: Bone 134, 115296 (2020).
18. Watts M.R., Ellims A.H., Eccleston D.S.: Med. J. Aust. 200, 150 (2014).
19. Choi N.K., Solomon D.H., Tsacogianis T.N., Landon J.E., Song H.J., Kim S.C.: J. Bone Miner. Res. 32, 611 (2017).
20. Yazici O., Aksoy S., Ucar O., Ozdemir N., Demir M., et al.: Med. Oncol. 30, 609 (2013).
21. Kanda J., Izumo N., Kobayashi Y., Onodera K., Shimakura T., et al.: Biol. Pharm Bull. 40, 1934 (2017).
22. Simko J., Karesova I., Kremlacek J., Eva Z., Horacek J., et al.: Pharmacol. Rep. 71, 1213 (2019).
23. Kilic F.S., Sirmagul B., Yildirim E., Oner S., Erol K.: Indian J. Med. Res. 135, 630 (2012).
24. Yoon M.H., Choi J.I.: J. Korean Med. Sci. 18, 478 (2003).
25. Jeon Y.T.: Korean J. Anesthesiol. 58, 115 (2010).
26. Yan B.C., Wang J., Rui Y., Cao J., Xu P., et al.: J. Neuropathol. Exp. Neurol. 78, 157 (2019).
27. Samra Y.A., Amin M.N., Said E.: Int. Immunopharmacol. 90, 107125 (2021).
28. Muratsu D., Yoshiga D., Taketomi T., Onimura T., Seki Y., et al.: PLoS One 8, e67906 (2013).
29. Anfuso C.D., Olivieri M., Fidilio A., Lupo G., Rusciano D., et al.: Front. Pharmacol. 8, 9 pages (2017).