Original Paper

Different Age Related Neurological and Cardiac Effects of Verapamil on a Transgenic Mouse Model of Alzheimer's Disease

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ABSTRACT: Dementias are the third cause of the disability-adjusted life-years (DALYs) worldwide with Alzheimer's (AD) having the highest prevalence. Despite ample research in the field, therapeutic options are limited. However, with the increase in lifespan, a larger number of AD patients will receive other medication for the evermore-increased number of comorbidities that such patients face. The purpose of this study was to evaluate the neurological and cardiac effects of verapamil, on C57BL/6J-TgN (Thy1-APPKM670/671NL; Thy1-PS1L166P (APP) mice. The daily administration of 3.5mg/kg of verapamil for 28 days revealed different effects on young and aged APP mice. While young animals showed less anxiety and improved short-term memory with minimal cardiac effects (an increase in the duration of ventricular depolarization), aged ones did not present behavioral improvements, but with a decrease in the duration of ventricular depolarizing. Repolarization effects of verapamil were similar in young and aged animals, except for the duration of the ST segment that was longer in aged animals. Considering our results, the use of calcium blockers in AD patients should take into consideration the stage of the disease, as different effects could be seen at different stages of AD, in our model.

KEYWORDS: *Calcium blockers, Alzheimer's, age.*

Introduction

Neurodegenerative diseases begin to have a higher impact on the disability-adjusted life-years (DALYs) worldwide, dementias being the third cause of DALYs [1].

From all dementias, Alzheimer's has the highest prevalence, with a major impact on the quality of life for both patients and their family members [2].

Despite already made efforts, the therapeutic methods used in Alzheimer's disease (AD) are still limited.

This can be attributed to a lack of understanding the fundamental mechanism that underline AD.

For a long time, the emphasis was on the genetic factor in the onset and evolution of the disease, with several genes been identified as key players in AD.

Genes like APP, PSEN1, PSEN2 [3], TREM2 [4], ApoE [5,6] have been described as the genetic triggers of AD, but they are responsible for a relatively small number of patients that have early onsets AD (before 65 years of age) [3,7].

Regardless of the onset age, some pathophysiological hallmarks of AD can be universally observed.

The accumulation of extracellular amyloid beta (Aβ) aggregates and pairs of intracellular filaments of tau protein formed in a process of hyper-phosphorylation can be seen in AD patients [8,9].

However, the so-called hallmarks were described in normal, age match individuals with no clinical signs of AD [10].

As such, the hypothesis of other factors involved in the appearance and evolution of the disease appeared.

Within the list of factors identified systemic diseases as diabetes, high blood pressure, obesity, dyslipidemia and cerebrovascular disease, were shown to increase the risk of developing AD [11].

Regardless, most of these comorbidities are present and need to be addressed. AD patients receive several classes of medication meant to alleviate different associated pathologies.

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As such, an interesting effect emerges, as some of them are ion channels blockers.

With certain central nervous system cells (such as microglia) heavily impacting AD, observations that microglial activity can be influenced by modulating sodium [12,13], calcium [14-16] or potassium [17,18] or ion channels, the use of such drugs needs to be carefully analyzed, especially if those recommended in other organ pathologies and a central nervous system effect is not obvious.

Here, we investigated what is the impact of modulating the heart function in an animal model of amyloid toxicity in young and aged mice.

Materials and Methods

Animals

Young (14-16 weeks of age) and old (110 to 116 weeks of age) C57BL/6J-TgN (Thy1-APPKM670/671NL; Thy1-PS1L166P (APP)) [19,20] mice were used for this study.

All animals were born and housed (in individual ventilated cages, under standard room temperature, humidity and day/night cycle) in the Animal Facility of the University of Medicine and Pharmacy of Craiova.

At least 24 hours before the beginning of the experiment, the animals were moved to the testing rooms where they were kept until the end of the experiment.

The weight of each animal was measured at this time.

Before the treatment, all animals underwent behavior testing and ECG recording (see below).

All procedures were in strict compliance with the recommendations of European guidelines for animal welfare and were approved by the University Welfare of Experimental Animals committee (2.11/29.10.2020).

Recording the electrical activity of the heart (ECG)

In order to obtain ECG recordings with the best possible quality, the measurement was made on anesthetized animals.

For anesthesia, a cocktail of Ketamine (100mg/kg) and Xylazine (10mg/kg) was used. This prevented most ECG artifacts.

Depth of anesthesia was established by pinch reflex, before starting the recording.

The electrical activity of the heart was recorded using the Emka Matrix to which several signal capture pads were connected, facilitating the simultaneous recording of several animals.

The Emka easyTEL telemetry system, which supplied signal to the Matrix was positioned externally to record the DI derivation, which transmitted the recorded signal to the pads.

The recorded signal was analyzed with IOX2 software.

For each animal, two recordings of a minimum 10 minutes were made: on day 0 and day 28 after treatment.

The day 0 recordings were considered as baseline.

After this, animals were then randomly assigned to control (n=3 for both young and aged animals) or treated (n=9 for young and n=6 for aged animals).

Animals assigned to the treated groups received a daily intraperitoneal injection of 3.5mg/kg verapamil for 28 days, while the controls received serum.

After removing the artifact sequences, the IOX2 software was able to provide the heart rate and the duration of the R-R interval.

Additionally, P wave (duration and amplitude), QRS (duration and amplitude), QT interval duration, ST segment (positioning and duration) and T wave (amplitude) were manually tracked.

Behavioral testing

Behavioral tests were performed on all mice before the treatment and 28 days after.

In order to reduce the interference between tests, open filed was performed first followed by the novel object recognition test.

All surfaces were wiped with 75 % ethanol before each trial to remove odors.

For the open field test, mice were placed in the open field maze, which measure 50cm (length) x 33cm (width) x 15cm (height). In each test, the mouse was positioned in the center of the arena and was allowed to move and explore freely for 10min.

The recording was automatically analyzed (EthoVision XT 14, Noldus Technology) and several parameters (time spent in the center arena, traveled distance and speed) were determined.

For short-term memory, the novel object recognition test was used.

As previously described, animals could freely explore the maze for 6 minutes; two identical objects were placed at a distance of 15cm from the side walls in two opposite corners of the apparatus.

After 60min, one of the objects was replaced by a new one, and the tested mouse was allowed to explore the maze again.

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For each animal the recognition index (D2) (the percentage of the time exploring the novel object among the total time of exploring both objects) was determined.

Results

The daily intraperitoneal administration of either the calcium blocker or the serum generated a stress factor for the animals, but no body mass difference was recorded at the end of the experiment, the animals loosing less than 10% of their initial weight.

Calcium blockers improve anxiety and short-term memory in young but not aged animals.

The presence of Aβ can generate different behavior changes in known animal models.

As such, we wanted to investigate how calcium blockers, commonly used in elderly, can also influence the behavior of APP animals.

While no difference in the distance and speed of young animals before and after the treatment was recorded, for aged animals both parameters decreased (Figure 1A and 1B), those animals spending more time on the edge of the open filed and less exploring the arena (Fig 1C).

Young APP mice seem to benefit from the treatment as they spend more time exploring (Figure 1C), and when tested, they showed an improved short-term memory after the treatment, by spending more time exploring a new object in their environment compared to baseline (P=0.0003) (Figure 1D).

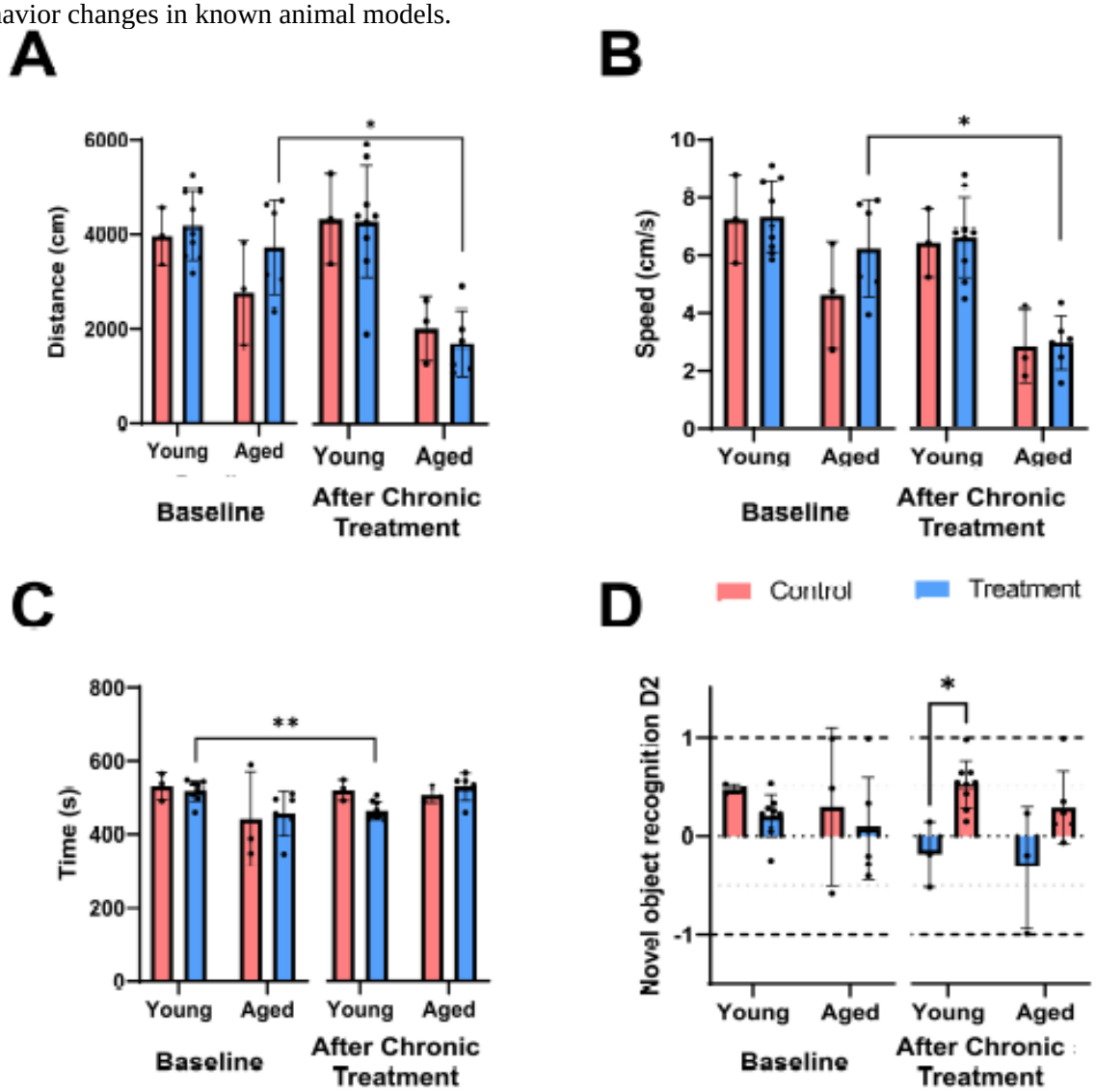

Figure 1. Behavioral tests in APP mice. Open filed test emphasizes an increase in anxiety in aged animals after chronic verapamil treatment, with older APP mice moving less (A) and at a lower speed (B) compared to baseline. The same treatment decreased the time that younger animals spend at the age of the open filed arena (C). After treatment, only young animals exhibited a higher interest in the new object, while aged ones seem to be indifferent to it (D). * P ≤0.05, ** P ≤0.01.

Calcium blockers can generate small depolarizing difference in young and aged animals.

Although, one of verapamil's main effect is on the L-type calcium channels, that are active in the plateau phase of the cardiomyocyte action potential, the chronic treatment of APP mice, showed some difference in depolarization.

As expected, no difference in the duration of atrial depolarization was observed (Figure 2A), however aged controls had a longer ventricular depolarization compared to baseline, that was lost in treated animals (P=0.015, Figure 1G).

Interestingly, some age-related amplitude changes could also be seen in both atrial and ventricular depolarization.

The amplitude of the P wave decreases with time, with aged animals having a lower amplitude compared to young ones (P=0.04, Figure 1B).

However, the chronic administration of calcium blockers seems to attenuate this difference, as young and aged treated animals have no difference in the amplitude of the P wave.

The opposite effect was seen in ventricular depolarization.

While the amplitude of ventricular depolarization under calcium blocker treatment, measured by ECG, increased in young APP mice this value dropped in aged animals (P=0.034, Figure 2H)

Calcium blockers affect the duration of ventricular activity in both young and aged animals

The global effect on the heart rate, after chronical use of verapamil was similar in both young and aged animals, but only for the latter the increase in R-R interval was significant (P=0.044, Figure 2E).

This increase seems to be consistent for both age groups, with a longer QT being observed in all treated animals (Figure 2I).

For aged treated animals however, the initial phase of repolarization (ST segment) was significantly longer (P=0.005, Figure 2C).

Verapamil did not have a major impact on the repolarizing amplitude, with no difference between groups in the ST amplitude.

However, the treatment decreased the amplitude of the T wave in young animals (P=0.035, Figure 2F).

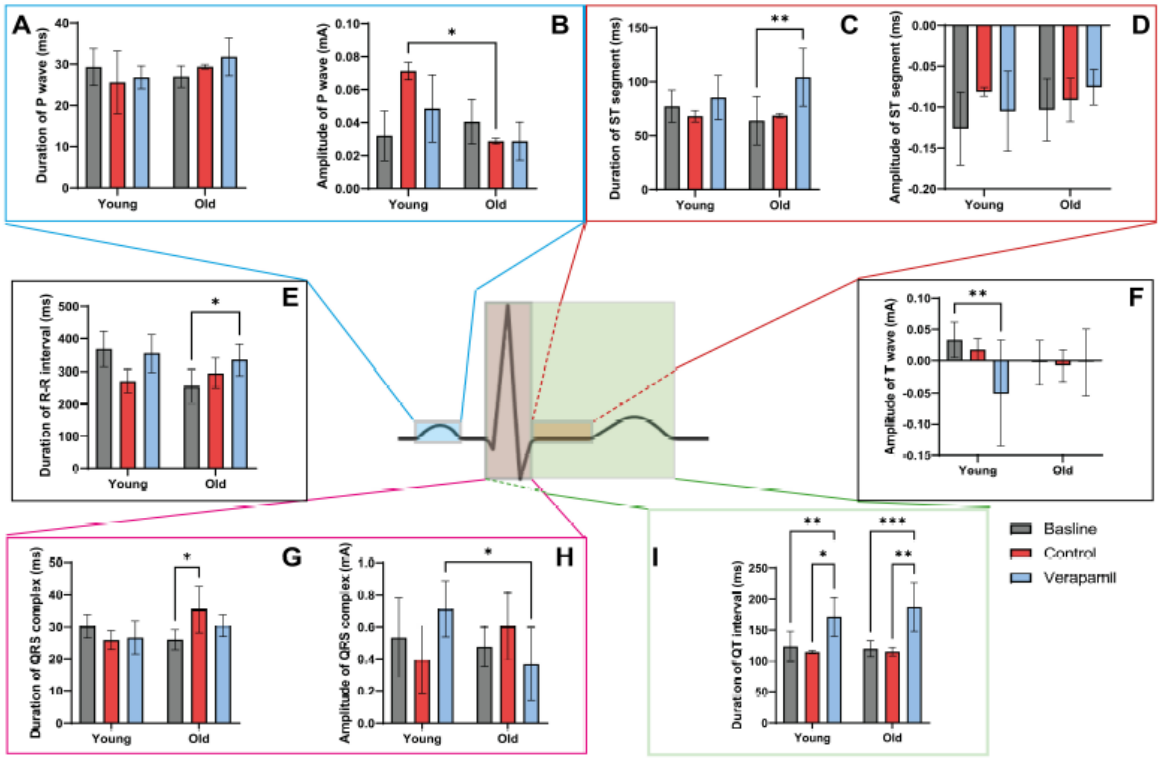

Figure 2. Electrical activity of the heart. Calcium blockers do not influence the atrial depolarization time (A) but can generate small difference in young and aged APP mice regarding the amplitude of the P wave (B). The total duration of ventricular depolarization was brought back to normal after Verapamil treatment for aged animals (G), but the amplitude of the QRS complex remained smaller (H). The main effects of verapamil were on the repolarization period with both young and aged animals showing an increase in the QT interval duration (I), but only the old APP mice had longer R-R intervals (E). * P ≤0.05, ** P ≤0.01, *** P ≤0.001

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Discussion

As lifespan increases, debilitating diseases will generate more DALYs worldwide, with AD being one of the biggest challenges.

As the number of patients increases, people will need additional treatments targeting ever more comorbidities per individual.

Some of the comorbidities will only be associated with age (high blood pressure, type 2 diabetes mellitus, dyslipidemia etc.) while other have a significantly increased prevalence in AD patients (osteoporosis, urinary incontinence, degenerative joint disease etc.) [21,22].

Despite all the efforts made over the years, progress in therapeutic means has been modest. However, a large amount of molecular processes that can influence the evolution of AD have been discovered [5,6,23-26].

With different classes and doses of drugs being prescribed to one patient in different pathologies, the interaction between molecules should be investigated.

For example, a study done on Wistar rats showed that doses lower than 2.5mg/kg/day of verapamil potentiated the effect of scopolamine, improving memory, while over 5mg/kg/day had an opposite effect, caused by the interactions between calcium homeostasis and the receptors on which scopolamine acts [27].

In a mouse model of AD, injected with intracerebroventricular streptozotocin, animal that received 1mg/kg/day of verapamil for 3 months, had improvement in memory testing, suggesting that low doses of verapamil over a long period of time may attenuate the progression of AD [28].

In the present study, although some improvements were observed in young APP animals, no memory improvement was observed in aged animals (Figure 1D).

This difference could be explained either by the length of the treatment as, in our case, verapamil was given for only 28 days, or by the fact that in the previous article the most advanced age tested was around 52 weeks, while we tested the effect on older animals.

In the present study, we found similar heart-rate verapamil-induced results on C57Bi/6J mice as seen in the literature, under anesthesia [29].

However, most parameters reported in other studies are just for acute treatment and short intervals after administration, while here, we focused on the chronic effect of therapy.

As such, the heart rate should not be the only ECG parameter monitored. We observed that, in our case, the R-R interval was much more accurate in depicting verapamil changes on the heart rate (Figure 2E).

Aged controls had a decreased P amplitude; however, the same was not true for the QRS complex (Figure 2H).

This changed in treated animals.

For young mice, this could be explained by a better ventricular filling secondary to the prolongation of R-R and as such a longer ventricular diastole, which results in improved perfusion and better myocardial activity.

In contrast, after chronic administration of verapamil, the observed decrease seen in older animals could be explained strictly related to age and to the degenerative changes that may occur.

On the contrast, all changes in duration can be explained by locally induced hypocalcemia, as a direct verapamil effect, thus prolonging the duration of the plateau phase in the action potential of the myocardial fiber [30] or by the fact that atrioventricular cells are depolarized at a higher frequency that is considered to be normal for them, in contrast with the sinoatrial cells, thus a different dromotropic effect of verapamil can be seen in the ventricles compared to the atrium [31].

In contrast with humans, where QT interval is related to heart rate, in mice it seems not necessarily to be related to it [32].

The main ECG disadvantage of this study is the ECG analysis system, as in small animals is almost impossible to obtain an overview as close as possible to the recording that is obtained in humans.

This is due to the limited number of derivations but also to the large amount of artifacts that can cause changes in the electrical activity of the heart, minimized in this case with the help of the anesthetic [33].

Conclusion

This study has shown that verapamil, a calcium channel blocker, commonly prescribed for various cardiovascular conditions, could also have an effect on the nervous system in patients with AD.

Depending on the age of the duration of the Aβ accumulation, the behavior of APP animals can improve anxiety and short-term memory, in the early stages, or have a negligible result in advance ones.

Although most of the age-related ECG changes were diminished after verapamil

treatment, the QRS complex amplitude was significantly smaller compared to young animals receiving the same treatment.

Although more studies are needed, based on our observations, in patients with early stages of AD and cardiovascular problems that can be resolved with a calcium blocker, its administration could improve the short-term life quality of such patients.

Conflict of interests

None to declare.

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