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Exploring the Potential of Riluzole in Stimulating Brain-Derived Neurotrophic Factor Release from Human Platelets: Implications for Depression Treatment

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ABSTRACT
This study explores the potential of riluzole, a drug used in depression treatment, to stimulate the release of brain-derived neurotrophic factor (BDNF) from healthy human platelets. BDNF plays crucial roles in neuronal survival, activity modulation, and neurotransmitter regulation within the central nervous system. While the source and peripheral function of BDNF remain unclear, its presence in serum has been established. Pharmacological interventions have shown that circulating BDNF, stored in platelets, can be released through treatment. However, the origin of this protein within platelets remains unknown. Notably, reduced serum levels of BDNF have been implicated in the pathophysiology of depression, underscoring its relevance in mood disorders. Riluzole, known for its ability to lower extracellular glutamate levels and increase BDNF expression, has emerged as a potential antidepressant agent. The investigation aims to elucidate whether riluzole treatment can directly stimulate the release of BDNF from human platelets at therapeutic concentrations. Such findings could enhance our understanding of riluzole's mechanism of action in depression treatment, shedding light on its role in modulating BDNF levels.

INTRODUCTION

This neurotrophic factor acts as a modulator of neurotransmitter levels and plays a key role in neuronal plasticity. Neuronal survival and activity are influenced by BDNF during brain development. [1 - 4]. Neurotrophins BDNF are also abundant in human serum, although their sources and functions are unknown. Though BDNF crosses both ends of the blood-brain barrier (BBB) and is circulating in the blood, investigators have mentioned the brain as its source [5 - 10]. BDNF is released at significant rates by peripheral tissues, such as different epithelia, where its amounts may be higher than those found in the central nervous system. The production of BDNF is primarily attributed to neurons and glia, however epithelia are also capable of releasing significant quantities of the substance [11]. In addition to white cells, platelets are also known to contain significant quantities of this circulating neurotrophin. These two sources may be important sources of this neurotrophin [12–14]. Pharmacological treatment can release BDNF proteins from blood platelets into the serum, which constitute more than 99% of blood BDNF proteins [15, 16]. Psychological diseases, such as depression, have been linked to changes in BDNF levels in serum [17–20]. Several studies indicate that decreased BDNF levels contribute to depression pathophysiology [21–25]. It has been suggested that antidepressant drugs could target reduced levels of BDNF in lymphocytes and platelets in depressed individuals. A number of antidepressants are known to increase BDNF expression in cells, but may also cause platelets to release BDNF after direct treatment in vitro, in a dose-dependent manner [26, 27].

Depression symptoms are increased when antidepressants are administered intravenously, owing to the rise in serum levels of BDNF. BDNF levels in the peripheral blood are related to the release of this

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neurotrophin from platelets. Mood disorders can be treated with glutamatergic modulators. There are several drugs that could be used to treat psychiatric conditions that are associated with glutamate excess, one of which is riluzole, an anticonvulsant developed specifically for treating convulsions, but which is also effective against psychiatric disorders in which excess glutamate is implicated in the pathophysiology. The complex pharmacological effects of riluzole may be explained by its many mechanisms of action. An antidepressant effect of glutamate has been suggested to be at least partially mediated through stimulation of glutamate uptake at low glutamate concentrations. The antidepressant properties of riluzole may also depend on other mechanisms, as it increases BDNF expression as well. BDNF serum levels in patients treated with riluzole are significantly increased. Since BDNF is believed to be produced by platelets and is evoked by antidepressants, we explored whether riluzole could increase its release from human platelets.

METHODS

Subjects

An anonymous blood collection was conducted at the Clinical Hospital's Hemotherapy Service on 54 healthy male volunteers. According to the World Medical Association's Code of Ethics for experiments involving humans, the study was conducted. Donors provided their informed consent, and their privacy rights were respected.

Procedures

Using a vacutainer containing K3-EDTA, two 4 mL samples were drawn from each donor's antecubital vein. A hematology analyzer, ABX Micros ES 60, was used to calculate the platelet count in each blood sample after gently inverting it 10 times. Previously described techniques were used to isolate platelets. A platelet-rich plasma (PRP) was obtained by centrifuging the vacutainers at 300 g for 5 minutes at 4 °C. Plastic pipette tips were used to carefully remove the supernatant (PRP), without disturbing the leukocyte layer. Based on the volume of each sample, PRP was transferred to a microcentrifuge tube. A centrifuge was then used to spin PRP for 10 minutes at 4C with 7000g of force. Using phosphate buffered sucrose (pH 7.3), pellets were reconstituted with heparin-enriched phosphate buffered sucrose (heparin rich). Until visible aggregates disappeared, the suspension was repeatedly passed through a 1 mL plastic pipette tip. Inversions of the suspension were followed by the addition of a buffered sucrose solution. After centrifugation, the PC was resuspended in a 0.32 M phosphate-buffered sucrose (pH 7.4) after centrifugation. The centrifuged PRP was resuspended in 0.32 M phosphate-buffered sucrose (pH 7.4) after a fifth of the original volume had been removed. In a second measurement, the PC suspensions of both subjects were blended and platelet counts were calculated. Following the washing of the platelets, the PC should be used to count the platelets. We measured the mean platelet volume (MPV) and platelet distribution width (PDW) of both whole blood and PC to evaluate potential variations in platelets during processing. A final volume of 150 μL was used in each well to dilute drugs in tris-citrate buffer.

Protein Assay for BDNF

An ELISA kit for measuring brain derived neurotrophic factor (BDNF) was used according to the manufacturer's instructions to measure the level of BDNF in fresh supernatants. For each BDNF measurement, we performed three identical experiments in triplicate on 96-well plates, each with a standard curve. A phosphate buffer solution (pH 7.4) was used to dilute samples from the supernatants for measurement of BDNF. Based on the BDNF result obtained by dividing the total platelet count from the same individual by the BDNF result, platelet BDNF content was calculated as pg BDNF 10−6 platelets. Microplate readers to 450 nm were used to measure optical density; Galapagos software was used to analyze the optical density data. There was no cross-reactivity between the assay and other nerve growth factor members, with a sensitivity of 7.8pg BDNFmL.

Assay for MTT

After 4 or 24 hours of drug exposure, riluzole or sertraline were tested for effects on platelet viability using the MTT assay. In this experiment, the absorption was measured at 570 nm, which gave the result (UAbs) as UAbs 20 x 108 platelets.

STATISTICS

Statistical analysis was performed with SPSS, and values are reported as mean ± SEM. Multiple comparisons were performed on the BDNF (one-way ANOVA) or MTT (two-way ANOVA)-derived data, determining their normal distribution.

RESULTS

The BDNF release was directly stimulated by riluzole acting directly on washed platelets. First, the cell count, MPV, and PDW of whole blood were measured and then once the PC was obtained, in order to assess whether these parameters changed during the processing. A normalization of the BDNF quantity in

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each experiment was then performed using the platelet number quantified in each PC. Since the number of cells was reduced during the process of obtaining the PC, we recommend determining the platelet count after the PC is obtained. PC analysis revealed no contaminant cells, and MPV and PDW indices did not change following cell harvest due to platelet loss. MPV and PDW, which showed levels of respectively, did not show significant differences compared to PC. Based on our analysis, we found that the platelet count achieved in the PC was significant. Our experiment showed that BDNF was released from donors' platelets at basal values between 9.0 and 220.2 pg 10−6 platelets. The effect of riluzole treatment on BDNF production from human platelets was tested in light of recent evidence that the drug significantly increases BDNF levels in patients' serum. At room temperature, riluzole was applied to platelets from healthy volunteers for a period of four or twenty-four hours. BDNF levels were significantly increased after four hours of riluzole treatment (P 0.05) in comparison to the controls. Treatment with riluzole stimulated the release of BDNF even in platelets from donors with lower basal levels.

Our model showed reproducibility in BDNF release after riluzole treatment, despite individual differences. We compared each set of experiments with a control group to account for these differences in baseline BDNF levels. BDNF was also released from platelets in the presence of sertraline (0.3 M) as a positive control. Sertraline has been reported to be a potent inducer of BDNF release from platelets in a recent study. As expected, sertraline failed to significantly stimulate the release of BDNF in our experiments. Incubating platelets with riluzole for 24 hours did not result in an increase in BDNF release. Riluzole was exposed during the MTT assay to determine whether it affected platelet viability. As a result, we evaluated the oxidoreductases of the cells after four and twenty four hours of incubation. After two incubation periods, no difference was observed in the viability of the untreated platelets, although slightly less formazan was generated after 24 hours.

DISCUSSION

As platelet function is reported to be correlated with MPV and PDW, we evaluated these indices. Platelet volume is measured by MPV, which describes platelet size and indicates activated platelets, and platelet volume is measured by PDW, which measures variability in platelet volume. In this study, we used platelets that had not been activated at the time of exposure to riluzole because MPV and PDW changed neither before nor after they were purchased and processed. The PDW did not reveal a significant difference between these cells, so a subpopulation was not selected. BDNF was quantified using only platelets, and, therefore, contamination by leukocytes cannot affect the quantification of BDNF. Our study demonstrated that riluzole directly induced the release of BDNF from human platelets. Despite low doses (from 10 m), riluzole had a stimulatory effect, potential clinical implications. While there were variations between donors, the stimulatory effect was reproducible when compared to that of another antidepressant, though not as large as that of another antidepressant. Because we used human cells instead of rat platelets, sertraline had no effect on BDNF release. The basal BDNF levels that Watanabe and coauthors obtained were not reported, which would have enabled us to compare them to ours. When riluzole was incubated for longer periods, it did not affect platelets acutely. It appears that riluzole activates neurotrophin release from the platelet pool rather than stimulating its synthesis when BDNF is released acutely (4 h) but not later (24 h). Based on data showing that BDNF mRNA levels are extremely low in human platelets, this finding is supported. BDNF is released from human platelets by riluzole, a novel finding that may occur peripherally as well as in deep CNS regions where platelets are very close to astrocytes and BDNF can penetrate the BBB. The effects of this neurotrophin on the CNS, especially regarding the glutamatergic system, may be complex and significant beyond the peripheral consequences of riluzole-mediated release. In glutamatergic synaptic transmission, BDNF enhances excitatory synaptic transmission via pre- and postsynaptic mechanisms. Furthermore, it is also known that it stimulates the expression of glutamate transporters in astrocytes and increases glutamate uptake capacity. In hippocampal neurons, BDNF has been shown to upregulate vesicular glutamate transporter expression, supporting its role as a modulator of glutamatergic synapses. Psychiatric conditions where glutamate excess is thought to contribute to the pathology have been treated with riluzole in clinical trials. In addition to its antidepressant properties, it may also be anxiolytic in nature. It reduces extracellular glutamate levels in these conditions and stimulates BDNF expression through its stimulatory effects. It is therefore important to study platelets from patients treated with riluzole in vivo in order to demonstrate that it causes BDNF release at low

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concentrations. A study in our laboratory is currently investigating how BDNF is released from human platelets.

CONCLUSIONS

This study provides valuable insights into the potential mechanism of action of riluzole in the treatment of depression. By exploring its ability to stimulate the release of BDNF from human platelets, we have uncovered a potential pathway through which riluzole exerts its antidepressant effects. The findings suggest that riluzole may enhance BDNF levels, which are known to play crucial roles in neuronal function and mood regulation. This further strengthens the rationale for using riluzole as an adjunctive or alternative therapy for depression, particularly in cases where BDNF levels are compromised. Future research could delve deeper into the specific molecular mechanisms underlying the interaction between riluzole and BDNF, potentially uncovering novel targets for pharmacological intervention in mood disorders. Overall, this study contributes to our understanding of the complex neurobiology of depression and offers new avenues for the development of more effective treatment strategies.

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