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INHIBITION OF NOVOCAINE HYDROLYSIS IN HUMAN SERUM BY HESPERIDIN IN VITRO

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Despite the development of modern approaches to the design and modification of new molecules, the nomenclature of local anesthetics has remained constant over the past two decades. The difficulty of developing new molecules with anesthetic effect is explained by the relative complexity of their mechanism of action, which in turn determines specific requirements for the physicochemical properties of these compounds. An alternative is to optimize existing anesthetic drugs to control pain effects of various origins and treat chronic neuropathic pain syndromes.

Novocaine is a common local anesthetic that is a reference anesthetic in ambulatory practice, which is well studied, with known sympatholytic, anti-inflammatory, and moderate toxic effects. However, the short duration of analgesic action of novocaine is a significant disadvantage compared to other alternatives, such as lidocaine. The relatively short duration of action is due to the structure of the molecule: novocaine is an aminoester, which, unlike amides, is metabolized by plasma esterases, namely butyrylcholinesterase [1-3]. The possibility of increasing the duration of local anesthetic action necessitates the search for inhibitors of the process of decomposition of novocaine by butyrylcholinesterase, as the main enzyme that prevents long-term anesthesia. The flavonoid hesperidin can be considered as a potential inhibitor of butyrylcholinesterase.

The efficiency and prospects of using hesperidin to create a new pharmaceutical composition based on novocaine were studied by determining the kinetics of novocaine hydrolysis by human serum butyrylcholinesterase. The study was based on the spectrophotometric kinetic method *in vitro*.

The working solution was a mixture of phosphate buffer (pH=7.6) and lyophilized blood serum solution (Czech Republic, Erba Lachema s.r.o.) in the ratio of 1400 μL: 50 μL, respectively. Dimethyl sulfoxide was used as a solvent for flavonoids. 30 μL of the required concentration of hesperidin solution was added to the working solution and incubated for 5 min at 37 °C. Hesperidin was tested at concentration points of 5, 25, 50, and 100 μM. After incubation, 50 μL of novocaine solution (10 μM) was added to obtain a total volume of 1530 μL of reaction mixture. As a control solution, 1530 μL of a mixture identical in composition to the working solution in the test samples, but not containing the flavonoid, was used. Immediately after the addition of novocaine, measurements were made using a SPECORD 200 UV spectrophotometer (Analytic Jena, Germany) at 290 nm for 30 min. The signal was detected at intervals of 75 seconds. Each concentration point was tested 3 times.

The rate of decomposition of novocaine by butyrylcholinesterase in the presence of a flavonoid was determined by calculating the first-order rate constant (K_H^1). The data were calculated and visualized using standard methods in Microsoft Excel for Microsoft 365 software.

Hesperidin was found to inhibit human serum butyrylcholinesterase at all test concentrations. The greatest inhibition effect was found when hesperidin was added to the mixture at a concentration of 100 μM, at which the rate constant of novocaine hydrolysis significantly ($p \leq 0.05$) decreased by 1.6 times ($K_{H(100)}^1 = 0{,}82 \pm 0{,}03 \times 10^{-3}$ 1/s) compared to the value of the corresponding rate constant of novocaine hydrolysis in the mixture without the flavonoid ($K_{H(0)}^1 = 1{,}39 \pm 0{,}01 \times 10^{-3}$ 1/s).

Hence, hesperidin can be considered a promising active pharmaceutical ingredient of a long-acting pharmaceutical composition based on novocaine.

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