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Human and Animal Physiology

Development of Luteolin Orodispersible Tablets Using Artificial Intelligence Technology and Assessment of their Antihypertensive Activity in Rats

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(Presented by Academy Member David Mikeladze)

The antihypertensive effect of luteolin orodispersible tablets (ODTs) created by artificial intelligence (AI) technology was investigated in hypertensive rats. It was shown that luteolin ODTs-1 during certain intervals of observable period (24h) provided significant hypotensive action, reducing arterial pressure and cardiac rhythm. Such alterations are more markedly expressed and prolonged after administration of luteolin improved formulation-ODT-2. The reduction in blood pressure caused by ODT-2 was correlated with significant increase in baroreflex sensitivity. The recent advance in novel drug delivery system aimed for the development of dosage forms convenient to manufacturing and administration, offering immediate release drugs within a few seconds without the need of water and increased bioavailability. Difficulty in swallowing of solid pharmaceutical forms is sometimes common among aged patients and children. In this study, a two-step experimental design was performed and databases were successfully modeled using AI technology as an innovative method to get optimal and stable ODT. © *2024 Bull. Georg. Natl. Acad. Sci.*

artificial neural networks, orodispersible tablets, luteolin, hypertension, baroreflex, cardiohemodynamic parameters

Many researchers in resistant forms of arterial hypertension (AH) indicate about necessity of a new targets, playing significant role in vascular homeostasis and consequently in the regulation of arterial pressure (AP). A number of evidence [1] suggests that flavonoid origin natural phenolic compounds may prolong epoxyeicosatrienoic acids (EETs) vasorelaxing action by inhibition of soluble

epoxide hydrolase (sEH) action [2]. The EETs may provide cardio-renoprotective, vasodilating and natriuretic action facilitating reduction in AP. To achieve resulting desirable effect of any drug along with their efficacy pharmaceutical formulation is also very important [3]. Drugs administered via the oral route represent 70% of the total pharmaceutical dosage forms in the market, because to easy receiving a correct dose, lowest cost and stability of all oral dosage forms, as well as convenient to carry and easy to swallow. Despite the listed advantages, the administration of solid dosage forms suppose a problem for pediatric, geriatric or psychiatric patients, due to difficulties to swallow them [4,5]. Orodispersible formulations represent a solution to all these problems, since they disperse or dissolve in seconds in the saliva [6]. Artificial Neural Networks (ANN) are computer technologies able to detect data trends and relationships and learn from experience, mimicking the learning process of the human brain. Neurofuzzy Logic (NFL) is a hybrid technology that combines the data learning capabilities of ANN with the ability of NFL to express concepts in a simplified way, as linguistic „IF-THEN“ rules [7,8]. The use of this technology could be a useful tool on formulation optimization process of NLC [9,10].

The objective of this work was to use the mixed Artificial Neural Network software and additional AI technology to obtain an optimal formulation of luteolin ODT with significant antihypertensive action.

Materials and Methods

Formulations preparation. An experimental design for 4 variables (binder 1 (%) – Prosolve SMCC HD90, binder 2 (%) – Mannitol, superdisintegrant (%) – Kollidon® 30 and compression level at 3 levels (30 kg/cm^2, 25 kg/cm^2, 20 kg/cm^2) was established. As a result 27 direct compressed formulations were prepared and characterized [11].

Evaluation of ODTs. Hardness/Crushing strength: Crushing strength parameter was measured following USP Pharmacopoeia <1217>. Friability: Tablet friability was determined following USP Pharmacopoeia <1216>. Disintegration time: The disintegration time was evaluated following USP Pharmacopoeia <701>, distilled water was used as a disintegration medium. Weight variation: Each tablets (50 pcs) were weighted individually to check weight variation using analytical balance [12].

***In vivo* study.** Experiments were performed in 64 Wistar male rats weighing 200-250 g. Rats were divided into 4 groups: I-Control C) normotensive, II-Hypertensive (H), III-H rats receiving oral disintegrating tablets (ODT-1) of luteolin, IV-H rats receiving (ODT-2) of luteolin (see Table). AH was created by subcutaneous administration of methyl prednisolone acetate MPA – (20 mg/kg week) for two week [13]. Systolic (S) and diastolic (D) was obtained using non-invasive “tail-cuff” sphygmomanometric method in non-anaesthetized rats. In part of experiments in rats of different groups under anesthesia polyethylene catheters were preliminary implanted into right carotid artery and right jugular vein for measuring blood pressure (mmHg) by electromanomentic method and drugs administration, respectively. Heart period (ms) was assessed by cardiotachometer. Baroreflex parasympathetic component sensitivity (BRS) was evaluated [14,15] by developing bradycardia in response to pressor effect after phenylephrine intravenous injection (10 mcg/kg). Baroreflex regression coefficient was calculated by methods of correlative and regression analysis.

Statistical analysis. The SPSS software was used for statistical analysis measurement data to mean ± standard deviation (SD) using t test and single factor analysis of variance for group comparison. $P<0.05$ indicates significant difference using Student’s test.

Modelling by artificial intelligence tools. Database obtained from the results of the experimental design assays were modelled using two commercial AI software: FormRules® v4.03 (Intelligensys Ltd, UK) and INForm® v5.01 (Intelligensys Ltd., UK). FormRules® is a Neurofuzzy Logic (NFL) technology that combines artificial neural networks and fuzzy logic, allowing to understand the effect of the addition of the different components and the operation conditions on the properties of the tablets by direct compression.

The quality of the predictive model for each parameter was evaluated using the determination coefficient of the training or test sets (R^2) [16] expressed in percentage (predictability) and the analysis of variance (ANOVA) (accuracy) as described previously [17].

Appropriate desirability functions were created to find the variables to obtain the tablets with hardness higher than 4 Kg, disintegration time less than 20 seconds and friability lower than 0.9%.

Results

FormRules model was assessed positively because provides an opportunity to establish optimal quantities of experimental batches, using of an adequate parameters, high predictability of expected results (more than 70%), the exact correlation between alteration of each parameters and predictability, proposing the best variant to achieve desirable goal, the relationship between variables and resulting of physicochemical parameters, giving experimental design. High predictability ANN models were also generated by INForm®, to obtain optimal ODT composition.

Based on the experimental results, "FormRules" given following information about hardness, friability and disintegration time of ODT. Increasing the binder 2 concentration causes a reduction of hardness and increasing binder 1 – prosolve SMCC HD90 leads to a increase hardness.

In the case of friability, when the amount of superdisintegrant increases this causes reduction of friability (%), in case of binder 2 concentration on up to 9.9% decreases friability of ODT and over this concentration it causes an increase friability (%).

Decreasing compression level results in a reduction of disintegration time and increasing the binder 2 concentrations also leads to a decrease the disintegration time.

High predictability ANN models were also generated by INForm®, it represents predictive quantities of variables (Prosolve SMCC HD90, Mannitol, Kollidon 30, Pruv and Pressure) for the best formulation of ODTs (Table).

Luteolin ODT-1 of was prepared using the predicted quantities variables. Luteolin 1mg mixed in predicted formulation and tableting was carried out using a predetermined pressing force. After the evaluation of luteolin ODT, the results did not fit within the acceptable limits of the parameters. In order to improve the physical characteristics of the luteolin ODT, the obtained results of evaluation

Table. The best formulation for ODT and its predicted properties and acceptance results

Best formulation of ODTs (Value)						Predicted properties/ acceptance results			
ODT	Prosolve SMCC HD90 (%)	Mannitol (%)	Kollidon 30 (%)	Pressure kg/cm^2	Luteolin (mg)	Weight variation (%)	Hardness (Kg)	Friability (%)	Disintegration (sec)
Model ODT	47.5	45.0	6.5	30	-	1.6	6.7	0.2	25.6
Luteolin ODT-1	47.5	45.0	6.5	30	0.5	1.8	10.7	0.08	58
Luteolin ODT-2	38	55.8	5.2	20	0.5	0.9	5.9	0.3	27

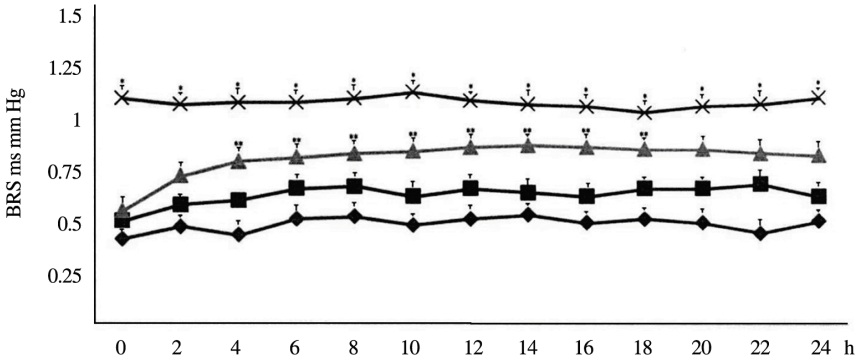

Fig. Time dependent changes of baroreflex sensitivity (BRS) in normotensive (N) and hypertensive (H) rats receiving luteolin different oral disintegrating tablets (ODT) X - N animals (n=6), ♦ - H animals (n=6), ■ - luteolin ODT-1 (n=6), ▲ - luteolin ODT-2 (n=6), *,** $p<0.05$ and 0.01 vs H rats respectively.

were reprocessed by artificial intelligence. A new predicted formulation was created and luteolin ODT-2 was prepared repeatedly.

Hemodynamic and baroreflex sensitivity study. The analysis of cardiovacular parameters obtained by "tail-cuff" method showed marked differences in baseline daily mean values of systolic (168.2±5.8 mm Hg, P<0.05) and diastolic (78.6±5.6 mm Hg, P<0.05) arterial pressure of hypertensive rats (HR) vs. the same indices in normotensive ones (NR), (122.6±5.3 mm Hg) and (63.8±5.4 mm Hg), respectively. High blood pressure in HR was associated with significant increase of mean cardiac rhythm (445±14 beats/min, P<0.05) in comparison with NR (392±18 beats/min).

During 24 h monitoring period it was revealed that administration of 1 mg luteolin ODT-1 into the mouth cavity in HR produced- slight hypotensive effect in 0.5-1.0 h interval, which was significantly increased after 4 h of ODT-1 administration, reducing S blood pressure by 17.4±3.8% (P<0.05) and D by 11.5±2.2% (P<0.05). Luteolin hypotensive effect gradually decreased after 12 h of receiving ODT-1 and returned to initial level after approximately 16-18 h of ODT administration. In comparison with ODT-1 formulation, ODT-2 improved luteolin efficacy associated with the more short onset and prolong hypotensive action, which reached its maximal value after 2 h of luteolin administration, S pressure (-27.1±4.8%, P<0.05) and D pressure (-27.5±5.2%, P<0.05). AP attained initial level after 16 h of receiving ODT.

Increased level of AP in HR was correlated with decreased BRS (Figure) and significant reduction of its regression coefficient (0.48±0.08 ms mm Hg) vs NR (1.15±0.1 ms mm Hg, p<0.05). Luteolin ODT formulation along its hypotensive effect in HR significantly increased BRS (mean value-0.72±0.15 ms mm Hg) especially after receiving ODT-2, conforming the involvement of baroreflex cardiochronotropic component in the antihypertensive effect of luteolin.

Discussion

The present work shows the usefulness of artificial intelligence tools to delimit the spaces of knowledge and design of ODT from results that include a small number of experiments.

In addition, the ANN model and genetic algorithms favor the estimation of the optimal conditions to formulate of the desired characteristics [7,8]. This work gives a possibility to manufacturers to change ingredients. Due to the known dependence of ingredients properties on ODT composition, further studies should be carried out to ensure the ability of ANN and genetic algorithms

to analyze the existing interactions between components and to predict formulation parameters, when using other compositions than the one employed on the current study.

The addition of luteolin in the model formulation got worse the characteristics of ODT, by reprocessing the obtained results and taking into account the identification of the influence of variables on the characteristics of the tablets. The program gave us a new formulation with minor changes of variables for the preparation of luteolin orodispersible tablets with the desired characteristics after the optimization of luteolin ODT dissolution time was significantly improved and sat within FDA requirement of *in vitro* disintegration time – less than 30 seconds.

The development of optimal pharmaceutical forms for alleviating bioavailability and rate of absorption of different drug with concomitant reduction of possible disturbing factors during their administration is a great challenge in modern pharmacy [4,5]. Using proper route of drug administration is most important in the elderly, children, mentally returded and uncooperative patients [3,4]. In present study was investigated the antihypertensive effect of ODT-1 and ODT-2 developed by artificial technologies, containing 1 mg luteolin being phenolic compound of natural flavonoids [2,14]. In comparison with ODT-1 improving by artificial intelligence ODT-2 more markedly decreased S and D arterial pressure and HR with faster onset of action, providing peak reduction in blood pressure after 1 h of ODT-2 introduction and more prolong hypotensive action. Such hemodynamic changes were correlated with significant increase of BRS being blunted in H rats.

It is suggested that luteolin ODT can be considered as promising pharmaceutical form for improving absorption rate and bioavailability especially in pediatric and old patients for avoiding difficulties associated with swallowing of drugs.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ლუტეოლინის ოროდისპერსიული ტაბლეტების შემუშავება ხელოვნური ინტელექტის გამოყენებით და მათი ანტიჰიპერტენზიული აქტივობის შეფასება ვირთაგვებში

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(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

ნაშრომში წარმოდგენილია ჰიპერტენზიულ ვირთაგვებში ხელოვნური ინტელექტის გამოყენებით დამზადებული ლუტეოლინის ოროდისპერსიული ტაბლეტების (ოდტ) ანტიჰიპერტენზიული ეფექტის კვლევის შედეგები. ნაჩვენებია, რომ ლუტეოლინი ოდტ-1 ობსერვაციის პერიოდის (24 სთ) გარკვეულ ინტერვალში ავლენდა სარწმუნო ჰიპოტენზიურ მოქმედებას, არტერიული წნევისა და გულის რითმის დაქვეითებით. აღნიშნული ცვლილებები უფრო გამოხატული და პროლონგირებული იყო ლუტეოლინის გაუმჯობესებული ფორმულაციის - ოდტ-2-ის შეყვანის შემდეგ. ოდტ-2-ით არტერიული წნევის დაქვეითება კორელირებდა ბარორეფლექსის გაძლიერებასთან. წამალთა ორგანიზმში ტრანსპორტირების ახალი სისტემა გულისხმობს დამზადებისა და შეყვანის თვალსაზრისით მოხერხებული დოზირებული ფორმების განვითარებას, წამალთა დაუყოვნებელი გამოთავისუფლებით (წამებში), წყლის მიღების საჭიროების გარეშე და მაღალი ბიოშეღწევადობით. მკვრივი ფარმაცევტული ფორმების პერორალური მიღება ზოგჯერ გაძნელებულია ასაკოვან პაციენტებსა და ბავშვებში. ჩვენს კვლევაში ინოვაციური მეთოდის სახით გამოყენებულ იქნა 2-საფეხურიანი ექსპერიმენტული დიზაინი ხელოვნური ინტელექტის ტექნოლოგიის საფუძველზე მიღებული შედეგების წარმატებული მოდელირებით ოპტიმალური და სტაბილური ოდტ-ს მისაღებად.

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