Coordinating metal Co$^{2+}$ and Zn$^{2+}$ compounds with acetylsalicylic acid: the influence on the cardiorespiratory system

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Abstract. The paper researches the effect of cobalt (ACCo$^{2+}$) and zinc (ACZn$^{2+}$) acetylsalicylates in doses of 5 and 10 mg/kg on the characteristics of the rats’ cardiorespiratory system. It is ascertained that coordinating metal compounds with the acetylsalicylic acid have a more pronounced biological effect in comparison with a monocompound of the acetylsalicylic acid; this makes the further search for coordinating compounds’ effects more prospective.

1. Introduction

Significant success in different spheres of medicine, achieved in the recent years, to a great extent is obliged to the introduction of new technologies of obtaining highly active medications into pharmaceutics. One of such technologies is complexing with metals, which is a very promising method of increase and modification of the biological activity of well-known medical substances. The research of coordinating compounds of s, p and 3d-metals with the acetylsalicylic acid, which is a traditional medical substance, is of a special interest. Different evidences of possibility of using complexes on the basis of aspirin derivatives as antineoplastic, anti-inflammatory and antimicrobial drugs have appeared recently [1-3]. It is necessary to point out that the residual of the salicylic acid is a very suitable object for complexing, and this gives new synthetic opportunities for obtaining compounds with an unusual combination of the biological activity.

According to a number of authors, the creation of complex salicylate compounds with bivalent metals is a perspective approach in this area [4]. The works of the above mentioned authors have shown that such complex derivatives, in the basis of which there are biometals (Fe, Zn, Co, Cd, etc.), surpass aspirin in anti-inflammatory effect and have less side effects.

Consequently, the creation of complex compounds with a coordinating center of Co, Zn, Fe, Mn and another range of other metals is a perspective area for the researches of biologists, chemists, and phamaeuitists. The introduction of newly synthesized chemical substances into the clinical practice is possible only providing the detailed study of their specific pharmacological activity and safety on the stage of experimental pre-clinic research. The complex approach with the use of physiological methods, comparison of the obtained results and mathematical biostatistic analysis of quantitative indicators is of great topicality.

In connection with this the aim of the current research was to identify the influence of newly synthesized compounds ACCo$^{2+}$andACZn$^{2+}$ in doses of 5 mg/kg and 10 mg/kg on the main characteristics of the rats’ cardiorespiratory system.
2. Materials and methods

2.1. Animals

All the researches on the animals were carried out according to the principles set out in Directive 2010/63/EU of the European Parliament and of the EU Council of 22.09.2010 on the protection of animals used for scientific purposes.

Healthy sexually mature male laboratory Wistar rats, weighing 180-200 gr ("FSUE "Nursery of laboratory animals “Rappolovo”) and having been placed under quarantine not less than for 14 days, were selected for the experiment. The experiments were performed on 70 male rats, characterized by an average motor activity and low emotionality in the “open field” test, and which make up the majority of the population; that is why they develop the most typical reaction to the effect of different factors [5], including the tested chemical compounds.

The animals were kept under standard conditions of the vivarium at the temperature of 18–22°C on the bedding «RexofixMK 2000» (on the basis of ear shanks) with the natural 12-hour day-and-night cycle, with free access to water (State Standard 33215-2014 «The handbook of keeping and nursing laboratory animals. The regulation rules on housing equipment and procedure organization»), and to full-fledged granulated food of State Standard Р-50258-92.

The research was carried out at the Center for Collective Use of Scientific Equipment “Experimental physiology and Biophysics” of the Department of Human and Animal Physiology and Biophysics of V. I. Vernadsky Crimean Federal University.

2.2. Design of research on studying the reaction of cardiorespiratory system to the injection of the tested compounds, the used research methods and statistic methods

The synthesis, the research of the composition, structure and properties of the coordinating compounds of acetylsalicylates with Co (ACCo2+) and Zn (ACZn2+) were done at the Department of General and Organic Chemistry of V. I. Vernadsky Crimean Federal University under the supervision of Professor A. N. Gusev.

The research of the biological effect of the acetylsalicylic acid, ACCo2+ and ACZn2+ was carried out during their intraperitoneal injection into rats in doses of 5 mg/kg and 10 mg/kg. For this purpose the rats were divided into 7 groups (10 rats in each group):

- Group 1 is a biological control (K, n=10), they are the animals which were injected by the physiological solution of 0.9 % in the volume of 0.2 ml;
- Groups 2 – 3 are the animals which were injected by the acetylsalicylic acid in doses of 5 mg/kg and 10 mg/kg respectively (n=10), the volume of 0.2 ml;
- Groups 4 – 5 are the animals which were injected by ACCo2+ in doses of 5 mg/kg and 10 mg/kg respectively (n=10), the volume of 0.2 ml;
- Groups 6 – 7 are the animals which were injected by ACZn2+ in doses of 5 mg/kg and 10 mg/kg respectively (n=10), the volume of 0.2 ml.

The bioscreening was done 20 minutes later after the intraperitoneal injection in the stated concentrations as at this period their maximum concentration is observed [6]. Simultaneously the following characteristics of all the groups’ animals were registered: heart rate (HR), respiratory rate (RR), systolic blood pressure (SBP) and diastolic blood pressure (DBP). The pulse pressure (PP) was calculated on the basis of the SBP and DBP indices difference.

It is necessary to point out that HR, RR, SBP, DBP and PP are important characteristics of the functional state of the whole cardiorespiratory system and cardiac performance at a definite period of time, and are ones of the main and the oldest biomarkers of the body state in general.

BP, HR and RR of the rats were registered with the help of the system NIBP200A («BiopacSystems, Inc.», USA). BP and HR were registered on the tail artery by applying the cuff on the tail base. In order to record RR the sensor was fixed on the chest area. While registering the indices, the animals were put into an individual box and were carried to the chamber Biopac with a constantly kept temperature of 33°C for creation of comfortable conditions for the animal.
The record of the indices was done during 5 minutes from the moment of the sensor’s signals stabilization. This time is enough for the five-fold BP measurement; HR and RR were being registered continuously. The record and the data processing were done by the computer with the help of the software program «AcqKnowledge 4.2 for MP150» (Fig. 1).

![Figure 1](image)

**Figure 1.** The example of the recording of cardiovascular system indices of the rats’ BP, HR and RR under the effect of ACZn\(^{2+}\) in a dose of 10 mg/kg with the help of the software program «AcqKnowledge 4.2 for MP150».

The data of three repeated experiments were used for the statistical manipulation. The non-parametric statistic methods were applied as the distribution of variate values differed from the normal. The calculations, the statistical manipulation and graphic design of the obtained in the research data on the effect of the tested compounds on the physiological characteristics were done by using the program MicrosoftExcel and the program package StatSoft STATISTICA 8. The reliability of the statistic differences between the control group (intraperitoneal injection of the physiological solution) and experimental groups with different doses of injecting the acetylsalicylic acid and acetylsalicylates Co\(^{2+}\),Zn\(^{2+}\) was assessed by Mann-Whitney test.

### 3. Results and discussion

During the research it was found out that the animals of the control group, after being injected by the physiological solution, did not demonstrate reliable changes of the cardiorespiratory system characteristics (Table 1). At the same time the reliable lowering of HR in animals, being injected by the tested compounds in a dose of 5 mg/kg, was registered (Table 1): at the effect of the acetylsalicylic acid by 22.0 % (p≤0.05), and at the effect of ACCo\(^{3+}\)andACZn\(^{2+}\)by 14.1%(p≤0.05) and 17.0% (p≤0.05) respectively in comparison with such in the control group. The rest characteristics of the animals’ cardiorespiratory system during injecting the tested compounds in a dose of 5 mg/kg did not change reliably.

The increase of the compounds’ dose up to 10 mg/kg lead to the reliable lowering of HR of the animals in all the tested groups (Table 1). Thus, during the injection of the acetylsalicylic acid HR lowered by 21.8 %(p≤0.05), and during the injection of ACCo\(^{3+}\)andACZn\(^{2+}\)by 6.9% (p≤0.05) and 14.3% (p≤0.05) in comparison with such in the control group of animals.

The characteristics of the system blood pressure and respiratory rate of animals, being injected by the acetylsalicylic acid and ACCo\(^{3+}\), did not change reliably. At the same time the injection of ACZn\(^{2+}\) in a dose of 10 mg/kg lead to the reliable lowering of SBP by 5.1 % (p≤0.05), rise of DBP by 14.7 %, lowering of PP by 38.3%(p≤0.05) and rise of RR by 56.3% (p≤0.05) with respect to such characteristics of the animals in the control group (see Table 1).
Thus, the injection of the acetylsalicylic acid and acetylsalicylates $\text{Co}^{2+}$ and $\text{Zn}^{2+}$ lead to the changes in the laboratory animals’ cardiorespiratory system functioning. At the same time, when the dose was 5 mg/kg, the reaction of the cardiorespiratory system of animals to the interjection of the tested compounds had a unidirectional character, which was shown in the negative chronotropic action. The increase in the dose up to 10 mg/kg lead to an insignificant lowering of evidence of negative chronotropic influence of all the tested compounds on the heart rate, as well as to the display of new properties of acetylsalicylate $\text{Zn}^{2+}$: reliable growth of DBP and RR and lowering of SBP, PP and HR.

Therefore, we may come to conclusion that introduction of the metals into the structure of the acetylsalicylic acid modifies the biological properties of the obtained coordinating compounds.

**Table 1.** The characteristics of the rats’ cardiorespiratory system after injecting the acetylsalicylic acid and acetylsalicylates of $\text{Co}^{2+}$ and $\text{Zn}^{2+}$ in different concentrations.

| Group | SBP, mm Hg | DBP, mm Hg | PP, mm Hg | HR, b/min | RR, r.r./min |
|-------|------------|------------|-----------|-----------|--------------|
| Control (1) | 118.4±1.66 | 74.3±1.49 | 44.1±1.56 | 468.9±9.22 | 98.4±4.34 |
| The acetylsalicylic acid 5 mg/kg (2) | 114.2±1.86 | 76.5±1.05 | 37.7±2.33 | 363.5±10.72 | 98.2±3.27 |
| 10 mg/kg (3) | 117.7±1.89 | 79.2±1.03 | 38.5±1.85 | 366.5±10.73 | 100.5±3.23 |
| ACCo$^{2+}$ 5 mg/kg (4) | 114.73±4.0 | 72.07±1.02 | 42.6±1.14 | 403.00±7.92 | 97.5±5.15 |
| 10 mg/kg (5) | 111.67±0.6 | 68.13±0.26 | 43.5±1.31 | 436.87±2.75 | 95.7±1.46 |
| ACZn$^{2+}$ 5 mg/kg (6) | 114.73±0.7 | 78.40±1.60 | 36.3±3.5 | 389.40±6.14 | 99.5±2.43 |
| 10 mg/kg (7) | 112.47±0.3 | 85.27±0.38 | 27.2±1.05 | 402.27±0.34 | 153.87±0.46 |

Note: M±m is an average arithmetic value, M±m is an error of mean, $p \leq 0.05$ is the confidence level of indices difference according to Mann-Whitney test concerning the corresponding animal groups; SBP is a systolic blood pressure; DBP is a diastolic blood pressure; HR is heart rate; RR is a respiratory rate.
Figure 2. The characteristics of the cardiorespiratory systems of animals, injected by the acetylsalicylic acid and acetylsalicylates of Co\(^{2+}\) and Zn\(^{2+}\) in concentrations of 5 mg/kg and 10 mg/kg relatively to the values in the group of animals, injected by the acetylsalicylic acid, and taken as 100%.

Note: * is the confidence level of differences according to Mann-Whitney test relatively to the values of the indices in control; SBP is a systolic blood pressure; DBP is a diastolic blood pressure; HR is heart rate; RR is a respiratory rate.

Actually, the comparative analysis of the effectiveness of the coordinating compounds with the initial substance (the acetylsalicylic acid) showed (Fig. 2) that during the injection of the acetylsalicylates Co\(^{2+}\) and Zn\(^{2+}\) in a dose of 5 mg/kg the unidirectional character of the tested indices changes of the cardiorespiratory system was observed, this was proved by the absence of reliable differences in the characteristics of animals in the groups AC\(^{2+}\)Co and AC\(^{2+}\)Zn respectively to such in the group of the acetylsalicylic acid. With the increase in the dose up to 10 mg/kg AC\(^{2+}\)Co a reliable growth of HR by 19.2% (p≤0.05) and reliable lowering of DBP by 14% (p≤0.05) were registered, after the injection of AC\(^{2+}\)Zn a reliable growth of HR by 53% (p≤0.05), lowering of SBP by 5.2% (p≤0.05) and PP by 29.7% (p≤0.05) were observed relatively to the indices of the animals in the group, injected by the acetylsalicylic acid in this dose.

It is necessary to pay attention to the differences of cardiotropic effect of the two studied derivatives of the acetylsalicylic acid – acetylsalicylates Co\(^{2+}\), Zn\(^{2+}\) in a dose of 10 mg/kg, this is demonstrated through the differently directed influence on the pulse pressure (the tendency to the growth during the injection of AC\(^{2+}\)Co and reliable lowering at the effect of AC\(^{2+}\)Zn), significantly more pronounced growth of HR at the injection of AC\(^{2+}\)Co and HR at the effect of AC\(^{2+}\)Zn.

Thus, in the state of physiological rest of the rats because of the AC\(^{2+}\)Zn effect in a dose of 10 mg/kg we may observe the irregularity of the heart rate (bradycardia), the increase of the breath (tachypnoea) on the background of decrease of the blood volume, thrown by the heart at the systole moment, which can be the consequence of the high intoxication of the animal’s body.

4. Conclusion
The results of the current research allowed to determine that the coordinating compounds of the acetylsalicylic acid with such metals as Co and Zn not only have more pronounced biological effect in comparison with the acetylsalicylic acid, but demonstrate new properties; the most effect on the cardiorespiratory system was achieved by AC\(^{2+}\)Zn.
The similar conformity of the pharmokinetics is observed in our previous research, while studying neurotropic effects of ACCo and ACZn\(^{2+}\), which showed the activated-modulating effect on the electric activity of mollusc’s \textit{Helixalbescens Rossm} neurons in comparison with the acetylsalicylic acid, and more pronounced effects of ACCo\(^{2+}\) in comparison with ACZn\(^{2+}\) effects [7]. The similar results were obtained by other authors [4, 8-10], in the works of whom it is shown, that such complex derivatives, in the basis of which there are biometals (Fe, Zn, Co, Cd, etc.), surpass aspirin in the anti-inflammatory effect and have less side effects.

Thus, the results of the current research allow us to claim that the salts of the acetylsalicylates, having the metal-complexing Co\(^{2+}\) and Zn\(^{2+}\) in its molecules’ composition, have more pronounced and qualitatively new properties in comparison with the acetylsalicylic acid precursor’s properties; this makes the further search for biological and pharmaceutical activity of these coordinating compounds more perspective, as well as it makes the technology of complexing an inexpensive and highly efficient approach to the creation of new medicines.

5. References

[1] Rubner G., Bensdorf K, Wellner A., Kircher B, Bergemann S, Ott I and Gust R 2010 \textit{J Med Chem} 53

[2] Gilligan M M, Gartung A, Sulciner M L, Norris P C, Sukhatme V P, Bielenberg D R, Huang S, Kieran M W, Serhan C N and Panigrahy D 2019 \textit{PNAS} 116(13) 6292

[3] Altinoz M A, Elmaci I, Cengiz S, Emekli-Alturfan E and Ozpinar A 2018 \textit{Chemico-Biological Interactions} 291 29

[4] Bica K., Rijksen C., Nieuwenhuyzema M, et al. 2010 Phys. Chem 12 2011-7.

[5] Cheretaev I V, Ravaeva M Yu, Dzheldubaeva E R, Chuyan E N, Shulgin V F, Sheichmambetov N, Palaevskaya M V 2019 Scientific notes of the Crimean Federal University named after V.I. Vernadsky. Biology, Chemistry. 5 (71) 199

[6] LevyhA. Je.Mamchur V.I. 2015 \textit{Arterial’naja gipertenzija} 6 (44) 57-63

[7] Cheretaev I V, Korenyuk I I, Khusainov D R, Gamma T V, Kolotilova O I and Nozdrachev A D \textit{Neuroscience and Behavioral Physiology} 2016 46 (6) 644

[8] Aljohin E.K. 1999 \textit{Sorosovskij obrazovatel’nij zhurnal} 5 (7) 85-90

[9] Sokolik J., Tumova I., Blahova M. et al.2006 \textit{Acta Facult. Farm. Univ. Comenianae} 53 224-8

[10] Grigor’eva A.S. 2000 \textit{Mikrojelementy v medicine} 2(1) 17-22

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