The off-label use of drugs for parenteral nutrition as a solvent of substances slightly soluble in water in pharmacological research

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INTRODUCTION

Drugs for parenteral nutrition are an integral part of methods to correct nutritional deficiency.[¹,²] The feasibility and safety of prescribing drugs for parenteral nutrition are based on the results of clinical studies.[³-⁶] It should be noted that, to date, off-label usage is widely distributed for parenteral nutrition tools, including lipid emulsions. There is a problem to evaluate biological activity in water-soluble substances in all phases of preclinical and clinical studies, so an original solvent for poorly soluble compounds based on substances for parenteral nutrition was developed.[⁷] The main aim is to examine the impact of the original solvent based on substances for parenteral nutrition on biological systems exemplified by the hemostatic system, characterized by sensitivity and variability of the effects in response to any impact, and its comparison with the solvents that are conventional in pharmacological research.

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SUBJECTS AND METHODS

Target of research
Originally developed solvent of water-insoluble compounds represented by 10% oil emulsion for 1 liter of the solution
- soybean oil (refined) – 30 g
- triglycerides with medium chain – 30 g
- olive oil (refined) – 25 g
- cod liver oil purified – 15 g.

An analog to dissolve might be any ready-made commercial lipid mixture for parenteral nutrition, corresponding to the above-mentioned formula, such as Smoflipid® (Sweden, Fresenius Kabi).

To compare the effects on hemostasis system, the following solvents were selected: distilled water, 0.9% NaCl solution, ethanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and dioxane at concentrations of 50.0% (I), 10.0% (II), 1.0% (III), and 0.1% (IV).

Study design
All the experimental work is performed in compliance with the guidance on preclinical research of new pharmacological substances.[9] In vitro study was carried out on the blood of healthy male donors. The median age was 22 ± 2.7. The study was approved by Ethics Committee (No 247 dated from October 17, 2012). All research participants gave consent before blood sampling.

Blood sampling and centrifugation
Blood sampling from donor volunteers was carried out aseptically from cubital vein through vacuum blood sampling BD Vacutainer® (Dickinson and Company, United States). All tests were carried out on enriched and platelet-depleted dry blood. The work included centrifuge OPN-3.02 (Kyrgyzstan).

Determination of solvent abilities of the selected solvents
The solvency of distilled water, DMSO, DMF, dioxane, ethanol, and 10% fat emulsion was estimated by solubility of slightly soluble substance in water. Slightly soluble substance was chosen acetylsalicylic acid (2-acetoxybenzoic acid). Ability to dissolve acetylsalicylic acid has been studied for distilled water 95% (vol/vol) DMSO and ethanol, 10% (vol/vol) solution of fat emulsion. Concentration of acetylsalicylic acid after the dissolution was to be 2×10⁻³ mol/L. The volume of solvent is 1 ml. The dissolution took place in standard conditions (standard ambient temperature and pressure) at atmospheric pressure of 750.06 mmHg and temperature of 25°C.

Platelet aggregation
A study of influence of the solvents on platelet aggregation with laser analyzer of platelet aggregation “Biola 230 LA” (LLC “Biola,” Russia).[4] Aggregation inductor was used adenosine diphosphate (ADP) with a concentration of 20 µg/ml and collagen of 5 mg/ml.

Coagulation component of hemostasis system
When examining the influence of solvents on coagulation hemostasis component, the cuvette with platelet-depleted plasma was injected 10 µl of solution of the substance upon constant mixing and incubated for 5 min at 37°C. Further coagulation activity of solvents was determined in vitro with standard clotting tests on turbidimetric hemocoagulometer Solar CGL (Belarus).

Flow cytometry
Cytofluorimetric analysis was performed on the BD FACSCanto II (United States), using FACSDiva (BD Biosciences, USA) software. The research measured binding to platelets of blood in healthy donors of fluorescent-marked antibodies (MA) against CD41a, labeled phycoerythrin, CD61, labeled fluorescein isothiocyan and CD62, marked with allopheocyanin (United States). A marker of platelet activation was measured the expression of P-selectin on platelet surface by ADP of 20 µg/ml for 15 min. The number of positive cells was assessed (%) as per CD41a, CD61, and CD62.

Acute toxicity
A toxicological study was made on 85 white viripotent male rat mice, weighing 20–21 g, upon intravenously injection of the studied solvents. The solvents were injected intravenously at doses of 0.1, 1.0, 5.0, 10.0, and 15.0 g/kg. The injected solution was calculated by its volume, depending on body weight, taking into account the maximum allowable amount of liquid. The test groups were observed within 14 days.

Statistical processing
The findings are processed with Statistica 10.0 (StatSoft Inc., USA). The normality of the distribution of actual data was checked by Shapiro–Wilk criterion. The groups were described by means of a median and interquartile interval. Variance analysis was performed with Kruskal–Wallis test (for independent observations) and Friedman test (for repeated observations). Lethal dose 50 (LD 50) value was calculated with probit-analysis method using BioStat 5.9 (AnalystSoft Inc.). The relationship of signs was evaluated with calculation of the Pearson’s correlation coefficient (r) and the coefficient of determination (r²). Critical level of P significance for statistical criteria was taken equal to 0.05.

RESULTS

The findings show that in selected dissolution conditions, the acetylsalicylic acid precipitates when dissolved in distilled water. Ethanol is able to dissolve this number of acetylsalicylic acid only when heated, and when returning
to the original temperature indicators, the acetylsalicylic acid precipitates. Other solvents, including a mixture of lipids, effectively and tantamountly dissolved acetylsalicylic acid [Table 1].

The findings of the influence of solvents on plasma component of hemostasis system are in Table 2. Traditional solvents lengthened the time of plateletless plasma coagulation. 95% solution of DMSO lengthens the activated partial thromboplastin time on average by 50% compared with the control. 95% ethyl alcohol shows anticoagulation activity for all indicators, extending indicators defined by an average of 5%. 10% solution of the fat emulsion had no effect on indicators of plateletless plasma coagulation.

The findings of the influence of ethanol, DMSO, DMF dioxane on the processes of aggregation of platelet activation, and binding of receptor glycoprotein IIb-IIIa (GP IIb-IIIa) on integrins CD41a and CD61 are in Tables 3 and 4.

**Table 1: Dissolution of acetylsalicylic acid under standard ambient temperature and pressure conditions**

| Solvent                  | Acetylsalicylic acid sludge |
|--------------------------|----------------------------|
| 10% DMSO (vol/vol)       | -                          |
| 10% mixture of lipids (vol/vol) | -                  |
| 95% ethanol (vol/vol)    | +                          |
| 10% dioxane (vol/vol)    | -                          |
| 10% DMF (vol/vol)        | -                          |
| H2O                      | ++                         |

DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide. Sludge: \(+\), \(\mp\) - yes, \(-\) - no.

**Table 2: Anticoagulation activity indicators of the source solutions of dimethyl sulfoxide, ethanol, and lipid emulsions (n=7)**

| Indicator solvent | APTT elongation, percentage to the monitoring | PT elongation, percentage to the monitoring | Fibrinogen, percentage to the monitoring |
|-------------------|---------------------------------------------|--------------------------------------------|----------------------------------------|
| DMSO              | 54.7 (52.6-58.8)**                          | 41.7 (36.7-46.8)*                         | 96.3 (91.2-98.4)*                      |
| Ethanol           | 7.2 (5.7-9.5)**                             | 2.9 (1.1-4.5)**                           | 0.0 (0.0-0.0)**                        |
| DMF               | 78.6 (69.5-84.3)**                          | 50.7 (49.5-56.8)**                        | 48.3 (44.2-50.3)**                     |
| Dioxane           | 84.3 (79.3-86.1)**                          | 60.3 (58.6-64.1)**                        | 63.2 (59.3-70.1)**                     |
| Lipid emulsion    | 0.0 (0.0-0.0)**                             | 0.0 (0.0-0.0)**                           | 0.0 (0.0-0.0)**                        |

The level of statistical significance of the differences of indications in comparison with the observational group: *P<0.001, **P<0.01, ***P<0.05. DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide, APTT: Activated partial thromboplastin time, PT: Prothrombin time.

**Table 3: Influence of solvents on indicators of platelet aggregation and marked antibodies binding with receptor of platelet glycoprotein IIb–IIIa on integrins CD61 and CD41a, Me (25-75)**

| Concentration | Solvent          | Positive platelets by monoclonal antibodies, % | Maximal platelet aggregation, mm |
|---------------|------------------|-----------------------------------------------|----------------------------------|
|               |                  | CD61                                          | CD41a                            |
| -             | Control          | 99.9 (99.9-99.9)                              | 99.9 (99.9-99.9)                 | 51.4 (48.5-54.3)                       |
|               | Water distilled  | 99.9 (99.9-99.9)                              | 99.9 (99.9-99.9)                 | 49.7 (47.9-53.1)                       |
|               | Solution NaCl 0.9% | 99.9 (99.9-99.9)                         | 99.9 (99.9-99.9)                 | 50.1 (49.6-52.9)                       |
|               | 10% blend of lipids | 99.9 (99.9-99.9)                        | 99.9 (99.9-99.9)                 | 52.1 (47.9-54.8)                       |
| I             | Ethanol          | 97.4 (96.2-98.5)                              | 97.5 (96.2-99.9)                 | 44.2 (42.1-46.5)*                      |
|               | DMSO             | 21.0 (19.4-23.1)**                           | 94.7 (93.8-95.6)                 | 0.0 (0.0-0.0)**                        |
|               | DMF              | 9.6 (8.9-11.2)**                             | 0.28 (0.12-1.2)**                | 0.0 (0.0-0.0)**                        |
|               | Dioxane          | 2.7 (2.3-3.1)**                              | 99.9 (99.9-99.9)                 | 0.0 (0.0-0.0)**                        |
| II            | Ethanol          | 99.9 (99.9-99.9)                              | 99.9 (99.9-99.9)                 | 50.9 (48.3-52.4)                       |
|               | DMSO             | 85.5 (83.2-87.4)**                           | 99.8 (99.4-99.9)                 | 13.6 (11.2-16.3)**                     |
|               | DMF              | 78.5 (76.4-79.2)**                           | 77.5 (75.4-78.9)*                | 0.0 (0.0-0.0)**                        |
|               | Dioxane          | 21.2 (19.9-23.6)**                           | 99.9 (99.9-99.9)                 | 0.0 (0.0-0.0)**                        |
| III           | Ethanol          | 99.9 (99.9-99.9)                              | 99.9 (99.9-99.9)                 | 53.7 (51.5-55.1)                       |
|               | DMSO             | 97.4 (96.5-98.3)                              | 99.9 (99.9-99.9)                 | 40.6 (38.8-42.6)**                     |
|               | DMF              | 83.4 (82.1-84.6)**                           | 99.2 (98.7-99.9)                 | 26.9 (24.7-28.5)**                     |
|               | Dioxane          | 51.6 (49.4-54.8)**                           | 99.9 (99.9-99.9)                 | 0.0 (0.0-0.0)**                        |
| IV            | Ethanol          | 99.9 (99.9-99.9)                              | 99.9 (99.9-99.9)                 | 52.6 (50.1-54.9)                       |
|               | DMSO             | 98.5 (96.4-99.9)                              | 99.9 (99.9-99.9)                 | 49.6 (48.7-53.8)                       |
|               | DMF              | 95.4 (93.2-97.4)**                           | 99.4 (93.1-98.9)                 | 39.7 (37.4-40.5)**                     |
|               | Dioxane          | 61.7 (67.1-72.5)**                           | 99.9 (99.9-99.9)                 | 9.9 (8.3-11.6)**                       |

The level of statistical significance of the differences of indications in comparison with control: *P<0.05, **P<0.001 (n=7). DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide.
Saline and sterile distilled water in these volumes do not affect the morphology of the platelets, platelet activation, and aggregation.

Ethanol at a concentration I shows antiaggregation activity that is equal to 10.0%. The values of binding of platelets MA CD41a and CD61 remain at the level of the reference values. However, concentrations of I and II showed full suppression, and concentration III showed reducing of ADP-induced expression of P-selectin by 48.9%. It should be noted that the concentration II and III of antiaggregational activity according to the Born method is no longer registered with ethanol.

DMSO in concentration I completely inhibits platelet aggregation and antiaggregational effect shows till concentration III. Solution III DMSO has no effect on ADP-induced platelet aggregation, however, even this concentration shows effective inhibition of the expression of P-selectin and binding disorder of binding with receptor GP IIb-IIIa on integrins CD41a and CD61 [Figure 1a-b].

DMF suppresses platelet aggregation induced by ADP, in all the studied concentrations. However, concentrations I and II show complete lack of response of platelets to addition of ADP. Solution III suppresses platelet aggregation by 48.9% and solution intravenous (IV) by 24.8% [Figure 1c-d].

The most apparent antiaggregational effect was registered with dioxane. Antiaggregational activity in concentration IV amounted to 85.8%. All the studied concentrations reported reduced expression of P-selectin and platelet, positive on integrins CD41a and CD61 [Figure 1e].

The findings of correlation analysis [Figure 2 and Table 5] show high significant inverse correlation between antiaggregational activity and the remaining free receptors of platelets GP IIb-IIIa on integrins CD61 on platelets for dimethyl sulfoxide, dimethylformamide, and dioxane.

Acute toxicity parameters are presented in Table 6. DMSO LD50 at IV injection to mice is 11.4 g/kg, which is almost 2 times the value of ethanol, which amounted to 5.7 g/kg. Most toxic were DMF and dioxane with indicators of LD50, 0.48 and 0.74 g/kg, respectively. The study of toxicity of lipids did not show any loss of laboratory mice at a dose of 15 mg/kg.

**DISCUSSION**

The findings show that with the exception of sterile distilled...
Table 4: Impact of solvents on the spontaneous and adenosine diphosphate-induced expression of P-select in, Me (25-75)

| Concentration | Substance                        | CD62ADP− | CD62ADP+ |
|---------------|----------------------------------|----------|----------|
|               | Control                           | 1.3 (1.1-1.4) | 17.9 (16.5-19.3) | 0.007 |
|               | Water distilled                   | 1.2 (1.0-1.3)* | 16.4 (15.9-18.6) | 0.008 |
|               | Solution NaCl 0.9%                | 1.3 (1.0-1.4)* | 17.1 (16.5-19.4) | 0.006 |
|               | 10% blend of lipids               | 1.2 (1.1-1.5)* | 17.2 (15.8-20.3) | 0.006 |
| I             | Ethanol                           | 1.2 (0.8-1.4)* | 1.2 (1.0-1.3)** | 0.6 |
|               | DMSO                              | 0.9 (0.7-1.2)* | 0.7 (0.5-0.9)** | 0.4 |
|               | DMF                               | 1.1 (0.9-1.3)* | 1.2 (0.9-1.3)** | 0.8 |
|               | Dioxane                           | 1.3 (1.1-1.5)* | 1.0 (0.8-1.2)** | 0.3 |
| II            | Ethanol                           | 1.3 (1.2-1.7)* | 1.3 (1.1-1.4)** | 0.6 |
|               | DMSO                              | 1.1 (0.9-1.3)* | 1.2 (1.0-1.4)** | 0.5 |
|               | DMF                               | 1.2 (0.9-1.4)* | 1.1 (0.9-1.3)** | 0.4 |
|               | Dioxane                           | 1.1 (1.0-1.3)* | 1.0 (0.8-1.2)** | 0.8 |
| III           | Ethanol                           | 1.2 (1.0-1.4)* | 10.4 (9.5-12.1)** | 0.4 |
|               | DMSO                              | 1.1 (0.9-1.3)* | 1.1 (0.8-1.2)** | 0.6 |
|               | DMF                               | 1.0 (0.8-1.3)* | 1.3 (1.1-1.5)** | 0.8 |
|               | Dioxane                           | 1.1 (0.9-1.2)* | 1.2 (1.0-1.4)** | 0.4 |
| IV            | Ethanol                           | 1.2 (1.0-1.4)* | 17.6 (15.2-19.3)* | 0.004 |
|               | DMSO                              | 1.3 (1.1-1.5)* | 4.6 (3.8-5.4)** | 0.001 |
|               | DMF                               | 1.1 (1.0-1.3)* | 8.7 (7.6-9.2)** | 0.002 |
|               | Dioxane                           | 1.1 (0.8-1.3)* | 15.8 (12.3-17.6)* | 0.005 |

The level of statistical significance of the differences of indications in comparison with control: *P≥0.05, **P≤0.001; P2: Level of statistical significance of differences in group indicators after the activation of the ADP (n=7). DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide, ADP: Adenosine diphosphate.

Table 5: Indicators of correlation of antiaggregation activity and free platelet receptors of glycoprotein IIb–IIIa on integrin CD61

| Solvent | r     | r²    | P     |
|---------|-------|-------|-------|
| DMSO    | −0.785 | 0.616 | 0.0007 |
| DMF     | −0.778 | 0.605 | 0.0008 |
| Dioxane | −0.794 | 0.603 | 0.007 |

DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide

Table 6: Indicators of acute toxicity of dimethyl sulfoxide, ethanol, and lipid emulsions

| Solvent | DMSO | DMF | Dioxane | Ethanol | Lipid emulsion |
|---------|------|-----|---------|---------|----------------|
| LD50, g/kg | 11.4 | 0.48 | 0.74 | 5.7 | >15.0 |

DMSO: Dimethyl sulfoxide, DMF: Dimethylformamide

water, saline solution, and 10% mixture of lipids, all the selected solvents affect hemostasis system.

**Dimethyl sulfoxide**

DMSO at concentration of 1%–10% inhibits platelet release reaction induced by collagen, arachidonic acid, and thrombin and inhibits platelet adhesion, due to inhibition of cyclooxygenase-1.[11,12] DMSO inhibits aggregation of platelet activity even in 950-fold dilution (0.1%). At a concentration of 0.1% (vol), the impact on platelet aggregation of DMSO is not recorded, but the expression of P-selectin reduces significantly. Thus, the use of DMSO as solvent under conditions of preclinical research regarding hemostasis in concentrations of >0.1% (vol) will not allow objectively evaluate their biological activity.

**Dimethylformamide and dioxane**

DMF and dioxane equally aggressively suppress physiological response of platelets in response to aggregation agonists.[13] Despite good solubility and DMF dioxane, using them as a solvent for preclinical studies on hemostasis system is unacceptable at concentrations exceeding 0.1% (vol/vol). Even in this concentration, there is an apparent inhibition of processes of P-selectin expression and antiaggregational activity associated with the binding of CD61.

**Ethanol**

The most studied one of the hemostatic systems in vitro and in vivo is ethanol.[14] In the year 2002, a number of researchers for the first time established direct impact of ethanol on the main receptors of platelets.[15] Dose-dependent inhibition of GP IIb/IIIa and the expression of P-selectin, CD63 receptor, and CD107a were observed in ethanol-treated whole blood samples. The dependence of the effects of ethanol on hemostasis system from concentration suggests the presence of concentration with minimal effect on the functional activity of platelets. The study, aimed at finding a concentration of ethanol that does not affect the
performance of platelet aggregation of ADP and collagen convincingly demonstrated that the use of ethanol in solution of <50% (vol) has no effect on platelet aggregation and probably, is the way out for the dissolution of slightly soluble substances for preclinical studies. However, the findings show that even in a concentration of 1.0% (vol) expression of P-selectin is reduced virtually twice.

**Lipid emulsions**

Lipid emulsions are characterized by good solvent capacity comparable to analog solvents. The widespread use of lipid emulsions as tools for nutritional support has enabled to verify biological inertness, security, and the absence of biological activity both in bolus and long-term use.

Lipid emulsions do not affect the performance of coagulational component of hemostasis and adhesive platelet function of platelets and are less toxic than ethanol and DMSO.

**CONCLUSION**

The solvents, possessing its own biological activity, should not be used as a solvent medium to study potential effects of medicines. The original mixture of lipids on the results of our own research and experience as a means of parenteral nutrition may be considered an adequate solvent at the stage of preclinical studies of new drugs that affect hemostasis system *in vitro*. Results of safety evaluation of IV fat emulsions in the applied dosages will allow eliminating the stage to assess the impact of the solvent on patients.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Casaer MP, Hermans G, Wilmer A, Van den Berghe G. Impact of early parenteral nutrition completing enteral nutrition in adult critically ill patients (EPaNIC trial): A study protocol and statistical analysis plan for a randomized controlled trial. Trials 2011;12:21.
2. Seres D, Way V, Charles W. Nutrition Support for the Critically Ill. Nutrition and Health. NY: Humana Press; 2016. p. 122.
3. Dhaliwal R, Cahill N, Lemieux M, Heyland DK. The Canadian critical care nutrition guidelines in 2013: An update on current recommendations and implementation strategies. Nutr Clin Pract 2014;29:29-43.
4. Pasinato VE, Berbigier MC, Rubin Bde A, Castro K, Moraes RB, Perry ID, et al. Enteral nutritional therapy in septic patients in the Intensive Care Unit: Compliance with nutritional guidelines for critically ill patients. Rev Bras Ter Intensiva 2013;25:17-24.
5. Oda S, Aibiki M, Ikeda T, Imaizumi H, Endo S, Ochiai R, et al. The Japanese guidelines for the management of sepsis. J Intensive Care 2014;2:55.
6. Ishitukov RR, Panteleev VS, Dorofeev VS. Analysis of early complications after using permacol bioimplant in combination with selective administration of the nicergolin in case of intestinal fistulas. Creat Surg and Oncol 2017;7:27-31.
7. Khalilullin FA, Kamilov FK, Timirkhanova GA, Samorodov AV, Samorodova AI, Khalimov AR. Solvent of Compounds Poorly Soluble in Water. Patent RU 2537260; 27 December, 2014.
8. Urakov AL. Development of new materials and structures based on managed physical-chemical factors of local interaction. Mater Sci Eng 2016;123:012008.
9. Urakov A, Urakova N. Rheology and physical-chemical characteristics of the solutions of the medicines. J Phys Conf 2015;602:012043.
10. Fisenko VP. Manual for Preclinical Studies of Medicaments. Part I. Moscow: Grif and C.; 2012.
11. Fratantoni JC, Poindexter BJ. Dimethyl sulfoxide: Effects on function of fresh platelets and on the viability of platelets in storage. Transfusion 1983;23:109-13.
12. Asmis L, Tanner FC, Sudano I, Lüscher TF, Camici GG. DMSO inhibits human platelet activation through cyclooxygenase-1 inhibition. A novel agent for drug eluting stents? Biochem Biophys Res Commun 2010;391:1629-33.
13. Imbriani M, Ghittori S, Prestinoni A, Longoni P, Cascone G, Gamba G, et al. Effects of dimethylformamide (DMF) on coagulation and platelet activity. Arch Environ Health 1986;41:90-3.
14. Salem RO, Laposata M. Effects of alcohol on hemostasis. Am J Clin Pathol 2005;123 Suppl:S96-105.
15. McKenzie ME, Bell CR, Horowitz ED, Oshrine BR, Atar D, Serebrany VL, et al. Effects of *in vitro* exposure of alcohol on surface receptor expression of human platelets. Clin Physiol Funct Imaging 2002;22:153-6.
16. Sharipov RA, Gizatulin RH, Leshkova VE, Latipov AM. Nutritive supports in the patients go through radical cystectomy with ileocystoplasty during early postoperative period. Creat Surg Oncol 2012;2:86-9.
17. Polyakov IV, Zolotukhin KN, Leyderman IN. Influence of artificial lung ventilation on real energy expenditure value of surgical Intensive Care Unit patients. Creat Surg Oncol 2017;7:16-21.
18. Guidance for Industry Q3C. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER); 2012.