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Applying theoretical premises of binary toxicity mathematical modeling to combined impacts of chemical plus physical agents (A case study of moderate subchronic exposures to fluoride and static magnetic field)

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Sodium fluoride solution was injected i.p. to rats at a dose equivalent to 0.1 LD50 three times a week up to 18 injections. Two thirds of these rats and of the sham-injected ones were exposed to the whole body impact of a 25 mT static magnetic field for 2 or 4 h a day, 5 times a week. For mathematical analysis of the effects they produced in combination, we used a response surface model. This analysis demonstrated that (like in combined toxicity) the combined adverse action of a chemical plus a physical agent was characterized by a diversity of types depending not only on particular effects these types were assessed for but on their level as well. From this point of view, the indices for which at least one statistically significant effect was observed could be classified as identifying (1) single-factor action; (2) additivity; (3) synergism; (4) antagonism (both subadditive unidirectional action and all variants of contradirectional action). Although the classes (2) and (3) taken together encompass a smaller part of the indices, the biological importance of some of them renders the combination of agents studied as posing a higher health risk than that associated with each them acting alone.

1. Introduction

The issue of assessing multifactor occupational health risks is rather complex and cannot be unambiguously resolved even in cases involving a combined effect of harmful agents of one and the same nature, in particular of two or more toxic substances, on the organism. Our research team has demonstrated in a range of experiments with binary combinations of salts of lead, cadmium, fluoride, manganese, nickel, chromium, and with manganese and nickel oxide nanoparticles (Varaksin et al., 2014; Minigaliyeva et al., 2014; 2015; Panov et al., 2015; Katsnelson et al., 2015) that the type of combined toxicity cannot be classified unequivocally for subchronic toxic exposure effects evaluated by a great number of indices for the status of different systems and organism as a whole.

Using different mathematical models, we have demonstrated that the three traditionally discussed types of combined toxicity (additivity, subadditivity and superadditivity of unidirectional action) exist along with a lot of their variants depending on exactly which effect is being considered and on its level and on the dose levels and their ratio, and besides that the modeling of opposite (contradirectional) effects of toxics deserves special attention.

It was theoretically and methodologically interesting to try and use the same methodological approach for analyzing the combined action of harmful factors that are *different in nature*, in particular that of a toxic substance and a physical agent, in order to elucidate whether the above conclusions could be generalized to include such action as well. In this paper, we consider this issue using as an example the combined effect of fluoride and static magnetic field (SMF) on the organism.

This case is interesting not only theoretically but also very much practically since this combination of agents is characteristic of working conditions at electrolytic aluminum smelters employing a

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lot of workers (Kvande and Drabløs. 2014). Admittedly, despite increasing electric currents up to several hundreds κA in order to increase the reduction cells capacity, the SMF induction at workplaces remains low (according to our own measurements, under 40 mT) compared with SMF employed in magnetic resonance (MR) technologies as a clinical imaging modality since the early 1980s (commonly from 0.2- to 3 T, with the most powerful modern systems operating at over 8–9 T). However, human exposure to such rather weak SMF in occupational contexts lasts much longer than in MR procedures. It should also be noted that they are higher by several orders of magnitude than the average strength of the Earth's magnetic field (0.05 mT at the latitude of 50°), the biological effects of which due to the so-called geomagnetic storms are well known.

The toxic effects of fluoride on the bone tissue but also on various internal organs have been well studied in humans in relation to occupational or endemic fluorosis and in animal experiments and are comprehensively described in a large number of articles, including reviews (e.g. IPCS , 2002; Katsnelson et al., 2006). There is a considerable body of literature on the effect of SMF on the organism as well, including authoritative review documents (Document, 2008; SCENIHR, 2015), devoted, though, mainly to the effects of stronger SMF than those characteristic of the above mentioned industry.

According to these reviews, researchers have proposed several feasible physical mechanisms of interaction between body tissues and static magnetic fields that could theoretically lead to pathological changes in the organism. However, numerous clinical and experimental studies seeking to identify various harmful effects of a short-term exposure even to powerful SMF (on DNA structure and gene expression, reproduction and development, blood brain barrier permeability, nervous activity, cognitive function and behavior, cardiovascular dynamics, hematological indices, temperature regulation, circadian rhythms, immune reactivity, and other biological processes) have largely provided contradictory results. Still less is reliably known about the effects of long-term exposures to relatively weak SMF.

As for the possible combined health effects of SMF and chemicals, a meta-analysis of experimental studies testing the hypothesis that extremely low frequency magnetic fields enhance the effects of environmental carcinogens has shown that the majority of the reviewed studies were positive (Juutilainen et al. 2006).

Thus the main goals of our study were to find whether: (1) moderate SMS impacts comparable with those characteristic of realistic workplace levels have any adverse effects on laboratory animals; (2) results of their combination with moderate fluoride doses may be described mathematically based on the same principles and methods as had been successfully applied to many cases of binary chemical combination.

2. Materials and methods

This experiment was carried out on outbred white female rats (from our own breeding colony) with 15 animals in each exposed and the control group. All rats were housed in conventional conditions (dry bulb temperature 20–22 °C, relative humidity 50–60%), breathed unfiltered air and were fed standard balanced food and clean bottled water. The study was planned and implemented in accordance with the "International guiding principles for biomedical research involving animals" developed by the Council for International Organizations of Medical Sciences (1985).

The model of subchronic intoxication was created by repeated i.p. injections of sodium fluoride to rats 3 times a week during 6 weeks (totally, 18 injections). The dosage of the salt corresponded to 0.1 LD_{50} and amounted to 3.19 mg F/kg. Animals in the control group were administered normal saline in the same volume (0.5 ml per rat).

Two thirds of the rats so injected (i.e. both receiving and not receiving NaF) were exposed to the whole body impact of a static magnetic field (SMF) for 2 or 4 h a day, 5 times a week. As can be seen in the photo (Fig. 1), this impact was delivered by placing an acrylic glass box with freely moving rats in it in the space between the irons of an electromagnet whose windings were fed with DC supplied from the electric power converter shown in the same photo. The magnetic field induction, measured with the help of the "Universal Portable Milliteslameter" (Metrological Centre of Radio and Magnetic Measurements, Mendeleyevo, Russia), first 3 times a week and then (given the practical constancy of the results) once a week, was equal to the exposure period average of 25 $\pm$ 0.05 mT. We assumed the doubling of a single exposure time to be equivalent to doubling the dose of magnetic energy.

The animals were randomly distributed among one control and five exposed groups. That this distribution provided basically equivalent groups was confirmed by the mean initial body mass indices that were virtually the same: 208.0 $\pm$ 3.6 g in the control group and from 208.3 $\pm$ 2.8 to 211.3 $\pm$ 2.7 g in the exposed groups.

We had no separate quasi-SMF device for a sham exposure of control group, while using to this end one and the same device would mean shifting sham exposures to the later hours of the day than those for the real ones. Thus we did not include sham exposures in to the experimental design, having presumed that a probable stress associated with moving the animals into the chamber of the exposure facility was moderate, and taking into consideration that it was equal for all the actually exposed groups. Thus we may consider its possible influence eliminated when we compare effects of separate agents or analyze the type of their combined action.

Thus we had 6 groups of rats:

1. Control (ip injections of normal saline);
2. Exposed to sodium fluoride ip injections;
3. Exposed to the SMF for 4 h per day;
4. Exposed to the SMF for 2 h per day;
5. Exposed to the SMF for 4 h per day and fluoride ip injections;
6. Exposed to the SMF for 2 h per day and fluoride ip injections.

After the exposure period, the following procedures were performed for all rats:

- weighing;
- estimation of the CNS ability to induce temporal summation of sub-threshold impulses – a variant of the withdrawal reflex and its facilitation by repeated electrical stimulations in an intact, conscious rat (Rylova, 1964);
- recording of the number of head-dips into the holes of a hole-board, which is frequently used for studying the behavioral effects of toxicants and drugs (e.g. Adeyemi et al., 2006; Fernandez et al., 2006);
- collection of daily urine for analysis of its density, urine output as well as lead, fluoride, coproporphyrin, delta-aminolevulinic acid (δ-ALA), and creatinine contents.

Then the rats were killed by decapitation and their blood was collected by exsanguination. The liver, spleen, kidneys, and brain were weighed. The biochemical indices determined from the blood included total serum protein, albumin, globulin, triglycerids, cholesterol, high and low density lipoproteins, bilirubin, ceruloplasmin, reduced glutathione (GSH), malonyldialdehyde (MDA), alkaline phosphatase, alanine- and asparate-transaminases (ALT, AST), catalase, gamma glutamyl transferase, creatinine, thyrotropic