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Original papers

Correlations between weather parameters, atmospheric hydrogen sulphide level and vitamin B12 administration to H_2S exposed workers

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Abstract

Hydrogen sulphide, a highly toxic gas, can be used in crenotherapy to balance all metabolic processes (minerals, fats and proteins). The main aims of this study were to correlate the weather characteristics with the atmospheric H_2S level and to evaluate the antidote activity of B12 Vitamin in the case of prolonged exposure to this compound. 46 volunteers, people from the medical staff of an important Romanian thermal water spring spa, with professional exposure at H_2S, were enrolled in this study; numerical data about their blood pressure, atmospheric H_2S concentration and about the weather conditions were collected every month for one year. The results indicate an improvement in the blood pressure of volunteers treated with Vitamin B12; no significant correlation between the concentration of total urinary sulphur and the concentration of atmospheric H_2S level was found.

Keywords: *antidote, cobalamin, detoxification, thermal spring waters, workers*

Introduction

Hydro sulfuric acid or dihydrogen mono-sulphide, known as hydrogen sulphide or H_2S, is a colourless, flammable and very toxic gas, heavier than the air, with a broken egg smell. H_2S exposure, usual occupational, may cause intoxication in laboratories and industry (sewage, sugar and paper factories), in canals, coal mines, tunnels and other workplaces, where it has been born through the proteins deposition [1]. The human body penetration of this toxic substance is done by the respiratory airway and the transdermal pathway. Its toxic effect is due to the action on the central nervous system. In acute poisoning, the patient suddenly loses consciousness, has an accelerated, irregular breath, followed by spasm, then stops breathing and death occurs in few minutes. Low toxic concentrations produce general weakness, restlessness, dizziness, nausea, vomiting, and acute pulmonary oedema. Conjunctival irritation, rhinopharyngitis, dysphagia, difficulty breathing, breath paralysis and blood pressure collapse occur in the case of prolonged exposures to low toxic concentrations [2]. The first aid in hydrogen sulphide intoxication consists in the immediate removal of the patient from the contaminated area and the achievement of artificial respiration associated with oxygen-therapy. The patient should be transported

rinsing the eyes with abundant warm water, washing with an isotonic NaCl solution and then application of an ointment with hydrocortisone and tetracycline. The patient regains his / her work capacity after several days in cases where the treatment was performed correctly. Healing occurs in hours (in mild cases) up to several days in case of complications of the respiratory and nerve pathways. In the industry, exhaust devices need to be supplemented with additional ventilation systems for massive releases of hydrogen sulphide. Individual protection is achieved by proper use of filter masks. In the case of working in wells, tunnels, pits, it is compulsory to use the safety belt and the signalling rope, under the permanent control of an external observer.

On the other hand, recent studies on H_2S benefits show that blood vessels maintain their integrity with this gas. In fact, they contain cystathionine-gamma-lyase, alternately known as cysteine desulphydrase, which participates in the production of the endogen H_2S. Professor Rui Wang (Lakehead University, Ontario) found that similar to nitric oxide (NO), hydrogen sulphide dilates the blood vessels [3].

Hydrogen sulphide activates a series of proteins that control the potassium ions circuit in smooth muscle cells [4]. This circuit generates an electrical current limiting the calcium ions that reach the cells, resulting in the dilation of the blood vessels and the relaxation of the muscles. Due to these properties, hydrogen sulphide has proven effective in lowering blood pressure. According to specialists, one of the causes of hypertension is the reduction in the level of enzymes that produce hydrogen sulphide in the body, with age [5, 6].

This paper describes the occupational exposure to hydrogen sulphide in the case of the medical staff engaged to the sulphur water baths from Băile Herculane, a very old spa resort from South-West of Romania, where the balneary treatments are similar to other resorts such as Essentuki, Sochi, Pyatigorsk, Sernovodsk-Caucasian, Kemerovo, and many more. These employees chronically exposed to low concentrations of H_2S over time they reported many health problems. Another important aspect of this research was to evaluate the potential of vitamin B12 as an antidote in H_2S intoxications.

Material and method

A. Subjects of study

During the last years, between October 2017 and September 2018, we studied 46 volunteers from the staff of Băile Herculane thermal spa; they were recruited through personal invitation and they knew the aim of this study, but they did not knew the quantification protocol of the study results. All principles of the Helsinki Declaration, of local jurisdiction have been respected, and the study was previously analyzed and approved by the Ethics Committee of "Victor Babeș" University of Medicine and Pharmacy Timișoara (Approval No. 7 / 2016); every volunteer read and signed an informed consent for his / her participation and for the publication of study results.

B. Study inclusion criteria

A group of 46 exposed volunteers (69.57 % women) was included in this study, all of them with a prolonged occupational exposure to sulphur mineral waters (minimum 8 years) - 58.7 % volunteers are still working there, while the others have been retired. Half of this group was treated with vitamin B12 (50 μg monthly) for one year and they were used as the treated group (TG), while the other subjects were used as control group (CG). Only 2 women and 3 men from TG, respectively 3 women and 1 man from CG had hypertension and they took medication to maintain the blood tension under control. Volunteers with renal diseases, diabetes mellitus, hypo- or hyper-thyroidism, heart failure and morbid obesity were not admitted in this research. Volunteers followed no special diet; everyone had a mixed and well-balanced dietary intake.

C. Protocol

We did the measurements of blood pressure randomly twice a day, at the beginning and at the end of the working program and differences between these values are comparatively presented; we performed each measurement three times and collected the average values.

We measured the weather conditions and the atmospheric H_2S concentration in the first Sunday of every month; a professional ROTH weather station installed in an open space in the centre of the city at the ground level evaluated the atmospheric pressure, the air temperature and humidity, together with an EMIMAT OH - 602 sampling pump (Radelkis, Hungary) that was used to draw air samples in order to measure the atmospheric hydrogen sulphide level.

H_2S concentration measurement was based on the spectrophotometric procedure (sensibility of method: 0.05 μg / mL) [7]: a volume of 2 liters of air was

aspirated (0.15 litters/minute) through two absorbers mounted in series, which contain 10 mL absorbent solution (4.3 g $CdSO_4 \cdot 5\ H_2O$ / 20 ml water mixed with 0.3 g NaOH / 20 mL water diluted to 1 liter). The absorbent solution was then mixed with a N,N-Dimethyl-p-phenylenediamine monohydrochloride solution (containing 0.5 g N,N-Dimethyl-p-phenylenediamine in 150 mL HCl mixed with 0.5 g $FeCl_3 \cdot 6$ H2O / 50 mL water) at 5:1 v/v mixing ratio. The extinction was measured in 1 cm plastic cuvette on a Jenway 6100 spectrophotometer at 667 nm (the molar extinction coefficient is 71,000 M–1 cm–1) [7]; we used Beer-Lambert law to determine the concentration of samples based on a calibration curve for standard solutions between 1 and 5 mg / mL H_2S ($R^2 = 0.991$).

Hydrogen sulphide is excreted in the urine as sulphate; it is first oxidized to thiosulfate and then to sulphite and sulphate inside mitochondria. The thiosulfate is not a specific marker of H_2S intoxication because it cannot make the difference between the inhalation of atmospheric hydrogen sulphide, a normal dietary intake with higher sulphur content or the endogenously generated H_2S [8]. We found the gravimetric method of Prof. Otto Folin to evaluate the urinary sulphate to have a reliability that remains unchallenged to the present day and to be entirely satisfactory [9]: we froze the urine from 8 volunteers (all women, half from TG and half from CG group; the same persons for the entire year) immediately after collection in order to assess the hydrogen sulphide level in humans; the samples were allowed to come to room temperature and we estimated the total sulphur in urine using samples of 25 mL urine treated with 15 mL oxidizing solution (40 g $Cu(NO_3)_2$ and 15 g $CuCl_2$ solved in 100 mL water), 20 mL HCl 2N, 400 mL distilled water and 15 mL $BaCl_2$ 5% solution.

D. Statistics

We performed the statistical analyze using a trial version of IBM SPSS software (SPSS Inc., Chicago, Il, USA). Comparisons between continuous variables were done with t test and F test and the correlations between different parameters were analyzed by the Pearson correlation coefficient. The significance level was set to 5% ($p<0.05$).

Table 1. Main characteristics of all volunteers

Characteristic	Mean value ± SD	Minimal value	Maximal value
Age, years	52.34 ± 7.19	41	63
Height, m	1.72 ± 0.28	1.59	1.86
Mass, kg	76.41 ± 6.73	62	91
BMI*, kg/m²	26.74 ± 2.21	23.56	31.71

* BMI = body mass index

Results

Personal information about the characteristics of volunteers (age, height and body mass index) was obtained using questionnaires (Table 1).

The initial blood pressures, at volunteers' inclusion in this study, are presented in Figure 1. The average values are 130.22±7.07 mm Hg for the systolic blood pressure and 80.30±4.82 mm Hg for the diastolic blood pressure.

Table 2 presents the weather characteristics such as temperature, humidity and atmospheric pressure, values determined for an entire year at Băile Herculane.

Atmospheric hydrogen sulphide level per month is presented in Figure 2. There were determined lower levels, under 1.5 mg/m3, in March, May and June, medium atmospheric H_2S levels, between 1.5 and 2.5 mg/m3, in January, February, July, October, November and December and increased H_2S concentrations, over 2.5 mg/m^3, in April, August and September.

The changes between the determined total urinary sulphur level and blood pressure at in volunteers treated with vitamin B12 - the treated group (TG) and the other volunteers - used as the control group (CG) from one month to another are shown in Table 3. Every blood pressure difference is expressed as the average value between the values recorded at the beginning and the end of a working day; almost 95% blood pressure evolutions showed a downward trend during the working program and this is the reason why all blood pressure differences are positives.

Discussions

The weather conditions are very important for the changes in the concentration of atmospheric toxic agents. The acid rain has an unusually low pH level, less than 5.6. It can have harmful effects on plants, marine animals, people or infrastructure. Acid precipitations usually occur in situations where large amounts of sulphur dioxide or nitrogen monoxide are emitted into the atmosphere. They react with the oxygen in the atmosphere to form sulphur trioxide and nitrogen dioxide, respectively.

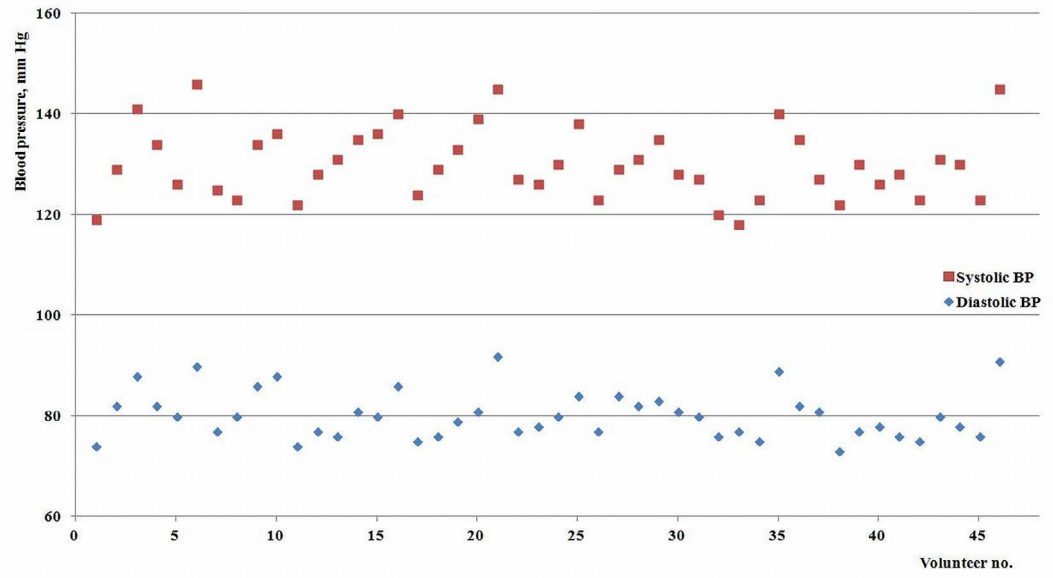

Figure 1.
The initial blood pressure values

Table 2. The weather characteristics between October 2017 and September 2018

Month	Day temperature, °C	Night temperature,°C	Humidity, %	Air pressure, hPa**	Average rainfall [11], mm
Oct. 2017	16	9	58	1024	102
Nov. 2017	9	5	71	1020	80
Dec. 2017	7	1	72	1019	71
Jan. 2018	2	-3	76	1021	61
Feb. 2018	3	-4	77	1015	142
Mar. 2018	9	3	80	1009	202
Apr. 2018	15	9	59	1017	41
May. 2018	20	15	64	1013	150
Jun. 2018	26	18	65	1011	192
Jul. 2018	29	21	53	1009	111
Aug. 2018	26	22	47	1002	36
Sep. 2018	20	17	44	1018	34
Annual averages	15.2	9.4	63.8	1014.8	101.8

** 1 hPa (hectopascal) = 1 mbar = 9.87 · 10-4 atm

Sulphur trioxide and nitrogen dioxide react with the rainwater forming sulfuric and nitric acid. The solution made up of water together with sulfuric and nitric acids is the acid rain [10]. On the other hand, the rainwater washes large amounts of volatile compounds including the atmospheric hydrogen sulphide and the result is more clear air.

H. Daldal et al. reported the danger of H_2S toxicity for individual bathing in thermal springs: a 26-year-old man was found unconscious and a 25-year-old woman was found dead in a hotel with access to a thermal spa [11]. The hydrogen sulphide concentrations in the pool indoor air of 22 thermal springs were monitored by M. Fazlzadeh et al.: they found a minim value equal with 12.66 mg/m^3, while Ghotour Souei hot springs (near Meshgin Shahr, Iran) had the highest concentration of H_2S with an average value of 40.86 mg/m^3 [12].

Table 3. The comparatively determined total urinary sulphur levels and blood pressure differences between October 2017 and September 2018

Month	The treated group (TG)			The control group (CG)		
	$BaSO_4$ from urine, g	Systolic B.P.*** difference	Diastolic B.P.*** difference	$BaSO_4$ from urine, g	Systolic B.P.*** difference	Diastolic B.P.*** difference
Oct. 2017	0.110	10	7	0.115	16	10
Nov. 2017	0.109	9	5	0.114	13	8
Dec. 2017	0.111	9	6	0.113	12	8
Jan. 2018	0.113	9	5	0.112	12	7
Feb. 2018	0.111	9	6	0.110	13	8
Mar. 2018	0.115	10	6	0.113	13	7
Apr. 2018	0.107	8	5	0.111	10	7
May. 2018	0.109	11	6	0.110	15	9
Jun. 2018	0.111	9	5	0.114	11	8
Jul. 2018	0.113	8	5	0.114	10	6
Aug. 2018	0.112	10	6	0.115	13	7
Sep. 2018	0.112	11	7	0.113	14	9
Annual averages	0.111	9.4	5.8	0.113	12.7	7.8

*** B.P. = blood pressure

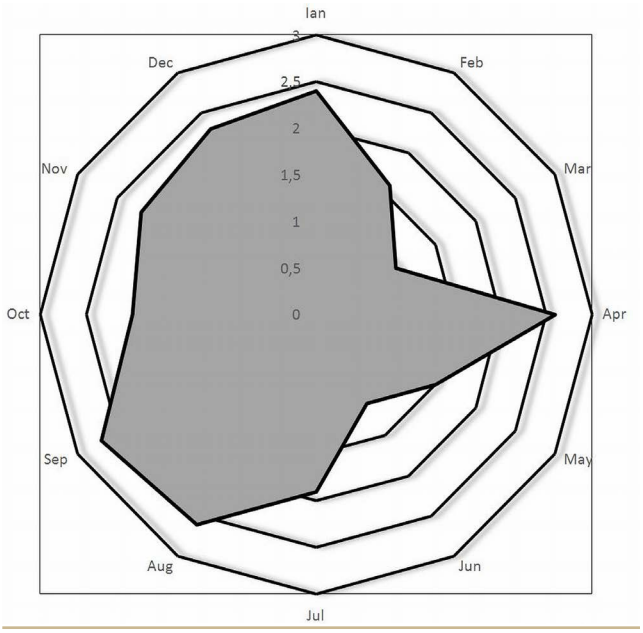

Figure 2. The changes of atmospheric hydrogen sulphide level (mg/m^3)

Hydroxocobalamin, known as the injectable form of vitamin B12, has been used in the treatment of cyanide poisoning for 35 years, with gradually expanding acceptance of its effectiveness and safety. The toxic has a greater affinity to bind with B12, rather than cytochrome-oxidase a3, forming cyanocobalamin, a compound that is renal excreted. Different retrospective and prospective studies have shown B12 effectiveness [12]. Similarly to cyanide poisoning, the cytochrome c prevents the H_2S cytotoxicity by its reduction: the methemoglobin prevented hydrogen sulphide cytotoxicity by trapping it and transforming it in sulphmethaemoglobin, which catalysed the H_2S autoxidation [13].

The results of this research indicate an important and inverse correlation between the average rainfall amount (Table 2) and atmospheric hydrogen sulphide level (Figure 2): when the rainfall amount is under 50 mm, the determined H_2S level is over 2.5 mg/m^3 (April, August, September), while when the rainfall amount is ≥150 mm, the determined H_2S level is under 1.5 mg/m^3 (March, May and June). Pearson correlation coefficients were calculated for all parameters using the data of Table 3 and the atmospheric hydrogen sulphide level (Figure 2). Our correlations were done based on the rules of T. Colton [14].

No evidence of a significant correlation between the atmospheric H_2S concentration and the total urinary sulphur level or blood pressure differences was obtained in this study. Probably the effects of this toxic on humans can be found in the measured parameters only after a certain period and this idea seems to be a fair assessment if we take into account that the highest H_2S levels (more than 2.5 mg/m^3) in August and September lead to the highest levels of the total urinary sulphur and blood pressure differences only in October. This theory is not respected when we

talk about the lowest atmospheric H_2S concentration (≤1.5 mg/m^3) in May and June; the total urinary sulphur level for the volunteers in the control group remains at an increased level (0.114 mg/mL) in June and July. On the other hand, the good correlations between the blood pressure of treated group (TG) and of the control group (CG) represent an indication that the volunteers have respected the treatment and that vitamin B12 is an active antidote in H_2S intoxications, as it is mentioned in the literature [12, 15].

Our team discussed in another scientific article the effects of hydrogen sulphide over the blood pressure [16]. We found important blood pressure differences between before and after the working program in volunteers exposed to H_2S and respectively between the volunteers of an exposed group and those of an unexposed group.

Conclusion

The role of endogen H_2S in cardiovascular physiology was often studied by animal exposure to different doses between 1 and 200 mg/m^3. Severe intoxications with hydrogen sulphide via the inhalation route were reported worldwide. More dangerous for the health of human beings is the prolonged exposure to this toxic because severe neurological and respiratory effects like incoordination, poor memory, hallucinations, personality changes, anosmia, sore throat, cough, and dyspnoea, were already reported. This study presents different correlations between some weather parameters and the level of atmospheric hydrogen sulphide, respectively the testing of vitamin B12 as an antidote for H_2S poisoning. The results indicate an important and inverse correlation between the atmospheric H_2S level and the average rainfall amount on the one hand and a non-significant impact of atmospheric hydrogen sulphide on the volunteers' blood pressure and their total urinary sulphur level on the other hand.

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