

Original article

National pholcodine consumption and prevalence of IgE-sensitization: a multicentre study

Background: The aim of this study was to test, on a multinational level, the pholcodine (PHO) hypothesis, i.e. that the consumption of PHO-containing cough mixtures could cause higher prevalence of IgE antibodies to PHO, morphine (MOR) and suxamethonium (SUX). As a consequence the risk of anaphylaxis to neuromuscular blocking agents (NMBA) will be increased.

Methods: National PHO consumptions were derived from the United Nations International Narcotics Control Board (INCB) database. IgE and IgE antibodies to PHO, MOR, SUX and P-aminophenyl-phosphoryl choline (PAPPC) were measured in sera from atopic individuals, defined by a positive Phadiatop® test (>0.35 kU_A/l), collected in nine countries representing high and low PHO-consuming nations.

Results: There was a significant positive association between PHO consumption and prevalences of IgE-sensitization to PHO and MOR, but not to SUX and PAPPC, as calculated both by exposure group comparisons and linear regression analysis. The Netherlands and the USA, did not have PHO-containing drugs on the markets, although the former had a considerable PHO consumption. Both countries had high figures of IgE-sensitization.

Conclusion: This international prevalence study lends additional support to the PHO hypothesis and, consequently, that continued use of drugs containing this substance should be seriously questioned. The results also indicate that other, yet unknown, substances may lead to IgE-sensitization towards NMBAs.

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Key words: anaphylaxis; cough mixtures; IgE-sensitization; international prevalence study; pholcodine.

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Accepted for publication 5 August 2009

Anaphylactic reactions during general anaesthesia are rare but dramatic events that have been reported with increasing frequency during the last decades. Neuromuscular blocking agents (NMBAs) represent the most

frequent causes, with suxamethonium (SUX) as the prominent culprit drug.

In most cases immunoglobulin E (IgE)-mediated mechanisms are involved. Since up to half of the patients

have not been exposed to NMBA previously, other drugs or environmental chemicals sharing the quaternary ammonium ion (QAI) epitope, regarded as the major NMBA allergenic epitope, are suspected of initiating the IgE-sensitization (1) which is the prerequisite for the anaphylaxis.

The reported frequency of reactions varies considerably between countries, starting at about one in 200 000 and reaching one in 5 000 anaesthetics in high prevalence countries (2). NMBA-related anaphylaxis seems to be about 10 times more common in Norway than in Sweden, a discrepancy that, at least partly, could be explained by different exposure and sensitization to PHO in the two populations (3).

The effect of possible IgE sensitizers, using morphine (MOR) as recommended for screening for IgE antibodies to QAI (4), was studied by testing 84 different chemicals collected from Norwegian and Swedish homes, of which several inhibited IgE binding to SUX and/or MOR. However, no difference in exposure to the above chemicals was found, except for the use of cough mixtures containing pholcodine (PHO) (3). These have until recently been purchased over the counter (OTC) in Norway but are not available in Sweden.

Further, the prevalence of IgE-sensitization to MOR, SUX and PHO in Norway and Sweden was investigated. In Bergen, Norway 0.4% of blood donors, 3.7% of atopic patients and 38.5% of patients with anaphylaxis to NMBA were sensitized to SUX, as were 5.0%, 10.0% and 66.7%, respectively, to MOR. IgE antibodies to SUX or MOR were undetectable in blood donors and atopics from Stockholm. Similar figures for IgE antibodies to PHO were found in Norwegian samples but, again, in none from Sweden (3).

Additionally, in a pilot study, two Norwegian individuals IgE-sensitized to PHO, MOR and SUX responded, after taking one single daily dose of PHO for one week, with a dramatic increase in total serum IgE and IgE antibodies to SUX, MOR and PHO reaching 60 and 105 times the levels prior to exposure (5). A similar increase has, so far, only been seen after bone marrow transplantation (6). In a recent follow up exposure study, 11 patients previously diagnosed with IgE-mediated anaphylaxis to NMBA and IgE-sensitized to PHO, were given a 20 mg daily dose of PHO for one week. IgE and IgE antibodies to PHO, MOR and SUX were raised highly significantly by 3 weeks after cessation of exposure (7).

To explain the observed differences between Norway and Sweden the pholcodine hypothesis was proposed (8), according to which the consumption of PHO-containing cough mixtures could be responsible for both the higher prevalences of IgE antibodies to PHO, MOR and SUX and also for the higher frequencies of anaphylaxis to NMBA. The manufacturer of the cough medicine subsequently withdrew the drug from the Norwegian market in March 2007, which already after 2 years

have led to a dramatic decrease in IgE-sensitization and successively tending to reduce the frequency of anaphylaxis to NMBA (unpublished observation). However, the question whether PHO exposure and IgE-sensitization could be of relevance for the different prevalences of peri-anaesthetic anaphylaxis reported from other countries than Norway and Sweden remains to be answered (8).

The aim of this international multi centre study was to take a further step in explaining anaphylaxis to NMBA by investigating the prevalences of serum IgE antibodies to PHO, MOR and SUX. Atopic individuals, attending allergy centres and/or allergy laboratories in nine high or low PHO-consuming nations, were chosen because they are likely to be high IgE responders. In addition, IgE antibodies to P-aminophenylphosphoryl-choline, (PAP-PC), proposed to detect IgE-sensitization to QAI, were also measured.

Materials and methods

Samples

Serum was collected at the collaborating centres in Stockholm (Sweden), Bergen (Norway), Copenhagen (Denmark), Helsinki (Finland), Nancy (France), Manchester (UK), Rotterdam (The Netherlands), Freiburg (Germany) and Lenexa, KS (USA). The sera, aiming at 200 samples per centre, were collected at each site either as superfluous volumes from routine allergy laboratories, or were drawn from patients referred for allergological examinations at allergy centres. The anonymized sera were collected during the years of 2005–2007 and stored at the respective centres at -20°C until analysed. Inclusion criteria were available serum volumes of at least 1.0 ml and, for reason of homogeneity, a positive Phadiatop[®] test (Phadia AB, Uppsala, Sweden) as the definition of atopy. The study was approved by the local Ethical Committees for medical research at each participating centre.

Immunoglobulin analyses

The serum concentrations of IgE (kU/l) were determined by ImmunoCAP[®] Total IgE (Phadia AB). Each serum sample was tested for IgE antibodies to a mix of common inhalant allergens, Phadiatop[®] (Phadia AB), and for IgE antibodies (kU_A/l) to pholcodine (c261), morphine (c260), suxamethonium (c202) and PAPPC (Rc777) by ImmunoCAP[®] Specific IgE (Phadia AB) according to the manufacturer's instructions.

Pholcodine consumption and exposure

Data on the national PHO consumptions summarized for the years 2001–2005 were taken from the United Nations International Narcotics Control Board (INCB) database (<http://www.incb.org>). Arbitrary levels were chosen for high (> 100 kg per million inhabitants) and low (< 40 kg) PHO consumption. In addition, the number of PHO containing drugs available in 2005 in the individual national markets was obtained (source: Drugdex). No information is available on the number of individuals actually exposed to PHO, neither in the general populations, nor among the individuals who donated serum samples.

Statistical evaluations

Based on the results from a previous publication (2), the present study design and the sampling criteria defined above, the sample size of approximately 200 sera collected at each of nine centres representing high and low consumption countries, respectively, would yield a statistical power sufficient to test the null hypothesis, i.e. no difference in PHO sensitization between high and low PHO consumption countries. nQuery Advisor® 5.0; Statistical Solutions Ltd, Cork, Ireland was used for the power calculations.

Comparison of number of sera containing IgE antibodies to PHO in the high and low consumption countries included both a complete dataset analysis (all countries) and a subset analysis (NL and USA excluded). Direct group comparisons were done by Pearson chi-square exact method. Further analysis of the association between PHO consumption in kg per million inhabitants and percent sensitized sera was performed with linear regression analysis.

Results

In the participating countries Sweden (SE), Denmark (DK), United states of America (USA), Germany (GE), The Netherlands (NL), Finland (FI), Norway (NO), United Kingdom (UK) and France (FR) the 5 year accumulated consumption of PHO during the period from 2001 to 2007 varied from 0 to 184.9 kg per million of inhabitants (Table 1). In the countries with high consumption like the UK and FR, as many as 14 different PHO-containing drugs were available, many of them in different commercial presentations, e.g. special preparations for children. The third highest PHO-consuming country, NO, had only one cough mixture, but it had been on the market since 1966 in different formulations, and an estimated 40% of the population has been exposed.

The percentages of atopic individuals IgE-sensitized to PHO, MOR and SUX varied from below 1% in low PHO-consuming countries (DK, FI, GE, SE) to between 2.4% and 7.0% for high consumption countries (FR,

Table 2. Number of sera collected from the participating countries and the respective percentages of sera with IgE antibody levels of 0.35 kU_A/l or higher to PHO, MOR, SUX and PAPPC

Country	City	No of sera	PHO %	SUX %	MOR %	PAPPC %
Sweden	Stockholm	213	0	0	0.5	0.9
Denmark	Copenhagen	179	0.6	0	1.1	0.6
USA	Lenexa	200	2.0	2.5	5.0	2.0
Germany	Freiburg	211	0	0.5	0.9	2.4
The Netherlands	Rotterdam	184	4.9	0	6.0	1.6
Finland	Helsinki	209	1.0	0	1.0	1.4
Norway	Bergen	199	7.0	1.0	5.5	0.5
UK	Manchester	209	2.4	0	2.4	0
France	Nancy	214	6.5	3.7	7.5	1.9

NO, UK) (Table 2). It is highly noteworthy that the samples from USA and NL did have a surprisingly high prevalence of IgE antibodies to PHO, 2.0% and 4.9%, respectively, but differed considerably for SUX, 2.5% and 0%, respectively. However, no PHO-containing drug is available in these countries according to the respective National Medicines Agencies. Examining the associations between PHO consumption and IgE-sensitization, two sets of statistical analyses were therefore performed, one including all countries and another all except NL and USA, respectively.

The countries were categorized according to PHO consumption (Table 1, Fig. 1) in high consumption countries (FR, NO, UK) and low consumption countries (DK, FI, GE, NL, SE, USA). Comparing high consuming countries with all low consuming countries showed a significant difference in the number of sera with IgE antibodies to PHO ($P < 0.001$) and MOR ($P < 0.001$), but neither to SUX ($P = 0.13$) nor to PAPPC ($P = 0.52$). Comparing high and low consuming countries after NL and USA were excluded, showed a significant difference in number of sera IgE positive to PHO ($P < 0.001$), MOR ($P < 0.001$) and SUX ($P = 0.01$), but still no difference was seen for PAPPC ($P = 0.63$).

Linear regression analysis including all countries demonstrated a statistically significant association between PHO consumption and IgE-sensitization to PHO ($P = 0.027$), but not to MOR ($P = 0.10$), SUX ($P = 0.11$) and PAPPC ($P = 0.59$) as shown in Fig. 2A. Excluding NL and USA, a statistically significant association between PHO consumption and IgE-sensitization to PHO ($P = 0.010$), MOR ($P = 0.004$) and SUX ($P = 0.032$), but not to PAPPC ($P = 0.89$) was demonstrated (Fig. 2B).

Discussion

The aim of this multi-centre, international study was to test the PHO hypothesis (8) originally suggested as an explanation for the reported different frequencies

Table 1. Accumulated PHO consumption in the nine participating countries and number of PHO-containing drugs on the individual national markets

Country	PHO consumption (kg) during 2001–2005	PHO consumption (kg) per mill. inhab. during 2001–2005	No. of PHO-containing drugs on the market
Sweden	0	0	0
Denmark	0	0	0
USA	0	0	0
Germany	45	0.5	0
The Netherlands	163	10.1	0
Finland	195	37.5	1
Norway	470	104.4	1*
UK	6478	108.0	14
France	11095	184.9	14

*Sera from Norway were collected before the drug was taken off the market in March 2007.

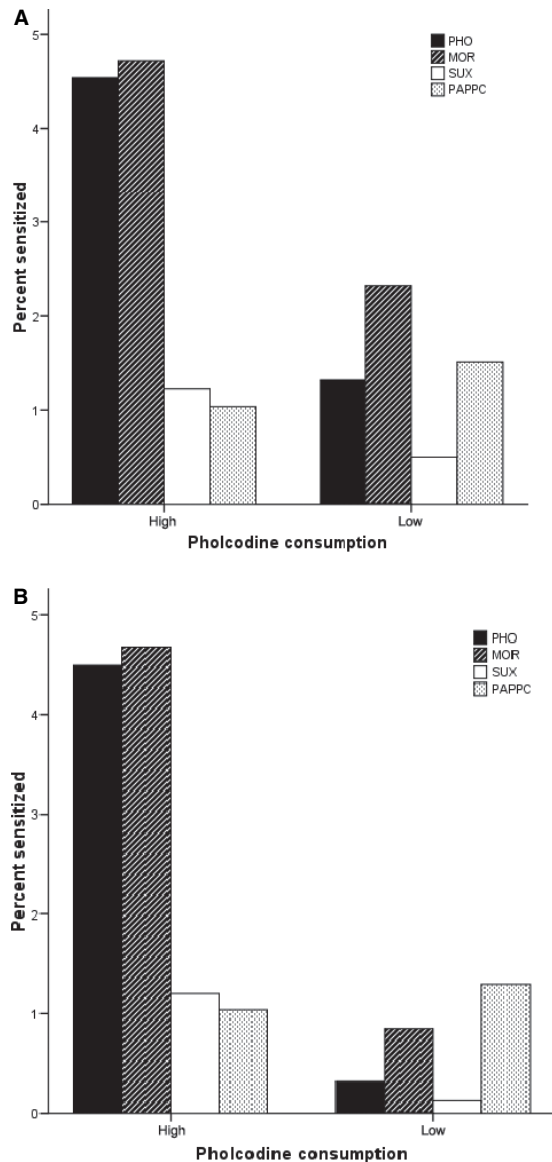


Figure 1. Total number of sera IgE-sensitized to PHO, SUX, MOR and PAPPC, (A) in all nine countries divided into high (> 100 kg PHO/million inhabitants, i.e. NO, UK and FR) and low (< 40 kg PHO/million inhabitants, i.e. SE, DK, USA, GE, NL and FI) consumption groups respectively, and (B) after excluding NL and USA where the low consumption group is represented by DK, FI, GE and SE.

of anaphylactic reactions to NMBAs in Sweden and Norway (9). In order to reduce number of serum samples needed atopics were selected since in Norway they have twice as high prevalence of IgE antibodies to PHO as healthy individuals (3). The results, at least partially, support the hypothesis by showing a statistically significant association between PHO consumption and prevalence of IgE-sensitization to PHO and MOR, but not to SUX when all nine countries were included. However, when NL and USA were excluded from the

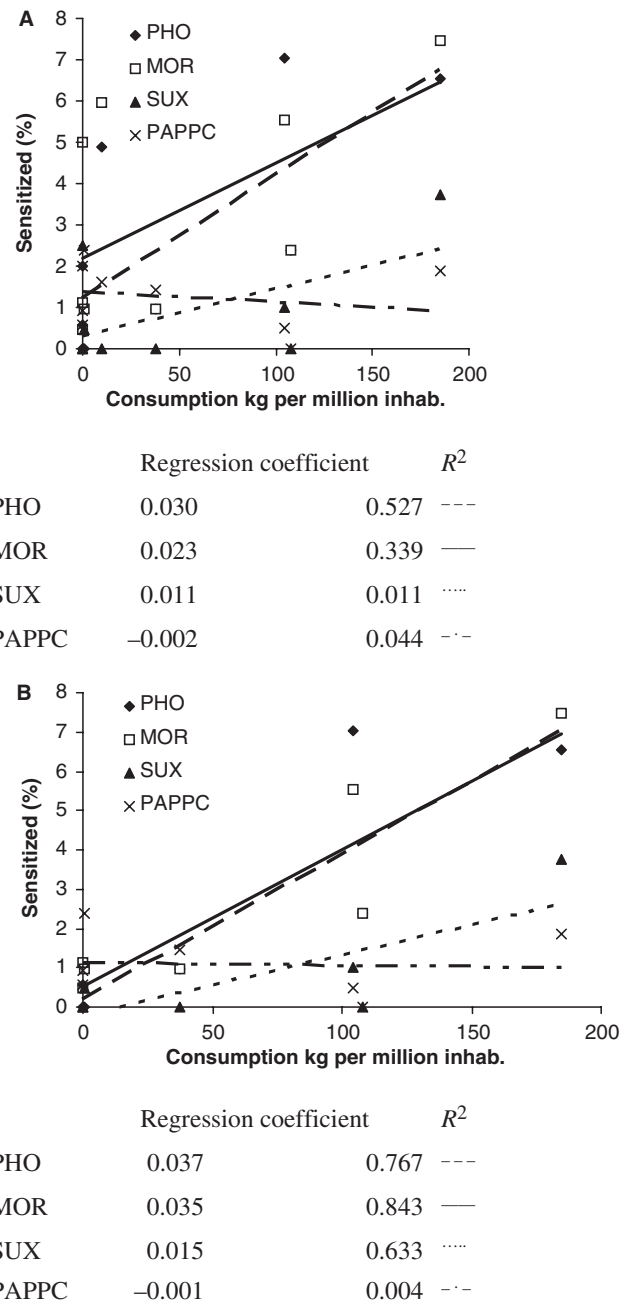


Figure 2. Linear regression analysis of the associations between PHO consumption and IgE-sensitization to PHO, MOR, SUX and PAPPC in all countries (A), and after excluding NL and USA (B).

analysis, the associations between consumption and sensitization became stronger and even the association to SUX reached statistical significance.

The information from NL is confusing since according to the United Nations International Narcotics Control Board (INCB) (Table 1) there is a considerable consumption of PHO but according to Dragex there are no PHO-containing drugs on the market. In the

USA there are, to our knowledge, no PHO consumption and PHO containing drugs on the market, although IgE-sensitization to PHO is prevalent. This does not fit with the PHO hypothesis and one explanation could be that other, yet unknown, environmental factors besides PHO may lead to the production of IgE antibodies to PHO and SUX. The presence of other, so far unknown, sensitizers is further supported by the discrepancy between NL and USA regarding IgE antibodies to SUX; none detected in NL while USA had the next highest prevalence figure, 2.5%, and deserves further studies.

IgE-sensitization to PAPPC was unrelated to PHO consumption both when all nine countries were included and when NL and USA were excluded. In fact in both cases the relationship showed a slight inverse trend (Figs 1 and 2). Thus PAPPC does not seem to be a preparation useful for detection of IgE-sensitization to QAI, although it does contain the QAI determinant.

The relation between PHO consumption and exposure must be viewed with caution. The data from the INCB (Table 1) actually represent amounts of PHO officially purchased by the individual nations as registered during the stated period. Thus they do not necessarily reflect exposure of the general population. Furthermore, it is not known how many of the anonymous allergic serum donors had actually been exposed to PHO. Another study design, for example, directly comparing PHO exposed *vs* nonexposed individuals would allow to assay the association between exposure and IgE-sensitization more directly than in this study.

Likewise, it can be questioned whether the number of PHO containing drugs on the respective markets necessarily give a reliable indication of PHO exposure. In Norway, for example, until recently there was only one drug, a cough mixture, containing PHO, to which the producer from the sales data carefully estimated that about 40% of the Norwegian population had been exposed. This drug was available in several formulations of which one was sold OTC for adults and children down to the age of 5 years. Similar exposure estimations for the other participating nations are not available. PHO is monovalent for two noncross reacting allergenic determinants (3) which might explain why allergic reactions to PHO seem to be very rare. However, one of the allergens is the QAI epitope and IgE-sensitization to PHO thus could predispose for an IgE-mediated anaphylaxis to NMBA.

Due to the differences in prevalences of IgE sensitization to PHO in Norway and Sweden, which related to incidences of NMBA anaphylaxis, and to the unique effects on IgE synthesis (3, 5, 7), it was taken off the market in Norway by the producer in March 2007. Studies in progress indicate that 2 years later the prevalence of IgE sensitization has dropped considerably and

the frequency of NMBA anaphylaxis shows a descending tendency.

The above responses to withdrawing PHO-containing cough suppressants in Norway will clearly be of interest from a preventive point of view, not least when taken together with recent historic exposure response data reported from Sweden. During the 1970s and 1980s a PHO containing cough syrup (Tussokon; Pharmacia AB, Uppsala, Sweden) was available in Sweden. Analyses of allergics' sera sampled from 1970 to 2000 revealed that as many as 5–6% had IgE antibodies to PHO during the years Tussokon was available (10). However, only 2% of the sera from the 1990s were positive. Among 300 sera from 2002 (3) and the 213 sera in the present study no PHO positive sample was found. Variations in IgE-sensitization to PHO during these years followed the number of reported cases of anaphylaxis to NMBA.

It was attempted to obtain cumulative data on the reported cases of anaphylactic reactions to NMBAs in the participating countries during the study period. Even through considerable efforts from the Norwegian Medicines Agency it was not possible to get complete, homogenous and reliable data from all participating countries. Thus, unfortunately, we were not allowed the option of investigating the associations between PHO consumption, IgE-sensitization and the frequencies of NMBA related anaphylaxis. However, in a few countries routines for reporting anaphylaxis during anaesthesia are established and in NO, FR and UK a high prevalence of IgE-sensitization to PHO was combined with a high prevalence of anaphylaxis and in DK the opposite was found (unpublished observation).

In conclusion, this multi-centre, international prevalence study in part supports the pholcodine hypothesis, but also supplies evidence for the probability that other, yet unknown, environmental substances may lead to IgE-sensitization to PHO and SUX. Further studies should be initiated on national levels to collect corroborating information allowing the respective drug agencies to consider withdrawal of PHO containing drugs. Good indications are now coming up that such interventions will result in a dramatic decrease of IgE-sensitization and descending frequencies of NMBA-induced anaphylaxis.

Acknowledgments

The authors thank Torkel Harboe, Bergen, Norway and Mogens Krøigaard, Copenhagen, Denmark for discussions on the protocol. The following persons are thanked for helping us collect serum samples: Sune Sved, Phadia Europe, Michael Land, Phadia US Inc., Gerhard Burow and Corinna Steigert, Phadia, Germany, AW van Toorenenbergen, Erasmus Medical Center, Rotterdam, The Netherlands, Dr Richard Pumphrey, Manchester, UK. Ingegerd Ågren-Andersson is acknowledged for performing the ImmunoCAP® analyses.

References

1. Baldo BA, Fisher MM. Substituted ammonium ions as allergenic determinants in drug allergy. *Nature* 1983;**306**: 262–264.
2. Mertes P-M, Aimone-Gastin I, Guéant-Rodriguez RM, Mouton-Faivre C, Audibert G, O'Brien J et al. Hypersensitivity reactions to neuromuscular blocking agents. *Curr Pharm Des* 2008;**14**:2809–2825.
3. Florvaag E, Johansson SGO, Öman H, Venemalm L, Degerbeck F, Dybendal T et al. Prevalence of IgE antibodies to morphine. Relation to the high and low incidences of NMBA anaphylaxis in Norway and Sweden, respectively. *Acta Anaesthesiol Scand* 2005;**49**:437–444.
4. Fisher MM, Baldo BA. Immunoassays in the diagnosis of anaphylaxis to neuromuscular blocking drugs: the value of morphine for detection of IgE antibodies in allergic subjects. *Anaesthesiology Intens Care* 2000;**28**: 167–170.
5. Florvaag E, Johansson SGO, Öman H, Harboe T, Nopp A. Pholcodine stimulates a dramatic increase of IgE in IgE sensitized individuals. A pilot study. *Allergy* 2006;**61**:49–55.
6. Ringdén O, Persson U, Johansson SGO, Wilczek H, Gharton G, Groth CG et al. Markedly elevated serum IgE levels following allogeneic and syngeneic bone marrow transplantation. *Blood* 1983;**61**:1190–1195.
7. Harboe T, Johansson SGO, Florvaag E, Oman H. Pholcodine exposure raises serum IgE in patients with previous anaphylaxis to neuromuscular blocking agents. *Allergy* 2007;**62**: 1445–1450.
8. Florvaag E. Anaphylactic reactions during general anaesthesia. *New Horiz* 2005;**2**:1–6.
9. Florvaag E, Johansson SGO. The Pholcodine Story. *North Am: Immunol Allergy Clin* 2009;**29**:419–427.
10. Johansson SGO, Öman H, Nopp A, Florvaag E. Pholcodine caused anaphylaxis in Sweden 30-years ago. *Allergy*, 2009;**64**:820–821.