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A serogroup A meningococcal polysaccharide vaccine

Studies in the Sudan to combat cerebrospinal meningitis caused by *Neisseria meningitidis* group A*

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Vaccination against cerebrospinal meningitis (CSM) has regained interest with the use of capsular polysaccharides (or polyosides) of the meningococcus as specific immunizing agents. These compounds proved to be effective in the USA against meningitis caused by Neisseria meningitidis *serotype C. This study considers whether the polysaccharides of the serotype A meningococcus, which is prevalent in the African CSM belt, could be protective in epidemic conditions. Taking advantage of the usual seasonal peak of CSM cases, controlled field trials were undertaken in the Sudan early in 1973. 21 640 persons were vaccinated, half of them with a meningococcal polyoside A vaccine and the other half with tetanus toxoid as a placebo. In the former group there were no cases of meningitis, whereas in the latter 10 cases were reported, of which 7 were confirmed by laboratory tests. These studies indicate that the meningococcal polyoside A vaccine is efficient in epidemic conditions and could be used to control outbreaks of meningococcal meningitis.*

A variety of meningococcal vaccines have been prepared and used in the past in attempts to control outbreaks of cerebrospinal meningitis (CSM), the first trial of such a vaccine in Africa having been carried out in the Sudan as long ago as 1915 (1). The results of such trials were generally poor or inconclusive, although in certain instances protection appeared to follow inoculation (2). More recently, specific immunization against meningococcal infections was recommended as a first priority in a research programme submitted to the World Health Organization (Lapeyssonnie, unpublished report, 1960).

In 1964, therefore, a whole-cell vaccine was prepared by the Institut Mérieux, Lyon, France, and in 1965 an enzyme-lysed vaccine was prepared by the Laboratory of Hygiene in the Department of National Health and Welfare of Canada (3). Both were monospecific to group A, but a trial in Upper Volta in 1966–68 failed to assess their value (4).

With improved understanding of the immunological properties of meningococcal antigens and with better techniques for their isolation and purification (5), the polyosides (i.e., the polysaccharides) of the outer layer of *Neisseria meningitidis* were considered, by analogy with the pneumococcal polyosides, as potentially suitable substances for immunization. Meningococcal polyoside C vaccine, first tried in the US Army, conferred protection (6), and it is possible that the present decline of meningococcal meningitis in the military population in the USA is related to the application of this vaccine to the recruits (7).

Fairly large amounts of meningococcal polyoside A were subsequently prepared and stored by the

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Institut Mérieux in cooperation with Rockefeller University, New York, in order to assess its value as a vaccine in the CSM belt in Africa. A trial in Nigeria in 1971 did not constitute a valid test of the efficacy of this type of vaccine because the difference between the immunized and control groups was statistically not significant (8).

Another trial was carried out in Egypt in the winter endemic CSM season of 1971–72 with a new batch of polyoside A vaccine. It involved a very large number of schoolchildren and the results, only recently published, confirm the safety of the vaccine and establish its value as a prophylactic agent (10).

In the Sudan, CSM has acquired a state of hyperendemicity with seasonal increases of incidence mainly from January to June, as is the case in other parts of the African meningitis belt (1). The present trial was therefore begun early in 1973 in order to evaluate the efficacy of the meningococcal polyoside A vaccine (PAV) under field conditions similar to those for its future use in the prevention of epidemic CSM.

MATERIALS AND METHODS

This study was performed as a controlled field trial utilizing the double-blind technique for vaccination. The protocol was prepared jointly by the Ministry of Health of the Sudan and WHO. The decisions on the conditions of the trial and on its execution, as well as on the ethical principles involved, were taken by the Sudanese Ministry of Health.

Population and locality

Two areas were chosen in accordance with the available epidemiologic information.

The first was near El Obeid in the Kordofan province, where 17 000 persons were vaccinated. On follow-up, however, no case of CSM occurred in the vaccinated group or in the population at large. This result was probably due to mass sulfonamide prophylaxis instituted just before the trial and to the fact that in many villages up to half the population received the meningococcal polyoside A vaccine. Although this result cannot contribute to the present study, this group might be used to determine the duration of immunity after vaccination.

The second comprised the crowded residential areas in Khartoum South and in the Omdurman extension of Mahdia with a total population of approximately 161 000. The areas of Khartoum South in the study included (*a*) the western and central parts of extension III, central and western Sahafa, Oshara, and Fellata with a population of approximately 60 000; (*b*) Mygoma, Saggana, and the adjoining parts of eastern Deims with a population of approximately 42 000; and (*c*) El Goz, Hilla Gadida, and Rumeila with a population of approximately 19 000. The Omdurman extension of Mahdia had a population of approximately 40 000.

The vaccine

The meningococcal polyoside A vaccine (PAV) was produced by the Institut Mérieux, Lyon, France, according to the method described and modified by Gotschlich et al. (5). The freeze-dried vaccine was supplied in vials with a buffer diluent for reconstitution to a volume of 25 ml. The dose of the vaccine was 50 μg contained in 0.5 ml of the reconstituted material.

Tetanus toxoid (TTC) was the control vaccine or placebo and was dispensed in a similar manner to give 50 doses, each of 0.5 ml, on reconstitution with the same diluent.

The freeze-dried vaccines were transported from Lyon by air in the cold and they were stored soon after arrival in Khartoum in a refrigerator for 4–7 days. Subsequently, some vaccine was transported to and returned from Kordofan province in ice. These periods of storage above freezing did not apparently affect the vaccine significantly. At the end of the trial, some vaccine samples were sent to Dr Gotschlich's laboratory in New York for determination of the molecular weight of the PAV. It was found to be about 75 000, both for the batch stored continuously at −20°C and for the batch transported and stored under field conditions.

Both PAV and TTC were put in the same type of vial labelled with yellow and green labels respectively with no other mark for identification.

Vaccination procedures

The vaccines were used immediately after reconstitution, which was effected only when a sufficient number of people were available. Meanwhile the dried vaccines were kept constantly in the cold even under field conditions. Vaccination was performed subcutaneously by means of Ped-O-Jet injectors.

Of the 161 000 total population, 21 640 individuals (13.4%) were vaccinated. Interference with natural transmission of the disease would be unlikely with this proportion (6). PAV (yellow label) was given to 10 891 (6.8%) and TTC (green label) to 10 749 (6.7%) individuals.

Table 1. Age and sex distribution of individuals vaccinated in Khartoum South and Omdurman

Age groups (years)	Meningococcal polyoside A vaccine		Tetanus toxoid control vaccine		Both vaccines		Totals
	male	female	male	female	male	female	
1–5	1 129	1 156	1 180	1 166	2 309	2 322	4 631
6–10	1 670	1 627	1 634	1 637	3 304	3 264	6 568
11–15	1 052	956	992	955	2 044	1 911	3 955
16–20	530	475	516	405	1 046	880	1 926
> 21	1 120	1 176	1 065	1 199	2 185	2 375	4 560
Totals	5 501	5 390	5 387	5 362	10 888	10 752	21 640

There were 57.1% males and 42.9% females in the total population. The numbers in each of the two vaccinated groups and the sex ratio were more even, however, as depicted in Table 1. Children up to the age of 15 years, in the age group of 6–10 years especially, formed the majority of those vaccinated. Older children and adults were fewer, all the adults being included in one age group, 21 years and over. Any differences in the sex ratios within age groups are negligible (Table 1).

People often came to the vaccination centres in family groups and vaccination with PAV and TTC was performed alternately, so that in no case did all the members of a family receive the same vaccine. Individual cards with serial numbers and the appropriate yellow or green colour code for the vaccine were filled in to give information on the locality, the person's name, age, and sex, and the date of vaccination. All cards were collected and kept for follow-up.

Follow-up of CSM cases

A constant vigil to pick up CSM cases was maintained in the casualty departments of all hospitals in Khartoum, Khartoum North, and Omdurman, as well as in the isolation units annexed to these hospitals. This continued from 10 April 1973 to the beginning of August 1973 when there were no more cases. All suspected CSM patients were examined clinically and bacteriologically. Individual record sheets were prepared for each patient with details from the card described above in addition to notes on the clinical symptoms, dates of onset and admission, results of bacteriologic and serologic examinations of CSF, and the case outcome.

Bacteriologic and serologic diagnosis

(*a*) Bacteriologic examination of CSF from all cases reported in Khartoum and Khartoum North was carried out in the Cross-Infection Laboratory in the Department of Medical Microbiology, Faculty of Medicine, Khartoum. CSF from cases in Omdurman Hospital was examined in that hospital's laboratory and, in addition, a second specimen from each patient was sent to the Cross-Infection Laboratory in Khartoum.

The bacteriologic methods adopted consisted in the usual microscopic examination of a Gram-stained film of the CSF specimen, and plating on Müller-Hinton agar. Thayer–Martin antibiotic inhibitor added to Müller–Hinton agar was also used in cases when contamination was suspected. Colonies were picked, tested for oxidase, and finally identified by fermentation reactions on glucose, sucrose, and maltose. The group identity was verified by slide agglutination tests. Sulfonamide and antibiotic sensitivity tests were also performed.

(*b*) Counter-immunoelectrophoresis (9) was carried out by the NAMRU-3 laboratories in Cairo for the detection of group A meningococcal antigen in the CSF of some cases.

RESULTS

During the follow-up period, 871 cases of clinical CSM were reported in the population under study giving an incidence of 5.4 per 1 000 in 16 weeks.

CSM did not occur in the PAV vaccinated group but 10 cases were identified in the TTC vaccinated group. The clinical picture in these 10 patients was that of cerebrospinal meningitis. In 9 cases the CSF was turbid, one patient having refused the lumbar

puncture. 7 cases were truly identified as meningitis caused by *N. meningitidis* group A, 5 of them by isolation of that strain and 2 by counter-immunoelectrophoresis (Table 2). Of the 2 remaining cases, the CSF of one was accidentally discarded and that of the other yielded negative results both by culture and immunoelectrophoresis.

Vaccination with PAV produced no side-effects apart from minor irritation at the injection site. However, a wheal-and-flare reaction was noted occasionally among those who received the TTC vaccine. No immediate adverse effects on pregnant women were noted. Poliomyelitis was reported in a 1-year-old boy who received the placebo.

Table 2. Number of serogroup A meningococcal meningitis cases in the vaccine and vaccine control groups

Area	Vaccine employed			
	PAV		TTC	
	No. vaccinated	CSM cases	No. vaccinated	CSM cases
Khartoum South	7 966	0	7 950	6
Mahdia	2 925	0	2 799	1
Total	10 891	0	10 749	7

DISCUSSION

The results of this study in the Sudan clearly demonstrate the protective effect of the meningococcal polyoside A vaccine, the χ^2 test indicating a significant difference in the incidence of CSM between the PAV and TTC groups ($P < 0.05$).

The field application of PAV may be limited by the instability of the polysaccharide in warm tropical temperatures. By keeping the vaccines in ice during transportation and avoiding delays, and by providing refrigeration or deep-freeze facilities at the central level, however, this difficulty may be overcome.

The safety of this vaccine is further confirmed by the absence of any serious side-effects soon after vaccination, or even of any late-developing adverse effects. For example, none of the vaccinated patients reporting to the Khartoum hospitals for reasons unconnected with this trial made any complaint that could be associated with the administration of the vaccine. The fact that 2 285 infants and young children received 50μg of PAV without side-effects is also noteworthy.

The duration of the immunity conferred by this vaccine is so far unknown, but a long-term follow-up of cases in this study, as well as those in Egypt, will attempt to provide an answer.

Also to be studied are the effects of a wider-scale immunization in controlling a rising outbreak of CSM in a limited focus and the effect of PAV immunization as a means of preventing the disease in particularly exposed groups within the general population.

The success of this trial and the one in Egypt suggests that other countries in the African CSM belt, where this disease remains one of the major health problems, should consider using this meningococcal polyoside A vaccine as a control measure.

RÉSUMÉ

VACCIN POLYSACCHARIDIQUE ANTIMÉNINGOCOCCIQUE DU SÉROGROUPE A: ÉTUDES AU SOUDAN AFIN DE LUTTER CONTRE LA MÉNINGITE CÉRÉBRO-SPINALE CAUSÉE PAR *NEISSERIA MENINGITIDIS* DU GROUPE A

La vaccination contre la méningite cérébro-spinale (MCS), qui avait été utilisée dans le passé avec des résultats habituellement peu satisfaisants, a donné lieu, sous l'impulsion de l'OMS, à de nouvelles recherches. Dans un premier temps (1964-68), des vaccins totocellulaires ont été étudiés en Haute-Volta. Ultérieurement, on a tenté de mettre en évidence pour le méningocoque du sérogroupe A, responsable de l'état endémo-épidémique qui règne dans la ceinture de la méningite en Afrique, l'effet protecteur qui avait été observé dans l'armée américaine après inoculation de la fraction polyosidique (ou polysaccharidique) du méningocoque du groupe C.

Aucun de ces essais ne permit de tirer de conclusion car la méthodologie employée ne permettait pas d'obtenir un nombre de cas de MCS dans le groupe vacciné et dans le groupe témoin suffisant pour atteindre le seuil de signification statistique.

On put réaliser en 1973 des essais satisfaisants au Soudan, où la MCS revêt l'aspect endémo-sporadique bien connu avec renforcement saisonnier au cours de la deuxième partie de la saison sèche. Deux zones d'essai

furent déterminées d'après les conditions épidémiologiques du moment. Seule la seconde, située dans le groupement urbain Khartoum-Omdurman produisit les informations désirées.

Au total, 21 640 personnes furent retenues pour cette étude, au sein d'une population exposée de 161 000 âmes: 10 891 reçurent 0,5 ml de vaccin polysaccharidique antiméningococcique A; 10 749 reçurent la même quantité d'anatoxine tétanique (placébo). Les deux produits étaient conservés au froid, reconstitués et injectés au Ped-O-Jet d'une façon identique. Les opérations durèrent du 12 au 25 avril 1973.

On a administré alternativement du vaccin antiméningococcique et l'anatoxine tétanique. La population étudiée comprenait 57,1% de sujets de sexe masculin et 42,9% de sexe féminin; 70% des sujets avaient moins de 15 ans. Aucun incident ou accident vaccinatoire ne fut observé.

Du 12 avril au début août 1973, il y eut 871 cas cliniques de MCS dans la population exposée (incidence: 5,4 pour 1000 sur 16 semaines). Parmi eux, 10 apparurent dans la fraction de la population faisant l'objet de l'étude. Tous appartenaient au groupe qui avait reçu le placébo. Aucun cas de MCS ne fut notifié dans le groupe qui avait reçu le vaccin polyosidique A. Parmi ces 10 cas cliniques de MCS, sept purent être retenus pour l'analyse statistique, remplissant simultanément les critères d'identification, d'anamnèse vaccinatoire et de confirmation bactériologique (5 fois par isolement de *Neisseria meningitidis* sérogroupe A, 2 fois par immunoélectrophorèse du liquide céphalo-rachidien). Ces dix malades guérirent simplement et sans séquelles sous l'effet du traitement sulfamidé.

La comparaison entre les deux groupes montre avec un degré de signification élevé que le vaccin polyosidique A protège l'individu contre l'apparition des signes cliniques de MCS en période épidémique et dans les conditions de cette étude.

La durée de l'immunité ainsi obtenue, la stratégie d'emploi de ce vaccin et sa place dans les moyens de lutte contre la MCS demeurent à déterminer.

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