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Sacred Disease of Our Times: Failure of the Infectious Disease Model of Spongiform Encephalopathy

Vivian McAlister, *The University of Western Ontario*

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OPINION

Sacred Disease of our times: Failure of the infectious disease model of spongiform encephalopathy

Vivian McAlister, MD

From the Department of Surgery, University of Western Ontario, London, Ontario, Canada

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Abstract

Background: Public health and agricultural policy attempts to keep bovine spongiform encephalopathy out of North America using infectious disease containment policies. Inconsistencies of the infectious disease model as it applies to the spongiform encephalopathies may result in failure of these policies.
Methods: Review of historical, political and scientific literature to determine the appropriate disease model of spongiform encephalopathy.
Principal findings: Spongiform encephalopathy has always occurred sporadically in man and other animals. Hippocrates may have described it in goats and cattle. Transmission of spongiform encephalopathy between individuals is too uncommon for it to be usefully considered an infection. Spongiform encephalopathy is a somatic disorder whose dissemination within a host or transmission between individuals is more like cancer than infectious disease. Spongiform encephalopathy transmission within a species is facilitated in comparison to transmission between species so that cannibalism may amplify the prevalence of the disease.
Conclusion: Agricultural policy should be directed toward an absolute prohibition on occult cannibalism and away from surveillance, quarantine and slaughter, the principal measures of infectious disease containment used to control bovine spongiform encephalopathy.

> "The truth of this is best shown by the cattle that are attacked by this disease, especially by the goats, which are the most common victims. If you cut open the head you will find the brain moist, very full of dropsy and of an evil odour, whereby you may learn that it is not a god but the disease which injures the body. So is it also with a man."
>
> *Hippocrates 5th century BCE*[1]

With these words the Hippocratic author sought to dispel the notion that the 'Sacred Disease' represented a random visitation from the gods. He claimed it had an organic basis, an understanding of which would permit the course of the disease to be altered. Two and a half millennia later, emerging infectious diseases have taken on many of the characteristics of random supernatural visitations. Clustering of different diseases such as Severe Acute Respiratory Syndrome (SARS), West Nile virus, Avian influenza and Bovine Spongiform Encephalopathy (BSE) has reinforced this notion in the mind of the public. A survey by the American Society for Microbiology showed that hand washing by users of the toilets of Toronto Airport was 95% compared with 60% in nearby airports of relatively unaffected US cities.[2] While hand washing is to be encouraged, the statistic probably heralds new phobias. Since the outbreak of BSE in Britain, the Canadian Food Inspection Agency and the US Department of Agriculture have used traditional infectious disease containment policies to "keep BSE out of North America". Application of these policies has resulted in considerable hardship for farming

families. Identification of isolated cases of BSE repeatedly undermines public confidence and contributes to an increasingly phobic environment. Although spongiform encephalopathy occurs sporadically in man and other animals, all cases of BSE are considered to be infectious. New cases of BSE in Canada or Britain are blamed on occult contamination of feed. But are the spongiform encephalopathies really infectious? Would other models of disease be more appropriately applied to them?

Medical, political and historical literature was searched using MEDLINE, Google and manual methods to find the origin of the classification of spongiform encephalopathy as an infectious disease. Relevant citations found in these articles were searched manually. Readings informed the opinion that is contained in this commentary, which tries to balance the evidence for the infectious nature of spongiform encephalopathy with that which suggests alternative models of disease.

Infectious disease model of spongiform encephalopathy.

In 1755 an outbreak of scrapie, a form of spongiform encephalopathy that affects sheep and goats, was discussed in the British House of Commons. It may have been present in Britain for some time before that, according to Brown and Bradley who recently reviewed the history of scrapie.[4] Although there were unsupported claims in 1759 that the disease was contagious, it was generally thought to be due to inbreeding and countermeasures appeared to be successful. In fact early 20th century experiments failed to show transmission of scrapie between animals, until extraordinary measures were taken such as the intra-ocular injection of infected nervous tissue. No direct link between scrapie and disease in man was suspected then or has been found since. Spongiform encephalopathy was first described in man by Alfons Maria Jakob in the 1921.[3] Creutzfeldt Jakob disease (CJD) is rare, affecting one per million population mainly in old age.

Carelton Gajdusek's discovery that Kuru was transmitted by cannibalism accompanied by the finding of scrapie-like lesions in the brains of Kuru victims strongly suggested an infectious basis to spongiform encephalopathy. Further research was restricted to the search for a viral agent.[5] The priority given that search almost cost Stanley Prusiner tenure when his research showed that a protein transferred the disease.[6] A paradigm shift to a non-nucleic infectious entity was required when the results were validated. Recent reviews explain how a prion protein transmits spongiform encephalopathy.[7] It wasn't until 1988 that the neuropathology of spongiform encephalopathy was properly described in cows.[8] The alarming amplification of BSE in the British herd heightened fear of transmission to humans and reinforced the belief in the infectious nature of spongiform encephalopathy. This was confirmed with the identification of a Kuru-like disease, called variant CJD, in humans exposed to BSE.[9] However, there are inconsistencies in the infectious disease model of transmissible spongiform encephalopathy (TSE).

Problems with the infectious disease model of spongiform encephalopathy

The incidence of variant CJD at the height of the outbreak in Britain was 10% that of sporadic CJD and the total rate of CJD has remained stable. An incidence rate of variant CJD in an exposed population of 1 in 10 million is many fold more rare than the rarest of infections. Codon 129 polymorphism cannot explain the rarity. Even for the methionine-homozygous high risk population, the incidence of variant CJD was 1:3,000,000, despite repeated exposure to BSE affected beef.[7] The overall incidence of CJD changed from 1 per million population to 1.1 per million. Carleton Gajdusek would have had to observe a tribe of hundreds of millions of Fore, or to have stayed in New Guinea for thousands of years, to have made his observations had they been about transmission of BSE to humans.

Clearly, transmission of TSE within a species by cannibalism is far more effective than across species. Widespread cannibalism results in extraordinary amplification of the incidence of the disease. Laboratory evidence confirms an incomplete species barrier.[10] The species barrier to TSE appears to be conserved in a manner similar to xenotransplantation rejection: the more disparate the species, the greater the barrier. Modification of the recipient to the type of the donor, increases transmission[11] whereas modification to a third species does not alter susceptibility.[12] The oral route favors the development of transplant tolerance.[13] This mechanism might explain the predilection for the oral route of cross-species transmission of spongiform encephalopathy. Cannibalism amplified BSE in the British herd by a factor between 100,000 and 1,000,000. If transmission is linear, this suggests that annual rate of variant CJD in a human population whose cattle are not fed cattle could be as low as one in a billion people.

Infectious diseases often become more virulent on jumping species. This is thought to be due to naiveté of the recipient's immunity to the new infection, which can then thrive. Cross-species infectious disease, once adapted to the recipient, spreads easily within the new species. Unlike emerging infectious diseases, transmission of TSE to close family or to sexual contacts is not known. No transmission has been found in the thousands of contacts of the recently identified BSE affected cows in North America. Farmyard quarantine, border closure and contact herd slaughter will not keep BSE out of North America. Spongiform encephalopathy is present in many native herds such as elk and deer. BSE-like symptoms have been noted in American dairy cattle over twenty years ago.[14] Richard Marsh of the University of Wisconsin wrote that as BSE was likely already present in North America, in "an unrecognized form…the major threat becomes feeding cows to cows."[14] Given the level of surveillance among Canadian cattle at present, the occasional discovery of a case of BSE is better explained by the spontaneous occurrence of spongiform in older mammals than by blaming a infectious feed which was common to herd

Spongiform encephalopathy as transmissible somatic proliferative disorder

Sporadic spongiform encephalopathy occurs in older subjects due to persistence and proliferation of an abnormal native protein. In this regard it is similar to neoplastic disease. Unlike traditional proliferative disorders that require cellular replication, the prion can transmit its abnormal conformation to a neighboring prion protein and thereby cause proliferation of the abnormality. Transmission of virtually every form of cancer from one human to another has been described in transplantation.[15] Fortunately this is rare but the fact that donor cancers can be transmitted to transplant recipients does not cause us to treat cancer as an infectious disease. The viral etiology of some cancers is another matter. For transplant transmitted cancers, an inoculum of donor malignant cells requires a suitable environment to survive and proliferate, just as the transplanted prion requires a suitable host environment to transmit its conformation to neighboring prion proteins. Public health policy would be better served if TSE were classified with cancer as a transmissible somatic proliferative disorder. Both diseases occur sporadically, probably due to failure of surveillance-repair mechanisms. Affected tissue propagates the abnormality within the native or recipient host, if the immunological barriers are overcome. The mechanism of conversion of the cellular form of the prion protein to the diseased form is becoming clearer with recent studies. Although genetic polymorphisms such as Codon 129 methionine homozygosity increase the risk of acquiring variant CJD, it is the recent finding that host RNA plays a role in prion protein conversion to the protease resistant form that particularly parallels cancer.[16]

Hippocratic description of spongiform encephalopathy

The Hippocratic author quoted above is clearly referring to epilepsy in describing the Sacred Disease in humans. However, in cattle and goats, he is referring non-specifically to neurological conditions that affect gait and behaviour. This may therefore be the first description of spongiform encephalopathy. It is not known if the ancients would have been able to see the gross pathological changes of spongiform encephalopathy. A differential diagnosis of brain abscess needs to be considered but the Hippocratics knew of pus and of abscess in other parts of the body and did not use those words or descriptions with respect to the brain of goats affected by a neurodegenerative disorder. We will never know if the quotation above represents the first reference to spongiform encephalopathy but the description and the species affected are certainly apt. More apt, however, is the Hippocratic author's advice that we should dispel the fear of random visitation and seek instead to understand and alter the organic basis of disease. Designation of spongiform encephalopathy as a transmissible somatic proliferative disorder will help divert public health policy away from surveillance, slaughter and quarantine toward the more necessary absolute prohibition of cannibalism.

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Address Correspondence to:
Dr. Vivian McAlister,
4-TU44, University Hospital,
London, ON, N6A5A5
vmcalist@uwo.ca

ORIGINAL RESEARCH

High rubella seronegativity in daycare educators

Theresa W. Gyorkos PhD*†
Claire Béliveau MD‡
Elham Rahme PhD*†
Cristin Muecke MD MSc*†
Serene Joseph MSc†
Julio C. Soto MD PhD'

From the Department of Epidemiology and Biostatistics, McGill University*, the Division of Clinical Epidemiology, Montreal General Hospital†, the Département de microbiologie, Hôpital Maisonneuve-Rosemont‡, Montréal, and l'Institut national de santé publique du Québec', Québec, Canada

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Abstract

Background: Congenital rubella syndrome, which is associated with severe malformations, can result from infants exposed in utero to maternal rubella infection. Health care workers and school-based educators are targeted for immunization, but evidence is scarce on rubella seronegativity in daycare centre educators who appear to be a high-risk occupational group. The purpose of the study was to generate new evidence on the magnitude of rubella seronegativity and associated risk factors in daycare centre educators.

Methods: Sera and questionnaires were collected between October and December 2001 from 481 female educators working in 81 daycare centres in Montréal, Québec. Rubella IgG serology was performed using ELISA.

Results: An overall seronegativity of 10.2% was found. The positive predictive value of previous rubella vaccination with seropositivity was high (92.1%). Ninety-one percent of the women were of childbearing age (=49 years). Only 1.3% (n=6) were currently pregnant, none of whom were seronegative. Significant predictors of seronegativity for educator- and daycare-level variables included lack of previous rubella vaccination (OR=3.60; 95%CI: 1.43, 9.01), not having own children (OR=3.76; 95%CI: 1.67, 8.55), age per 5-year increment (OR=0.81; 95%CI: 0.66, 0.99), and increased number of colds in educators in the daycare centre in the last two weeks (OR=1.15; 95%CI: 1.01, 1.31).

Interpretation: The high proportion of seronegativity, in addition to the potential increased transmission in daycare centres emphasize the need for a review of the rubella vaccination recommendations and health promotion interventions targeted to this occupational group.

The incidence of rubella in Canada, the USA, and other industrialized countries has decreased considerably since the adoption and successful implementation of routine immunization of infants.[1-4] Current guidelines in Canada for the trivalent measles-mumps-rubella (MMR) vaccine for children are one dose at 12 months of age and a second dose before school entry.[1,2] The decrease in endemic rubella transmission has been accompanied by a change in the epidemiology of rubella. In Canada, there is a trend towards more cases in young adults than in younger children.[5-6] Twenty-nine cases of rubella were reported in Canada in 2000 with the majority in the 10-39 yr range.5 However, an increased incidence rate is seen in pre-school age children (< 5 yr) compared with other age groups. Rubella incidence rates were 0.59 and 0.35 per 100,000 in 0 to 1 year olds, and 1 to 4 year olds, respectively, in 2000. This corresponded to two and five cases, respectively.[5] The average incidence rate in all other age groups was 0.09 per 100,000.[5]

Rubella infection is mild in children and is more often symptomatic in adults, with common symptoms such as headache, malaise and conjunctivitis.[7] However, a more serious concern is congenital rubella syndrome (CRS), which can result from in utero exposure to rubella infection during pregnancy.[8,9] The

TABLE 1. Cases of rubella infection and congenital rubella syndrome (CRS) for Canada, the USA and the UK (England, Scotland, Wales) from 1990 to 2001.

	1990	*1991*	*1992*	*1993*	*1994*	*1995*	*1996*	*1997*	*1998*	*1999*	*2000*	*2001*
Rubella												
Canada[3*]	402	704	2265	1028	237	300	302	4007	69	24	29	n/a
USA[11]	1124	1401	160	192	227	128	238	181	364	267	176	23
UK[12†]	705	9345	8857	11772	9242	7454	11530	4078	3953	2502	2002	1717
CRS												
Canada[13,14‡]	2	5	1	7	4	2	2	1	1	1	2	0
USA[11]	11	47	11	5	7	6	4	5	7	9	9	3
UK[15]	12	3	7	3	7	1	12	0	0	1	4	3

* 2001 numbers not published

† 1990 rubella cases for England and Wales only

‡13 1990-1995, 14 1996-2001

syndrome can cause intrauterine growth retardation, retinopathy and other congenital abnormalities, as well as long-term manifestations such as cataracts, sensorineural hearing loss and congenital heart disease.10 The risk of congenital defects is highest for exposure in the first trimester.[7] Although the decrease in rubella incidence in most industrialized countries of the world has translated into a decrease in CRS incidence, elimination has not yet been achieved (Table 1).[5,11-15] In addition, the ambiguity and misclassification in the diagnosis of CRS, and the lack of reporting of cases associated with spontaneous and therapeutic abortions leads to underreporting of this condition.[16-18]

Successful elimination of CRS can only occur if immunity in pregnant women is ensured. In Canada, rubella vaccination is recommended for all female adolescents and women of childbearing age who do not have documented proof of immunity.1 However, it is clear that a high proportion of women in these age groups remains seronegative (Table 2).[4,19-25] A recent study in Canada detected an 8.4% seronegativity at prenatal screening.[23] In addition to failure to vaccinate, waning immunity of previously vaccinated individuals has been implicated in the high seronegativity rates.[2,22] Risk factors for seronegativity are foreign-birth, non-married status, and not having own children.[19,21,24,26]

Women who are in contact with young children should be concerned due to the potential for increased exposure to infection. Daycare centres (DCCs) are one such setting that appears to be a high risk environment for disease transmission in general.[27-29] Large numbers of children together in one location and inadequate hygiene practices of young children are known to facilitate infectious disease transmission in the daycare setting.[30] The DCCs now consist of higher numbers and proportions of children of younger ages than in previous years, with many children being enrolled before full immunity from vaccination is acquired.[31] With the higher age-specific incidence rates of rubella in preschool age children,[5] there is the potential for horizontal transmission in the daycare centre.

The Canadian Immunization Guide currently recommends rubella vaccination for individuals in health care and educational institutions where exposure is high.[1] No recommendations are specifically made for individuals in the daycare setting, despite a consensus supporting immunization promotion for susceptible daycare employees of reproductive age.[27] As many individuals working in DCCs are women of childbearing age, it is imperative that their risk status for rubella be assessed. No previous studies on rubella seronegativity have been undertaken in the adult female daycare population.

The purpose of this study, therefore, was to generate new evidence on the risk factors for rubella seronegativity in daycare educators. Research was undertaken in Montréal, Québec, to determine the proportion of seronegativity of rubella and its associated risk factors among DCC educators.

Methods

Ethics approval Ethics approval for the study was given by the Research Ethics Committee of the McGill University Health Centre.

Study Population A sampling frame of all daycare centres in Montréal, Québec was used to obtain a random sample of centres for the study. The number of centres chosen was based on a predetermined goal of recruiting 480 educators. Eligible centres were those

TABLE 2. Seroprevalence studies of rubella in countries having routine immunization.

First author	*Year, Location*	*Study population (all adult women)*	*Sample size*	*Seronegativity (%)*
Dykewicz [19]	1988-1994, US	NHANES III * study	8,840	11.1
Zufferey [20]	1990-1991, Switzerland	Postnatal women	9,046	5.7
Duclos [21]	1991, Canada	Military recruits	36	2.8
Ratnam [22]	1991-2000, Newfoundland	PHL † screening service	100,000	8.3
Gyorkos [23]	1994-1996, Québec	Prenatal screening	2,551	8.4
Cheffins [24]	1995, South Australia	Antenatal population	9,442	3.3
Tookey [4]	1996-1999, North London	Antenatal population	137,398	2.5
Danovaro-Holliday [25]	2000, Alabama	Poultry-processing plant	148	12.8

* NHANES III = Third National Health and Nutrition Examination Survey
† PHL = Public Health Laboratory

that: 1) were registered with the government ministry, Ministère de la Famille et de l'Enfance (MFE) as of December 31, 2000, 2) were in current operation, 3) enrolled children aged < 36 mos, and 4) employed at least six educators.

Data Collection Contact was made with the director of each centre and, if verbal consent was given, further information was provided and distributed to educators. If more than three educators at one centre were willing to take part in the study, a visit to the daycare centre was made by the research team. Inclusion criteria for educators were: 1) > 18 yr, 2) employment for >15 h/wk, and 3) regular care of children < 60 mos. Information on educator- and daycare-level variables were obtained by self-reported questionnaires from educators and directors, respectively. Questionnaires included information on socio-demographic, employment and other lifestyle characteristics of the educators, as well as operational, physical and health and hygiene characteristics of the daycare centres. Previous rubella vaccination was determined by self-report only. Questionnaires, consent forms, and blood samples were collected during the on-site visit from participating educators.

Blood samples were transported to the microbiology laboratory of Hôpital Maisonneuve-Rosemont in ice-packed coolers. Presence of IgG antibodies was determined using an ELISA assay according to the manufacturer's instructions (Rubella IgG ELISA, Wampole Laboratories, Cranbury, NJ). An index value of = 1.10 was considered positive and = 0.90 was considered negative. In between values were considered equivocal, but there were no results in this category. ELISA has a high relative sensitivity and specificity for rubella IgG compared to hemagglutination inhibition (100% and 95.8% respectively) and other commercially available ELISA tests (100% and 97.1%, respectively) (Wampole Laboratories, Cranbury, NJ). It is also commonly used in seroprevalence studies for rubella.[24,25]

Statistical Analysis Logistic regression was used to compute odds ratios and confidence intervals for all potential educator- and DCC-level risk factors for rubella seronegativity. Those that were considered eligible in univariate analysis ($P<0.20$) were included in a multivariate logistic regression. Those that remained statistically significant ($P<0.05$) were included in the final model. All statistical analyses were performed with SAS (SAS Statistical Software, version 8.0).

Results

Out of 167 daycare centres that were randomly chosen for participation in the study, 152 were found to be eligible based on the inclusion criteria. Centres were ineligible if they had an insufficient number of educators (n=8) or enrolled only children older than 36 months (n=7). Of the eligible centres, 81 had full participation of directors and educators, and complete serologic results for the educators. Compared with 16 non-participating centres, the 81 participating centres were slightly more likely to be not-for-profit (67.5% vs. 62.5%), enrolled fewer licensed daycare places (61.6 ± 22.7 vs. 69.3 ± 29.1), have a greater number of children < 36 mos (48.8% vs. 41.8%), and be less likely to accept part-time attendees (61.0% vs. 73.3%).

Eighty-one daycare centres and 491 educators provided complete results for questionnaires and serology for rubella. As there were only 10 male educators, analysis included only female daycare educators (n=481). Overall seroprevalence was 89.8%, indicating 10.2% seronegativity. The positive predictive value of self-reported previous rubella vaccination with seropositivity was high (92.1%).

TABLE 3. Characteristics of 81 daycare centres (DCC) in Montréal, Canada, 2001.

Daycare centre characteristics	*Percent or Mean±SD**
Status-Not-for-profit	67.5
Daycare accepts children in diapers	95.1
Daycare accepts disabled children	72.4
Number of licensed places	61.6 ± 22.7
Number of nursery places	6.3 ± 6.7
Years of operation	18.9 ± 9.1
Number of hours open per week	55.5 ± 6.2
Number of children in daycare	59.2 ± 22.5
Educator to child ratio in daycare	0.2 ± 0.1
Minimum age of children at registration (months)	10.9 ± 8.6
Number of children absent due to illness in last 2 weeks	12.2 ± 18.5

*SD = standard deviation

Characteristics of daycare centres were examined (Table 3). Of the 81 participating centres, 67.5% operated on a not-for-profit basis. DCCs had been in operation for an average of 18.9 years (range 1-42 years) with an average staff of 11 educators and approximately 59 children enrolled per centre. A total number of 4,737 children were enrolled at all 81 centres. Of the total number of children, 52.7%, 36.2% and 11.1% were > 36 mos, 18-35 mos, and < 18 mos, respectively. The average minimum age of children at registration was 11 mos.

Proportions of seronegativity were calculated for various characteristics of daycare educators (Table 4). Most women were born in Canada and had a higher seronegativity than those born elsewhere. Ninety-one percent of women were of childbearing age (=49 yr), and seronegativity decreased with age. A higher seronegativity proportion was found in educators without any children. Six educators (1.3%) were currently pregnant and none were seronegative.

Educator- and DCC-level variables that were found to be associated with rubella seronegativity in univariate analysis are shown in Table 5. Crude odds ratios and confidence intervals were calculated for: age (per 5-yr increment), experience in daycare (per 5-yr increment), place of birth, marital status, not having own children, number of people living with educator, not planning a future pregnancy, lack of previous rubella vaccination, number of colds among educators in the last two weeks and the number of children absent. Younger age, not having own children, lack of previous rubella vaccina-

TABLE 4. Personal characteristics and rubella seronegativity in 481 daycare educators in Montréal, Canada, 2001.

Educator Characteristic	*Number of educators ** #	%	*Seronegativity (%)*	
Age				
<20 years	5	1.1	20.0	
20 to 29 years	150	31.5	18.0	
30 to 39 years	168		35.3	8.3
> 40 years	153		32.1	4.6
Born in Canada				
No	150		31.2	6.7
Yes	331		68.8	
11.8				
Income grouping of birth country				
High	372	77.3	10.5	
Middle	78	16.2	9.3	
Low	31	6.4	6.4	
School				
Primary or secondary	47	9.8	10.6	
Post-secondary	432	90.2	10.0	
Diploma in ECE † or related				
No	134	27.9	9.7	
Yes	310		64.6	9.7
In progress	36	7.5	16.7	
Gross household income				
Under $20,000	119	27.5	13.4	
$20,000 to $39,999	177	40.9	11.9	
$40,000 and over	137	31.6	6.6	
Marital status				
Single	171	35.6	15.8	
Spouse or common-law	310	64.4	7.1	
Have own children				
No	213	44.3	17.8	
Yes	268	55.7	4.1	
Currently pregnant				
No/don't know	474	98.7	10.3	
Yes	6	1.3	0.0	
Planning pregnancy in next year				
No/don't know	427	90.1	11.0	
Yes	47	9.9	4.3	
Experience in daycares				
<5 years	164	34.1	13.3	
5 to 9 years	132	27.4	12.1	
>10 years	185	38.5	6.0	
Age of children cared for				
<18 months	84	17.5	10.7	
18 to 35 months	139	29.0	10.8	
>36 months	202	42.1	10.9	
No particular age group	55	11.5	5.4	
Number of hours worked/week				
<25 hours	29	6.0	6.9	
25-35 hours	146	30.4	13.0	
>35 hours	306	63.6	9.2	

* Totals may not add up to 481 because of missing responses on the questionnaire

† ECE = early childhood education

TABLE 5. Crude and adjusted analyses for risk of rubella seronegativity for educator- and daycare-level variables, Montréal, Canada, 2001.

Variable	*Crude OR **	*95% CI †*	*Adjusted OR*‡*	*95% CI †*
Educator-level				
Age (per 5-year increment)	0.71	0.59, 0.86	0.81	0.66, 0.99
Experience in daycare (per 5-year increment)	0.77	0.58, 0.95		
Born in Canada	1.87	0.91, 3.85		
Single vs. married/common-law	2.44	1.35, 4.55		
Not having own children	5.00	2.50, 10.00	3.76	1.67, 8.55
Number of people in house	0.83	0.67, 1.02		
Not planning a future pregnancy	2.78	0.65, 11.11		
Lack of previous rubella vaccination	2.44	1.01, 1.09	3.60	1.43, 9.01
Daycare-level				
Number of colds among educators (last 2 weeks)	1.14	1.01, 1.29	1.15	1.01, 1.31
Number of children absent (last 2 weeks)	1.03	1.01, 1.05		

* OR = odds ratio

† CI = confidence interval

‡ Blank cells indicate non-significant variables ($p<0.05$) in the final multivariable model

tion and higher number of colds among educators in the previous two weeks all remained significantly associated with rubella seronegativity in the final multivariate model (Table 5). Results remained similar when the analysis was restricted to women of childbearing age only (data not shown).

Discussion

The results of this study support recent findings that a high proportion of women of childbearing age continue to be rubella-seronegative. The 10.2% seronegativity in this population is higher than that which was found by Gyorkos et al (1998) in a prenatally screened population in Québec and higher than proportions published in recent studies in industrialized countries.[4,20-22,24]

The association between younger age and seronegativity is consistent with recent studies.[19,22] The high seronegativity proportion in young adults correlates with the higher proportion of cases in this age group compared with other age groups,[5,6] and highlights the need for preventive action in this vulnerable population.

Not having own children was also found to be a predictor of seronegativity in our study population, which may represent fewer opportunities for vaccination from pre- or post-natal screening, or from acquired immunity from rubella exposure through fewer child contacts. Dykewicz et al (2001) found an increased risk of seronegativity in women without children, but this difference was only noted in unmarried women.[19] This was attributed to the success of pre-marital screening programs in the USA which target married women, regardless of parity, for vaccination. Similar programs are not in place in Canada.

The association between a lack of (self-reported) previous rubella vaccination and seronegativity has not been previously documented in seronegativity studies. This result highlights the need for active immunization of this group. The low seronegativity proportions in older age groups, and the association between seronegativity and lack of vaccination suggest that waning immunity may not play an important role in seronegativity in this population, as has been speculated.[2,22] However, time elapsed since immunization was not explicitly determined in this study and vaccination status was based on self-report only.

In contrast to previous results,[11,12,14,15,24,25] women in the current study who were born in Canada had a higher proportion of rubella seronegativity than those who were foreign-born, although the difference was not statistically significant in multivariate analysis. The lower proportion of seronegativity in foreign-born individuals may indicate immunity arising from natural rubella infection in areas without vaccination but with higher circulation of the virus. The lower proportion of seronegativity in this group may also be a result of the success of Canada-based rubella screening and vaccination programs post-immigration.

The only daycare-level variable that was associated with rubella seronegativity was the number of colds in educators in the daycare in the previous two weeks. However, this result does not appear to have any theoretical or clinical basis that we are aware of and may be a chance finding. As no previous studies have been conducted in the daycare setting, it is clear that potential daycare-level risk factors not examined in this study will require further investigation.

Strengths of this study include a large sample size and heterogeneity of the daycare educator population. The high sensitivity and specificity of the ELISA, and the absence of blood samples with equivocal assays, increase the validity of the serologic results. Although the results of the study may be applicable to other daycare educator populations in similar urban centres, these results may not be generalizable to other groups in the general population. Generalizability may also be limited by the fact that the majority of our daycare educators were born before the 1983 implementation of routine infant MMR vaccination nationwide.1 Therefore, younger educators may have different seronegativity and risk factors than the current population studied. The cross-sectional nature of the study does not allow for seroconversion or associated risk factors to be determined. In addition, as no comparison group was included, we are unable to make conclusions regarding the risk of rubella seronegativity relative to the general population or other occupational groups.

Overall, a greater than 10% seronegativity in this occupational group is of concern considering the large proportion of women of childbearing age who would be at potential risk of contracting rubella during pregnancy. A review of current guidelines for rubella vaccination in daycare educators during Early Childhood Education training, or at the time of employment at daycare centres may be warranted to ensure that this occupational group is appropriately targeted. In addition, health promotion interventions may be needed to reach this population.

The fact that reports of CRS are still made every year in industrialized countries whose vaccination programs have been a cornerstone of preventive medicine reflects missed opportunities to prevent serious disease burden.

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Address correspondence to:
Dr. T. W. Gyorkos
Division of Clinical Epidemiology
Montreal General Hospital
1650, Cedar Avenue
Montréal, Québec H3G 1A4
Canada
Email: theresa.gyorkos@mcgill.ca

ORIGINAL RESEARCH

Medical Student Career Choice and Mental Rotations Ability

Michael G. Brandt[1] BSc
Erin D. Wright[2] MDCM MEd FRCSC

From the Schulich School of Medicine[1] and the Department of Otolaryngology[2], University of Western Ontario, London, Ontario, Canada

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Abstract

Purpose: To determine whether innate visual-spatial ability influences medical student choice of a surgical career. In addition, the student's career interests on entering medical school (matriculation) predicted application and acceptance to a residency program.
Methods: Fifty-nine fourth year medical students at the University of Western Ontario completed a career choice questionnaire that identified the residency program(s) to which they showed interest at the time of matriculation, the program(s) to which they applied, and the residency program(s) to which they matched. The selections were compared with the student's score on the Vandenberg & Kuse Mental Rotations Test, a test of visual-spatial ability.
Results: Graduates initially interested in visual-spatially intense medical disciplines scored better ($P < 0.02$) on the Mental Rotations Test. The findings did not persist to the time of application and acceptance into residency training programs. There was no correlation between visual-spatial ability and selection of a visual-spatially intense specialty. Only 32% of graduates applied to their specialty of initial interest.
Conclusion: The ability to rotate an object in three dimensions mentally does not to play an important role in surgical career selection although it was a predictor of initial career interest upon entry to medical school. Initial career interest was not an accurate predictor of career choice in general. In contrast to the overall results, 71% of individuals initially interested in family medicine ultimately applied to this medical discipline.

Many factors contribute to the career decisions of medical school graduates. In recent years, the influence of a controllable lifestyle in choosing a career path has been suggested to play an increasingly important role[1-4]. This influence has been purported to have led to recent U.S. medical school graduates' career-choice trending away from surgical specialties[4]. Although U.S. medical school graduates appear to be selecting more controllable-lifestyle careers, Canadian medical school graduate statistics over the past eight years have remained stable, with a similar, if not increased, number of graduates selecting surgical careers[5]. Thus, a relative degree of competition persists amongst Canadian senior medical students in obtaining surgical residency positions.

With many medical school graduates still selecting surgical careers, attention has been focused on the factors that predict surgical aptitude and, from a practical perspective, the best applicant for a surgical career. One such factor, visual-spatial ability, has received increased attention[6-8]. Several studies have identified the influence that innate visual-spatial ability plays in the initial learning of a surgical technical skill[6,7] although the precise role that this particular factor plays in overall aptitude for a surgical career is a matter of contention[6]. The purpose of this study was to identify whether individuals with superior ability to manipulate an object mentally self-select a surgical career based on their innate visual-spatial aptitude.

Initial interest in Family Medicine and surgical careers amongst medical students upon admission to medical school (matriculation) has been reported to

play a role in ultimate career selection[9,10]. The maintenance of this influence up to the point of graduation and its impact on career choice has not been investigated. Hence, in addition to the primary goal of assessing the role of innate visual-spatial ability on career selection, this study seeks to assess if initial interest in a particular medical discipline influences ultimate career choice.

Methods

The study was approved by the University of Western Ontario Research Ethics Board. All graduating fourth-year medical students (Class of 2004) at the University of Western Ontario were invited to participate in this project via electronic mail and in-classroom announcements. Contained within the electronic mail was information regarding the study including: the study objectives, informed consent issues, and contact information. Students were asked to participate on a voluntary basis, and any student wishing to participate was to report to the designated classroom at a particular date and time.

Fifty-nine fourth year medical students participated in the study. All the student participants were greeted by the principal investigator and once again provided information on the project. Once all of their questions had been adequately addressed, the students were provided with a package containing a copy of the Vandenburg and Kuse Mental Rotations Test (MRT-A) and a career path questionnaire. The career path questionnaire provided students with three listings of all residency programs offered by the Canadian Residency Matching Service (CaRMS). The students were asked to circle the residency program that interested them when they applied to medical school, the programs to which they applied, and the program to which they ultimately matched.

The students were asked to place no identifying marks on either the career path questionnaire or the Mental Rotations Test. Once the career path questionnaire was completed, the Mental Rotations Test was administered per the protocol outlined by Peters and colleagues (1995)[11]. The principal investigator then collected both the career path questionnaire and the Mental Rotations Test.

Mental Rotations Test scores were quantified and recorded along with responses to the career path questionnaire. For analysis, residency programs were subjectively classified as "Visual-Spatial", or "Less Visual-Spatial" (TABLE 1). Although most medical disciplines utilize visual-spatial ability, the specialties identified as "Visual-Spatial" represent fields where visual-spatial ability serves as a central skill to the day-to-day function within the specialty. A breakdown of this classification is is loosely based on the subjective identification of radiological and surgical specialties as necessitating visual-spatial ability in their daily function[2].

TABLE 1: Canadian Residency Programs Classified By Visual-Spatial Intensity

Less Visual-Spatial	*Visual-Spatial*
Anatomic Pathology	Cardiac Surgery
Anesthesia	Diagnostic Radiology
Community Medicine	General Surgery
Dermatology	Neurosurgery
Emergency Medicine	Nuclear Medicine
Family Medicine	Obstetrics & Gynecology
General Pathology	Ophthalmology
Integrated Family and Community Medicine	Orthopedic Surgery
Internal Medicine	Otolaryngology
Laboratory Medicine	Plastic Surgery
Medical Genetics	Radiation Oncology
Neurology - Adult	Urology
Neurology - Pediatric	
Neuropathology	
Occupational Medicine	
Pediatrics	
Physical Medicine & Rehabilitation	
Psychiatry	

Comparisons of the mean mental rotations tests scores were made of graduating medical students showing initial interest in visual-spatially intense disciplines vs. those showing interest to less-visual spatially intense specialties, of graduating medical students applying to visual-spatially intense disciplines vs. those applying to less visual-spatially intense specialties and of graduates matching to visual-spatially intense careers vs those matching to less visual-spatial disciplines.

Qualitative comparisons were also made observing whether initial interest in a particular medical discipline lead to application and acceptance to that particular specialty.

Statistical Analysis

A Levene F-test was used to test the equality of the variability of the two samples being tested. The groups were then compared using Student's t-tests based on this variability. If the two samples had equal variability the value of the t-test for samples with equal variability was recorded. Conversely, the value of the t-test for

TABLE 2: Residency Programs Matched

	Number of Students Matched
Less Visual-Spatial Programs	
Anesthesia	4
Community Medicine	1
Emergency Medicine	3
Family Medicine	13
Integrated Medicine and Rural Family Medicine	1
Internal Medicine	16
Pediatrics	3
Psychiatry	4
Total	45
Visual-Spatial Programs	
Diagnostic Radiology	1
General Surgery	2
Obstetrics/Gynecology	5
Orthopedics	2
Radiation Oncology	2
Total	12

samples with non-equal variability was recorded for F-tests with $P < 0.01$.

Statistical analysis and data tabulation were performed using Microsoft Excel 2002 software.

Results

Fifty-nine graduating medical students participated in the study, representing 57% of the 2004 graduating class. Participating students varied amongst the future residency programs to which they matched (TABLE 2).

The residency programs matched to by our sample varied to a similar degree to that of the total population of 2004 medical school graduates successfully matching to residency programs (1227 graduates in total) (TABLE 3).

Of the 59 particpants, 41 identified interest in less visual-spatially intense medical disciplines at the time of matriculation. Conversely, nine graduates identified initial interest in visual-spatially intense medical disciplines. Those initially interested in the visual-spatially intense medical disciplines scored better on the Mental Rotations Test ($P< 0.02$) (Figure 1).

These findings did not carry through to the ultimate application and acceptance of the medical graduates into their respective residency training programs. Those applying to and gaining acceptance to less visual-spatially intense disciplines scored as well as those graduates applying and gaining acceptance to visual-spatially intense specialties.

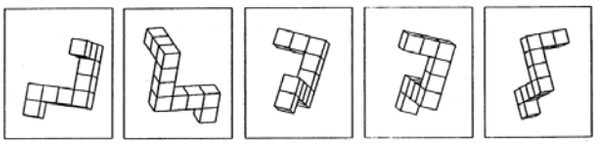

FIGURE 1: Sample from the Mental Rotations Test. Medical students were asked to identify which of the four items depicted on the right matched the reference object on the left. (Reprinted with permission from Peters et al.[10])

Of those individuals who reported an initial medical discipline of interest, only 32% applied to this residency program, and 21% ultimately gained acceptance (Table 4).

In contrast to this cumulative figure, 71% of graduates indicating initial interest in family medicine applied to this residency program, with 39% ultimately matching to this discipline. Of the individuals initially interested in less visual-spatially intense medical disciplines, 35% applied to the identified discipline, and 24% gained acceptance (Table 4). For those individuals who showed initial interest in visual-spatially intense specialties, 23% ultimately applied and 13% were accepted (Table 4)

Discussion

We found that the innate skill of mentally rotating an object in three dimensions, visual-spatial ability, appears to play a role in initial career interest. Of the 59 medical school graduates participating in the study, those with stronger visual-spatial ability were more likely to be initially interested in the visual-spatially intense medical disciplines. However, this initial interest in medical disciplines that foster innate visual-spatial ability, does not persist throughout medical school into residency program application and acceptance.

All of the graduates showed a similar degree of variation between their chosen specialty at the time of matriculation, and the disciplines for which they applied and to which they were ultimately matched. There was a consistent percentage of individuals applying for, and gaining acceptance to, the medical specialties to which they showed initial interest. This occurred equally among graduates initially interested in both the visual-spatially intense and less intense disciplines.

Our results support those of Garrett et. al (1991) which identified consistency between initial interest in Family Medicine and ultimate application and accep-

TABLE 3: Residency Position Matching

Residency Programs	*% of Total Residency Positions Matched To*	*% of Total Residency Positions Successfully Matched To By Our Sample*	*% of Total Residency Positions Applied To By All 2004 Medical School Graduates*	*% of Total Residency Positions Applied To By Our Sample*
Anatomic Pathology	0.7	0.0	5.4	0.0
Anesthesia	5.2	7.0	0.3	9.9
Cardiac Surgery	0.6	0.0	1.2	1.1
Community Medicine	0.6	1.7	0.5	1.1
Dermatology	0.5	0.0	1.3	0.0
Diagnostic Radiology	3.9	1.7	5.1	3.3
Emergency Medicine	2.0	5.3	2.4	4.4
Family Medicine	33.3	22.8	26.3	28.6
General Pathology	0.0	0.0	0.2	0.0
General Surgery	5.7	3.5	0.0	2.2
Integrated Family and Community Medicine	0.1	1.7	5.1	1.1
Internal Medicine	15.5	28.1	14.6	20.9
Laboratory Medicine	1.5	0.0	1.6	0.0
Medical Genetics	0.2	0.0	0.2	0.0
Neurology - Adult	1.4	0.0	1.5	0.0
Neurology - Pediatric	0.2	0.0	0.4	0.0
Neuropathology	0.0	0.0	0.0	0.0
Neurosurgery	1.1	0.0	1.2	0.0
Nuclear medicine	0.2	0.0	0.2	0.0
Obstetrics & Gynecology	4.2	8.8	4.2	6.6
Occupational Medicine	0.1	0.0	0.1	0.0
Ophthalmology	1.4	0.0	2.6	5.5
Orthopedic surgery	3.1	3.5	3.0	2.2
Otolaryngology	1.2	0.0	1.8	0.0
Pediatrics	6.1	5.3	7.0	4.4
Physical Medicine & Rehabilitation	0.7	0.0	0.8	0.0
Plastic surgery	0.9	0.0	2.7	0.0
Psychiatry	6.4	7.0	6.2	5.5
Radiation Oncology	1.7	3.5	2.1	3.3
Urology	1.4	0.0	2.1	0.0

TABLE 4: Percentage of 2004 medical school graduates who apply-to and are accepted-to programs for which they identified interest at the start of medical school

	% Interested That Apply	*% Interested That Match*	*% Difference*
All Specialties	31.9	20.9	11
Less Visual-Spatial Specialties	35.3	24.1	11
Visual-Spatial Specialties	23.4	12.8	11

tance to this residency program[9]. Our results do not suggest a similar consistent trend amongst those individuals initially interested in surgical specialties. Our findings of higher innate visual-spatial ability amongst those individuals initially interested in visual-spatially intense medical disciplines appears to suggest initial selection of career based on innate abilities. This initial career choice does not remain consistent and is most likely affected by the practical implications of pursuing a career in these disciplines. The numerous studies on the impact of a controllable lifestyle on career choice may provide some insight into this change from initial interest to ultimate application. The initial career choices of medical students may be less informed with respect to the practical implications of pursuing a demanding surgical career and, hence, their interests change after clinical exposure to these specialties.

The role of visual-spatial ability in predicting aptitude for becoming a technically proficient surgeon is a contentious issue. Several studies have illustrated the strong initial role visual-spatial ability plays in the learning of a novel surgical technical skill[6,7]. Despite this initial role in surgical skill acquisition, Wanzel and colleagues suggest that the role of visual-spatial ability

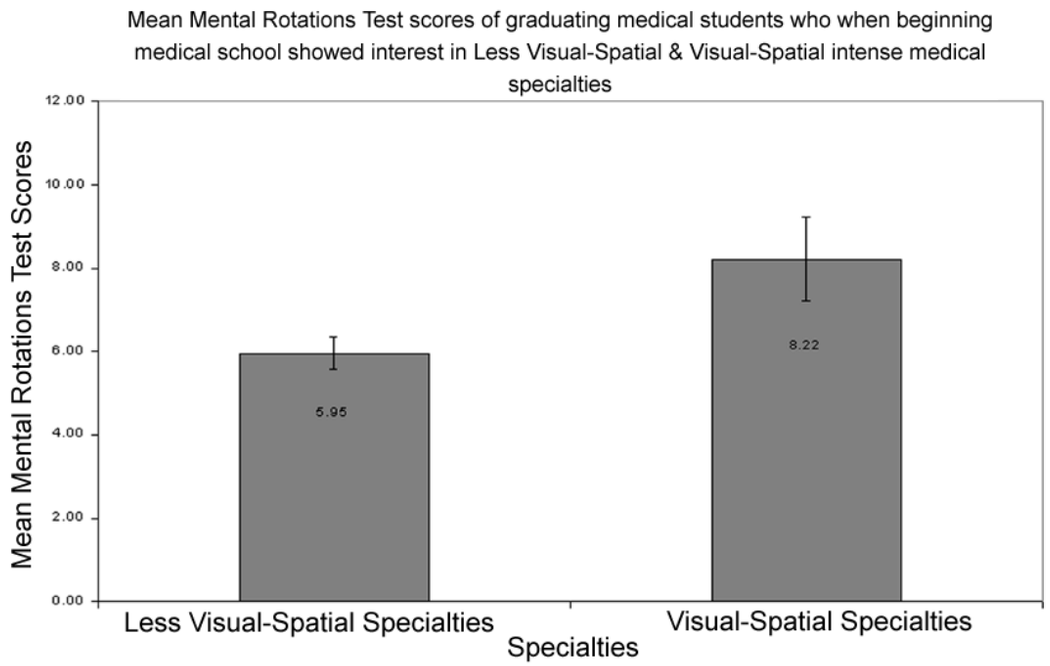

FIGURE 2: Mean Mental Rotations Test scores of graduating medical students who when beginning medical school showed interested in Less Visual-Spatial & Visual-Spatial intense medical specialties.

may not be related to a surgeon's ultimate technical competency and conclude that practice and repetition are more accurate predictors of overall technical expertise[6]. Extrapolating from these findings, it appears that, although overall expertise may not be predicted by visual-spatial ability, the speed by which one learns a new technique may be more strongly related. For individuals seeking a career in a visual-spatially intense discipline, the ability to learn novel spatially-complex techniques more readily may be an asset. Consequently, selecting a specialty based on innate ability may align individuals to be more successful in their careers. Our study suggests that, although individuals may initially select visual-spatially intense medical disciplines based on innate spatial aptitude, this does not carry forward to their ultimate application and acceptance to a visual-spatially intense medical discipline. If individuals are indeed better able to learn spatially-complex techniques based on innate visual-spatial abilities, identifying these individuals early in their medical school training and recognizing the other factors that impact on their career choice may aid in recognizing why these individuals do not follow career paths which highlight their innate abilities.

The results obtained in this study suffer from the inherit limitations of its retrospective design. Performing a longitudinal study over the course of the subjects medical school training would have provided more strength to our conclusion and is the basis for a future study. With respect to the study parameters, The Vandenberg and Kuse (1978) Mental Rotations Test[6,7,11,12] has been validated as a psychometric instrument for the assessment of visual-spatial ability (Figure 2). Superior scores on the Mental Rotations Test have been shown to correlate strongly with the proficiency of learning a spatially complex surgical task as well as the quality of the final product of that task.[6,7] Thus, the Mental Rotations Test was chosen as our visual-spatial ability assessment instrument.

In summary, identifying the factors that influence graduate career choice is of paramount importance at a time when the health care needs of many communities are not being met by the existing medical school graduate pool,. This study suggests, albeit retrospectively, that innate aptitudes may play an initial role in career selection. We also identified that initial career choices are altered as medical students progress

through their undergraduate medical training. Future investigations would benefit from a prospective analysis of medical students from the time of matriculation to the time of graduation, identifying the point at which innate abilities are set-aside for more important influences in career selection.

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Address correspondence to:
Dr. Erin D. Wright
Associate Professor, Department of Otolaryngology
University of Western Ontario
900 Richmond St., 3rd Floor
London, Ontario, Canada
N6A 4V2

E-mail: erin.wright@sjhc.london.on.ca

ORIGINAL RESEARCH

Angiotensin II Type 1 Receptor Blocker Inhibits Pulmonary Injury

G. B. John Mancini MD FRCPC FACC
Nasreen Khalil MD FRCPC FCCP

From the Department of Medicine, Divisions of Cardiology and Respiratory Medicine, University of British Columbia and Vancouver Coastal Research Institute, Vancouver, CANADA

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Abstract

Purpose: Pulmonary damage and fibrosis may be the result of diverse forms of injury and there is an association between pulmonary diseases and cardiovascular events. The purpose of this study was to evaluate the effects of an angiotensin II type 1 receptor blocker, valsartan, on systemic, cellular, and fibrotic consequences of pulmonary injury induced by the anti-neoplastic antibiotic, bleomycin.

Methods: Sprague Dawly rats were used in the classical bleomycin model of pulmonary fibrosis. Bleomycin (1 unit, n = 7) was administered intra-tracheally to induce lung injury. Valsartan (0.66 mg) was given either concomitantly (n = 9) or for two days prior to bleomycin (n = 8). A control group (n = 6) was given normal saline.

Results: Valsartan-treated animals showed abrogation of weight loss, suppression of release of total and active transforming growth factor beta-1 (TGF-ß1), and diminished connective tissue synthesis. In an explant, lung tissue culture model devoid of alveolar macrophages (saline control, n = 3; bleomycin, n = 6; bleomycin plus valsartan, n = 12), both total and active TGF-ß1 were suppressed in the valsartan-treated cohort.

Conclusions: Valsartan, known to have cardio-protective properties, was shown to be protective of bleomycin-induced pulmonary injury. Thus, ARBs may be beneficial in both cardiac and pulmonary diseases.

The association between smoking and cardiovascular disease is well recognized.[1]. There is also an association between diverse forms of pulmonary injury and cardiovascular events. For example, pulmonary injury from air pollution has been shown to enhance atherosclerosis[2] and to be closely associated with induction of acute cardiovascular events.[3-6] Furthermore, it has been known for decades that there is a relationship between poor lung function and both myocardial infarction and sudden cardiac death but that this relationship cannot be explained solely by the relationship between smoking and cardiovascular disease.[7-10] Although, there are therapies that have been shown to alter successfully cardiovascular prognosis, there is little available that modifies the long-term prognosis of pulmonary disease.[11,12] Since there is an association between pulmonary disease and cardiovascular disease, it encourages one to determine if drugs effective in altering cardiovascular diseases may also modify pathological processes within the pulmonary system and thereby improve the prognosis of patients with pulmonary disease.[13] The purpose of this investigation was to evaluate the effects of an angiotensin II type 1 receptor blocker (ARBs), a class of drugs shown to improve cardiovascular outcomes, in attenuating pulmonary injury responses. Specifically, we evaluated the effects of valsartan on systemic, cellular, and fibrotic consequences of pulmonary injury induced by the anti-neoplastic antibiotic, bleomycin which is a well described model.[14-17] The results indicate that valsartan improves the systemic and local effects of bleomycin induced lung injury. Since valsartan is an

effective cardioprotective drug, the results suggest that valsasrtan may be considered to have dual, cardiopulmonary protective properties.

Methods

Reagents. Transforming growth factor- beta1 (TGF-ß1) ELISA kits were purchased from R&D (Minneapolis, MN). Hydrocortisone, retinol acetate, agarose, insulin-transferrin-selenium X, and fetuin were purchased from Sigma (St. Louis, MO). Dulbecco's Modified Eagle Medium (DMEM) was purchased from GIBCO BRL (Burlington, ON, Canada). Bleomycin (Blenoxane) was purchased from Bristol-Myers Squibb (Evansville, IN). Anti- TGF-ß1 antibody was obtained from Genzyme (Cambridge, MA).

Rats. Female Sprague-Dawley rats, which were free of respiratory disease and weighed 200-250 g, were obtained from The University of British Columbia Vivarium. The Canadian Council of Animal Care approved all procedures on the rats and the numbers of rats used were restricted to the minimal number possible to address adequately the most relevant issues related to this work.

Bleomycin and Valsartan Administration. Rats were anesthetized using 0.4 ml ketamine (Biomedia-MTC, Cambridge, ON, Canada) and 0.2 ml rompun (Bayer, Etobicoke, ON, Canada), which were administered intra-peritoneally. With blunt dissection, the trachea was exposed and, using an 18 gauge needle, the rats were given either 200ml sterile normal saline or 1 unit bleomycin in 200 ml normal saline. A cohort of rats received valsartan (0.66mg/200ml saline, orally) two days before intra-tracheal bleomycin, while another cohort received valsartan on the day of intra-tracheal bleomycin. Once the oral valsartan was commenced, it was given daily at the same dose until the day of sacrifice. Rats were killed seven days after bleomycin administration. This time for sacrifice was chosen to harvest alveolar macrophages, on the basis of our findings that alveolar macrophages are maximally stimulated seven days after intra-tracheal bleomycin to secrete active TGF-ß1.[15,17] Weights and appearances of rats were recorded daily after receiving the various treatments.

Macrophage Cultures. After sacrifice, the trachea, thoracic and abdominal cavities were exposed using blunt dissection. The inferior vena cava and abdominal aorta were severed. To remove peripheral blood leukocytes from the pulmonary circulation, 10 ml normal saline were injected slowly into the right ventricle until the lungs turned white. The trachea, lungs, and heart were removed. To obtain alveolar inflammatory cells, the lungs were lavaged with 50-60 ml warm normal saline through the trachea. Two hours later the culture plates were washed to remove non-adherent cells, a method previously described to leave behind a pure culture of macrophages that remain adherent to the culture plates[14,15,17] Alveolar macrophages were maintained in serum-free media containing gentamicin (4 mg/ 100 ml; Roussel, Montreal, PQ, Canada), fungizone (100 µ1/ 100 ml; GIBCO BRL, London, ON, Canada), and 0.2% bovine serum albumin (National Biological Laboratory Limited, Dugald, MB, Canada). After 20 h of incubation at 37°C, 5% CO_2, the media were collected in the presence of protease inhibitors [leupeptin, 0.5 µg/ml (Amersham, Buckinghamshire, UK) and aprotinin and pepstatin, 1 µg/ml each, (both from Sigma, Oakville, ON, Canada)] and frozen at 80°C until ready for TGF-ß1 quantitation.[14-17].

TGF-ß1 assay by ELISA. TGF-ß1 is secreted in a biologically latent form because of a non-covalent association of latency associated peptide-1 (LAP-1) with the NH2-terminal 25-kDa portion of the protein. In this form, TGF-ß1 is called latent TGF-ß1 (LTGF-ß1). But, to convert LTGF-ß1 to a biologically active TGF-ß1, the LAP-1 must be removed.[14-18] This can be achieved by acidification of the culture media. The current ELISA detects TGF-ß1 only in its active conformation. Each sample was divided into two equal aliquots in which one aliquot had a neutral pH containing biologically active TGF-ß1. To detect total TGF-ß1 in each sample, the other aliquot was acidified by 1 N HCl for 10 min then neutralized with 1.2 N NaOH/0.5 M HEPES, and used in the assay. The DuoSet TGF-ß1 ELISA kit (R&D Systems, Minneapolis. MN) was used to determine TGF-ß1 in neutral culture media (representing active TGF-ß1) or culture media that were acidified and subsequently neutralized (representing total TGF-ß1) according to the manufacturer's instructions.[19-21] Anti-TGF-ß1 capture antibody was coated onto a 96-well microplate (Costar) overnight at room temperature. A working concentration of 2 µg/ml of the antibody was prepared and 100 µl of the preparation was added to each well. After being washed with wash buffer and blocked with block buffer, 100 µl of sample or the standard (rh-TGF-ß1), were added and incubated for two hours at room temperature. The plate was washed before adding 100 µl detection antibody, a biotinylated chicken anti-human TGF-ß1. The TGF-ß1 binding was colored by streptavidin-horseradish peroxidase

and the optical density was read with correction at 540 nm using a microplate reader at 450 nm.[19-21]

Western analysis to detect and quantitate connective tissue proteins, and connective tissue growth factor. The lung tissues were frozen on dry ice with ethanol and stored at -80°C until protein extraction. Lung explant protein extraction was performed as described previously.[20] The frozen lungs were pulverized in a mortar and placed in tissue lysis buffer containing 1 mM phenylmethylsulfonyl fluoride (Sigma) and the samples were further homogenized in the presence of 0.5% Triton X-100 and then centrifuged at 15,800 g for 10 min at 4°C. The supernatants were collected, and protein concentrations were determined by a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). The protein samples (25 µl) and protein molecular weight markers (Amersham) run in parallel were electrophoresed on 10% SDS-PAGE in a Mini-Protean II Electrophoresis Cell (Bio-Rad). Equal loading of protein was evaluated using silver staining (not shown). Additional methods to silver staining were done to validate equal loading of protein on SDS-PAGE gels using Ponceau S staining solution (Sigma) or Coomassie brilliant blue (Sigma) staining. The separated proteins were transferred at 50 V overnight onto nitrocellulose membrane (GIBCO BRL) in a Mini Trans-Blot chamber with transfer buffer (25 mM Tris · HCl, 192 mM glycine, and 20% methanol) and the nitrocellulose membrane was blocked for one hour using 5% instant skim milk in Tris-buffered saline (TBS). For detection of procollagen I and III (Rockland, Gilbertsville, PA), a 1:3,000 dilution of antibody was used; for collagen IV (Rockland), a dilution of 1:2,500 antibody was used; for collagen V (Cedarlane, Hornby, ON, Canada), a dilution of 1:3,000 antibody was used. A 1:1,000 dilution was used for fibronectin, and 1:500 dilution for connective tissue growth factor (CTGF). After washing, the nitrocellulose membrane was incubated with horseradish peroxidase linked with the secondary antibody (anti-rabbit or anti-goat immunoglobulin G; Bio-Rad), as recommended by the manufacturer. Finally, the washed blots were exposed to an enhanced chemiluminescence (ECL) detection system (Amersham) and recorded on an autoradiograph (Kodak X-Omat film). Before being re-probed, the nitrocellulose membranes were incubated at 50°C for 30 min with a stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris · HCl, pH 6.7). The blots were rinsed twice with TBS and to ensure the removal of antibodies, membranes were incubated with the ECL detection reagents and exposed to film (Kodak). No band was detected, confirming that all antibodies were stripped off the membrane. The same nitrocellulose membrane was blocked using 5% instant skim milk in TBS for detection of the other proteins. Relative absorbance was determined using the Quantity I imaging system (Bio-Rad) (21).

Explant Experiments of Cultured Lung Slices. Rats were anesthetized as described earlier,[20] the peripheral blood was removed as described above and the lungs were then infused with 5 ml of 40°C 0.4% agarose DMEM [2x solution of serum-free DMEM and 0.8% agarose solution (GIBCO) at 1:1 concentration at 40°C, supplemented with hydrocortisone (0.2 µg/ml), retinol acetate (0.2 µg/ml), and 2% insulin-transferrin-selenium X]. The trachea was closed by tying a thread, and the lungs were placed into six-well plates. The plates were left on ice overnight to further solidify the lungs. The lungs were separated from the heart and gently sliced manually from each lobe with a sterilized scalpel. Each pair of lungs yielded 40 slices that were 1-2 mm in thickness. Sterile gelform was made by adding 1.5 ml warm 0.4% agarose DMEM into six-well plates. After agarose DMEM was solidified, a sterilized scalpel was used to remove gelform from the well. Six lung slices were placed on the top of each gelform, and 1.5 ml of serum-free DMEM supplemented with hydrocortisone (0.1 µg/ml), retinol acetate (0.1 µg/ml), and 1% insulin-transferrin-selenium X were added on the bottom of the gelform. Six-well culture plates were incubated at 37°C, 5% CO_2. The slices were cultured with media, or bleomycin (1 unit per well), or bleomycin plus valsartan (0.66mg/ml). Lung slices were turned every other day and were collected on day 7 after culture. The overlying CM was removed, stored in sterile, siliconized Eppendorf tubes in the presence of the protease inhibitors [including 5 µg/ml of leupeptin (Boehringer Mannheim), 5 µg/ml of aprotinin (Sigma), 5 µg/ml of pepstatin A (Sigma), and 1 mM PMSF (Sigma)] and frozen at 86°C until ready for use.

Statistical Analyses

The Levene procedure tested that homogeneity of variance existed across multiple samples. The One-Sample Kolmogorov-Smirnov procedure tested that a sample came from a normal distribution. If assumptions were not greatly violated (population normality, homogeneity of variance and equal sample sizes), then one-way analysis of variance (ANOVA) was used to compare samples, followed by the t statistic when

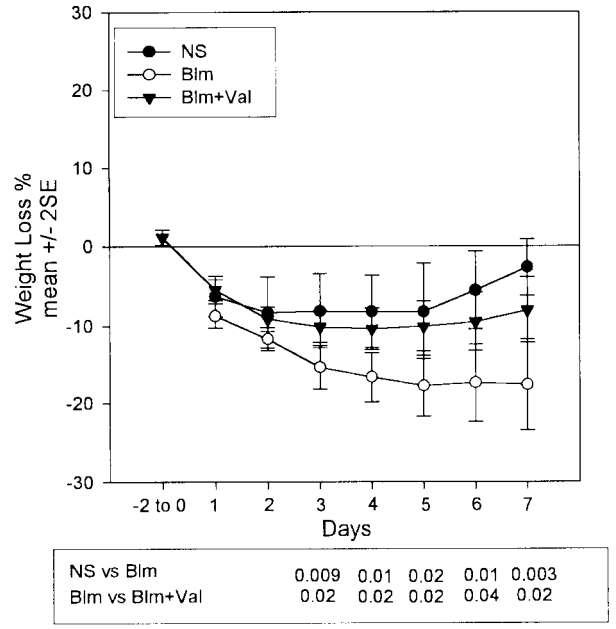

FIGURE 1 Change in weight is plotted on the y axis versus time after intra-tracheal instillation of agents on the x axis. Bleomycin induced a significant and progressive systemic effect as indicated by weight loss. This effect of intra-tracheal bleomycin was almost completely prevented in the valsartan-treated animals. NS = normal saline, Blm = bleomycin, Val = valsartan, numbers in box indicate P values.

appropriate. When distribution of data greatly violated the assumptions of ANOVA, the non-parametric equivalent, the Krukal-Wallis H test, was performed, followed by the Mann-Whitney U test when appropriate. Significance was defined at $\alpha = 0.05$. Statistical analysis and plotting were performed with SPSS (version 11.0.1) and SigmaPlot 2001 (version 7.101), respectively (SPSS, Inc., Chicago, IL).

Results

In-vivo Protocol - In all cases, there were no differences between the results obtained when valsartan was given as pretreatment for 2 days before or at the same time as the bleomycin administration. Accordingly, the data from the two valsartan-treated cohorts were combined. Figure 1 shows the changes in weight. Bleomycin induced a progressive weight loss and this was almost completely eliminated in the valsartan-treated animals. Figure 2 shows the results of the

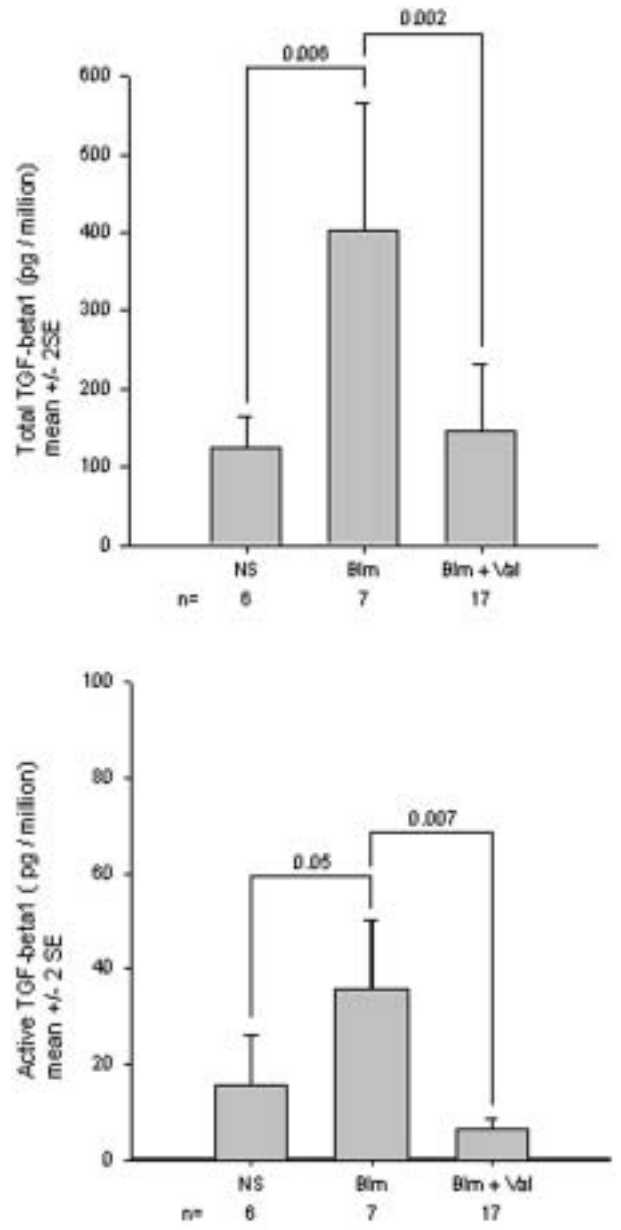

FIGURE 2 Total (upper panel) and active TGF-ß1 (lower panel) levels are demonstrated for the in-vivo series of experiments. The significant increase in both total and active TGF-ß1 induced by bleomycin is abrogated in the valsartan-treated animals. Abbreviations as for Figure 1.

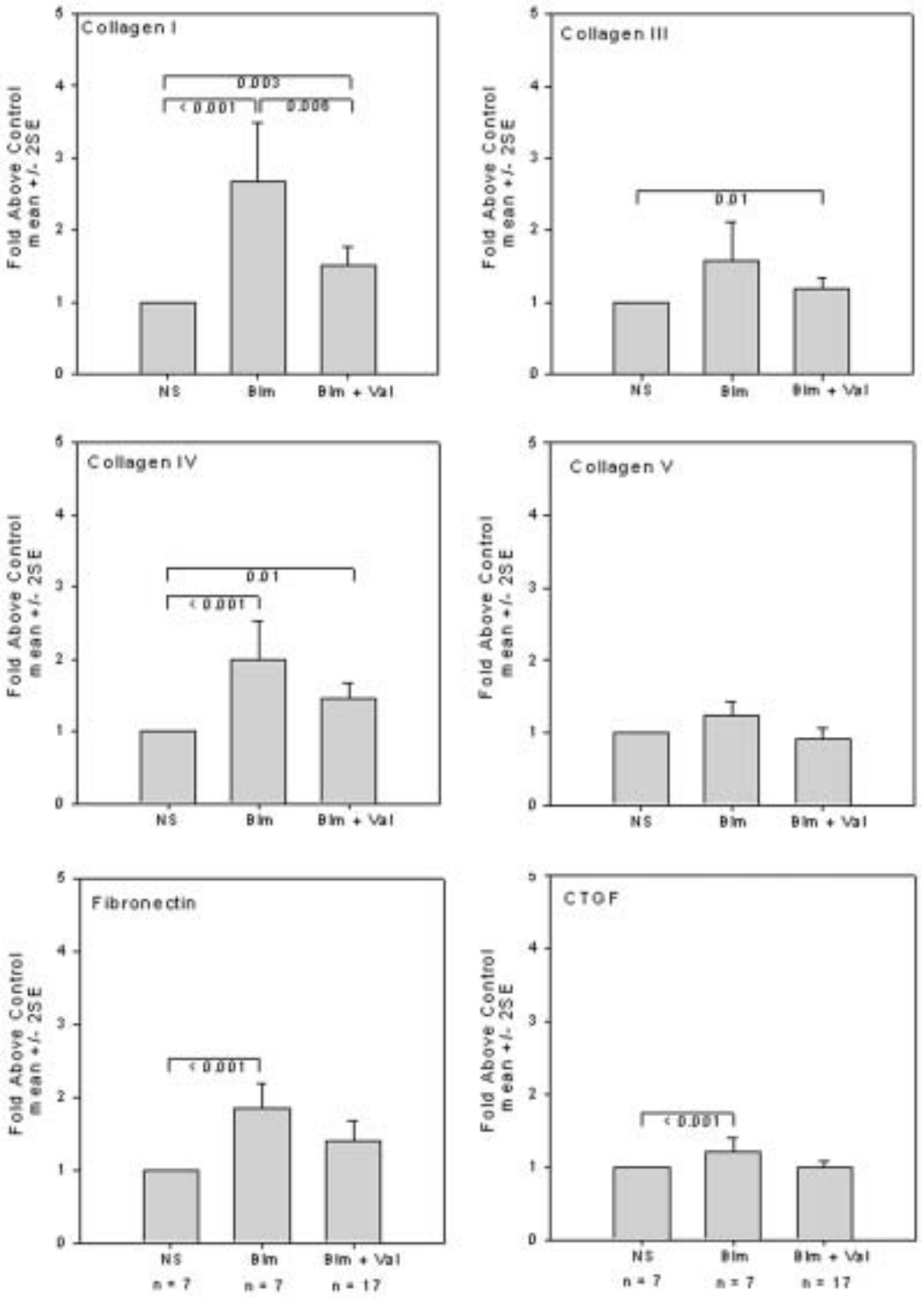

FIGURE 3 Changes in collagen I, III, IV, V, fibronectin and connective tissue growth factor (CTGF) are shown for the in-vivo series of experiments. The general pattern of augmentation induced by bleomycin and abbrogation induced by valsartan is seen in each panel but most dramatically in the upper left panel (Collagen I). Abbreviations as for Figure 1.

assays for total and active TGF-ß1 levels in CM overlying alveolar macrophages obtained from rats seven days after bleomycin administration. The increase in both total and active TGF-ß1 induced by bleomycin was abrogated in the valsartan-treated animals. The fibrotic responses are shown in Figure 3 for changes in collagen I, III, IV, V, and fibronectin. These were diminished with treatment by valsartan. The regulation of connective tissue by TGF-ß1 is mediated by connective tissue growth factor (CTGF),[20] which was also elevated after bleomycin administration and reduced in the valsartan treated rats. The most dramatic results were seen in the analysis of collagen I. The connective tissue expression after in-vivo bleomycin administration reflects the combined responses of epithelial cell injury induced by bleomycin and pulmonary alveolar macrophage activation to release TGF-ß1.[14-17]

Explanted Lung Tissue Cultures - Figure 4 shows the results of total and active TGF-ß1 levels released into the CM by the lung tissue explants that were free of resident and recruited alveolar and interstitial inflammatory cells but were cultured with bleomycin. Valsartan treatment led to a decrease in both total and active forms of TGF-ß1 compared with control and with bleomycin-induced concentrations. This is similar to the results noted above but indicate more specifically the response of the pulmonary epithelial cells to bleomycin injury.[15,16]

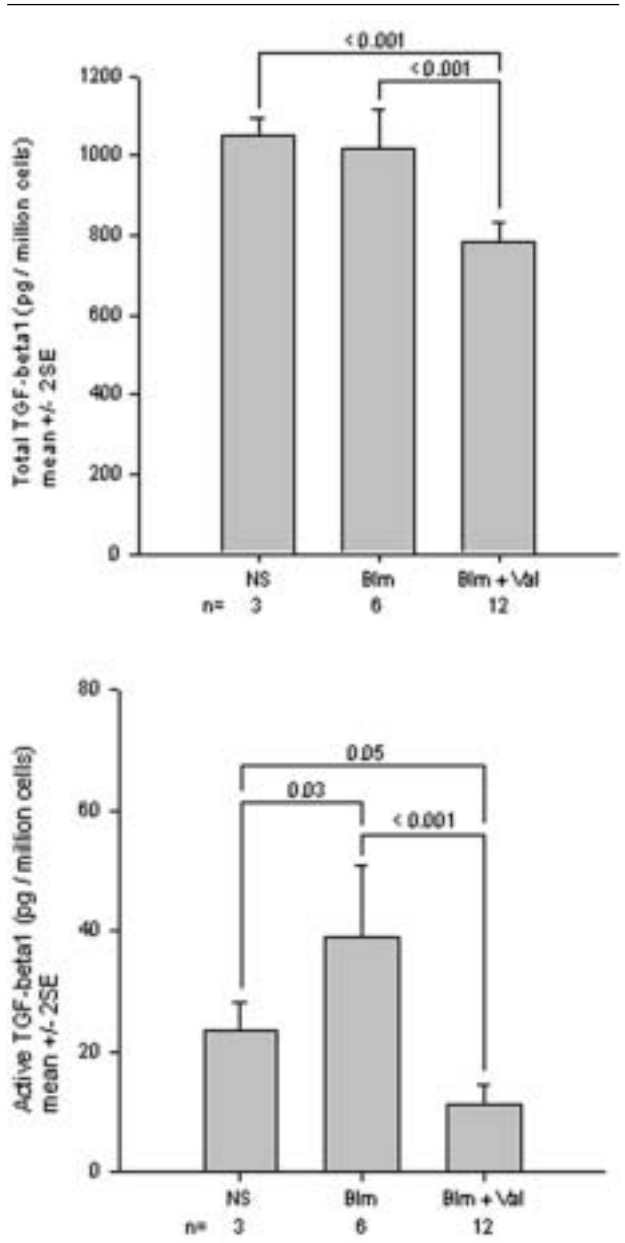

FIGURE 4 Total (upper panel) and active TGF-ß1 (lower panel) levels are demonstrated for the lung tissue explant experiments. Valsartan treatment led to a significant decrease in both total and active forms of TGF-ß1 compared to control and compared to bleomycin-induced levels. Abbreviations as for Figure 1.

Discussion

This study shows that valsartan abrogates a number processes involved in bleomycin-induced pulmonary injury. Valsartan inhibits the systemic effects of in-vivo bleomycin lung injury as shown by amelioration of weight loss seen after bleomycin administration. The in-vivo lung injury by bleomycin is mediated by epithelial cell damage in the presence of activated alveolar macrophages and other inflammatory cells.[14-17] We had previously shown that the release of active TGF-ß1 from alveolar macrophages was critical to the fibrotic response to bleomycin administration.[14,15,22] Alveolar macrophages from bleomycin and valsartan treated rats release less active and total TGF-ß1, which results in less connective tissue synthesis. In the explant model, bleomycin exclusively injures alveolar epithelial and capillary endothelial cells.[14,17] However, the endothelial injury by bleomycin results in the release of the biologically inactive form of TGF-ß1[23] while epithelial injury by bleomycin leads to the release of active TGF-ß1 as observed in this study and as previously shown by us.[16]

Thus, our series of experiments indicate that the pulmonary-protective properties of valsartan affects two key cell types involved in the pathogenesis of pulmonary damage by bleomycin, the pulmonary epithelial cells and activated macrophages. These observations, coupled with the fact that angiotensin receptor blockers have been shown to reduce cardiovascular events, suggest that this class has a unique, potential role as a dual, cardiopulmonary-protective class. For this reason we have coined the term "double dip hypothesis" to describe the conjecture that ARB's could modify both pulmonary morbidity and mortality as well as the cardiovascular morbidity and mortality that is associated with diseases of the lungs.[13]

These observations are concordant with earlier work indicating that angiotensin II is a mediator of lung injury. The angiotensin II type 1 receptor mediates apoptosis of the human lung epithelial cell in response to angiotensin II;[24] bleomycin induced apoptosis of lung epithelial cells requires de novo and in situ synthesis of angiotensin II;[25] and angiotensin II is mitogenic for human lung fibroblasts.[26] Additionally, earlier literature indicates that angiotensin converting enzyme inhibitors as well as ARBs can inhibit pulmonary endothelial dysfunction, epithelial cell toxicity and collagen deposition in response to diverse pulmonary insults in animal models.[27-33] The beneficial effects of angiotensin converting enzyme inhibitors in these models of lung injury appear to derive from the effect of the drugs in preventing elaboration of angiotensin II.[32] During creation of this report, Otsuka and coworkers published similar salutary effects of another ARB, candesartan, for the abrogation of bleomycin induced lung injury.[34] Our results and those of Otsuka et al indicate that the pulmonary protective effects may apply to many agents in this class.

The fibrotic response seen with angiotensin II occurs through induction of the potent fibrogenic cytokine, TGF-ß1 mRNA and protein.[18] When induced, TGF-ß1 is released in association with a precursor protein called the latency associated peptide-1 (LAP-1). The association of TGF-ß1 with the LAP-1 (L-TGF-ß1), causes the TGF-ß1 to be biologically inactive.[18] TGF-ß1 is acitved after removal of the LAP-1 by the actions of plasmin, a serine protease or after a conformational change induced by an interaction between LAP-1 and the integrin, αvß6.[18] Angiotensin II has been described to increase the conversion of L-TGF-ß1 to its active conformation.[35] In bleomycin induced injury the alveolar epithelial cells have an induction of the integrin, αvß6.[18,36,37] αvß6 has previously been demonstrated to convert the latent form of TGF-ß1 to active TGF-ß1 after bleomycin injury.[37] Thus, it is possible that the angiotensin released by bleomycin lung injury induces L-TGF-ß1, and this is then activated by αvb6 which is also induced by bleomycin injury. In several models, plasmin is critical to the release of active TGF-ß1.[17] Angiotensin II induces urokinase plasminogen activator[38], an enzyme that effectively converts plasminogen to plasmin. Therefore, in the presence of angiotensin II, there would not only be an induction of L-TGF-ß1 but the plasmin released by pulmonary cells might also rise and convert L-TGF-ß1 to active TGF-ß1.

In order to avoid excessive mortality, we used pre- and co-treatment in the context of acute lung injury and did not investigate the role of valsartan in a chronic animal model of lung fibrosis. This study is limited further by the fact that the effects of valsartan were evaluated in only one animal model and that bleomycin was chosen as the form of injury. But the effects of diverse, pulmonary insults, including tobacco smoke and ultra-fine particulate air pollutants, invariably involve the pulmonary epithelial cells and activation of alveolar macrophages and so the bleomycin-induced injury remains relevant for the demonstration of a potential protective effect of valsartan on pulmonary tissue that may well be more broadly applicable than merely in the context of bleomycin-induced injury. Indeed, previous work has demonstrated the induction of angiotensin or angiotensin converting enzyme in other forms of lung injury such as radiation or amiodarone injury.[24-33]

We conjecture that the possibility that ARBs have a dual, cardiopulmonary effect may have profound clinical importance. It is predicted that by 2020, COPD will account for the fifth leading cause of disease burden worldwide.[39] Current management of COPD is largely based on symptom relief with use of bronchodilators, steroids, and judicious use of antibiotics.[12] The only intervention shown to affect the prognosis of the disease is oxygen therapy but only in a subset of COPD patients with hypoxemia.[11] Furthermore, patients with COPD also experience cardiovascular events. Accordingly, if ARBs have dual, cardiopulmonary protective properties, they may be effective as preventive therapy for patients with COPD. This "dual dip hypothesis" warrants clinical testing.[13]

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Address correspondence to:
G. B. John Mancini, MD
Vancouver Hospital
3300-950 West 10th Avenue
Vancouver, British Columbia CANADA
V5Z 4E3
E: mancini@interchange.ubc.ca

ORIGINAL RESEARCH

Use of anti-thyroid drugs in euthyroid pregnant women with previous Graves' disease

Tammy McNAB, MD, FRCPC*
Jody GINSBERG, MD, FRCPC

Department of Medicine, Division of Endocrinology, University of Alberta, Edmonton, AB, CANADA
*Present address: Division of Endocrinology, Department of Medicine, University of Saskatchewan, Saskatoon, SK, CANADA

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Abstract

Purpose: Euthyroid pregnant women with a previous history of Graves' disease treated with radioiodine or surgery may have persistently elevated TSH receptor antibody (TRAb) levels, putting their offspring at risk for fetal hyperthyroidism (FH) and/or neonatal hyperthyroidism (NH).
Methods: We performed a literature review using a MEDLINE search to determine if and how anti-thyroid drugs (ATD) were utilized in euthyroid pregnant women with previous Graves' disease to prevent FH/NH.
Results: There are 11 published reports involving 13 pregnancies where ATDs were utilized to prevent FH in euthyroid mothers with a previous history of Graves' disease. Subjects were treated if high titres of TRAb (>5-fold above normal) were noted on either radioreceptor assay or various bioassays. Such intervention appeared beneficial. Thirteen live births were observed when previously these mothers collectively experienced six miscarriages, stillborn or infant deaths attributed to FH or NH. Developmental consequences such as craniosynostosis or dysmorphic features were not observed in the infants described. Both propylthiouracil and methimazole were used effectively. When utilized, cordocentesis (or periumbilical blood sampling) to determine fetal thyroid status and TRAb levels proved to be of value in establishing the diagnosis and guiding therapy.
Conclusion: Maternal ATD prevent the serious consequences of FH/NH and should be considered for euthyroid Graves' mothers with high TRAb titres.

Neonatal Graves' disease is a complication of 1% of pregnancies in Graves' mothers.[1] This complication occurs as a consequence of transplacental passage of TSH-receptor antibodies (TRAb).[2-5] Neonatal hyperthyroidism (NH) is associated with increased mortality, craniosynostosis, cognitive impairment and growth retardation.[6,7] As a consequence of fetal hyperthyroidism (FH), miscarriage, hyperkinesia, fetal tachycardia, intrauterine growth retardation, cardiomegaly with heart failure, non-immune hydrops foetalis, advanced bone maturation and pre-term birth have been observed.[7,8]. The rarity of FH and NH is partly due to the spontaneous decline in TRAb titers during pregnancy 3 as a consequence of alterations in the maternal immune system during pregnancy.[9] In women with active hyperthyroidism during pregnancy, the use of anti-thyroid drugs (ATD) will have the additional benefit of protecting the fetus from the effects of TRAb. However, in Graves' mothers rendered euthyroid or hypothyroid by prior treatment with radioiodine or surgery, the persistence of TRAb puts the fetus at risk. In such patients, the European Thyroid Association (ETA) recommends monitoring of TRAb levels in the third trimester of pregnancy if significantly elevated initially.[10] However, there are no

formal recommendations about ATD use in these patients. We performed a literature review to determine the benefits of ATD for euthyroid mothers with significantly elevated TRAb levels in preventing FH.

Methods

A MEDLINE search was employed using the following terms: fetal hyperthyroidism, neonatal hyperthyroidism, Graves' disease, pregnancy, hyperthyroidism in pregnancy. A case report was included if it was in English, the mother was euthyroid during pregnancy, given ATD to prevent FH or NH, and there was evidence of transplacental passage of TRAb in previous gestations.

Results

A review of the literature is outlined in the accompanying table. Our literature review reveals that in euthyroid mothers with Graves' disease who have elevated TRAb levels, there are 11 published reports where ATD have been given to prevent FH (Table).[7,11,16-24] This intervention appears to be beneficial. Thirteen live infants were born to 11 mothers treated with ATDs while collectively these mothers previously experienced 6 miscarriages, stillborn or infant deaths attributed to FH or NH. Another patient who had 7 previous pregnancies result in second-term abortions/still births due to non-immune hydrops, successfully carried her infant to term when ATD were introduced.[21] Developmental consequences such as craniosynostosis and dysmorphic features were not noted in offspring whose mothers took ATD during pregnancy, even if they experienced such before.[7,22] Of the 13 infants reported, in 4 cases where information is available, normal growth and development up to 2 years of age was observed.[7,16,19] One infant was diagnosed with spastic hemiplegia at 6 months of age.[20] The authors hypothesize that asphyxia at delivery due to thyrotoxicosis may have played a role.

Discussion

(i) Nature of TRAb Measurement

Neonatal hyperthyroidism is due to transplacental passage of TRAb and present recommendations suggest TRAb monitoring during pregnancy in euthyroid women previously treated for Graves' disease with radioiodine or surgery.[10] Recent studies have suggested TRAb >5-fold above normal are associated with NH.[11,12] The present ETA recommendation on TRAb monitoring during pregnancy indicates that either a radioreceptor assay or a bioassay may be effective.10 However, some 13 but not all authors 14 have observed the development of TSH-blocking antibodies (TsBAb) in Graves' mothers during pregnancy. Others have also noted TsBAb to be rare in Graves' disease.[15] In a report of a euthyroid Graves' mother in which TRAb was assessed by both a radioreceptor and stimulating assay, discrepancy between the TRAb measured by radioreceptor assay and in a bioassay for thyroid stimulating immunoglobulin (TSI) was noted.[16] Fetal thyroid tests confirmed hyperthyroidism and maternal PTU was commenced. Neonatal hyperthyroidism was confirmed at day 2 of life. Based on this experience, we would presently advise confirmation of significantly elevated radioreceptor TRAb levels with TSI measurement in order to avoid unnecessary ATD usage.

(ii) Choice of ATD

Among the 13 pregnancies in which ATDs were used, 9 mothers were treated with propylthiouracil (PTU) and 4 with carbimazole or methimazole (MMI). The reasons for the choice of ATD were not stated. Both ATDs appear to be equally effective in this circumstance. Although MMI is stated to cross the placenta more effectively and thus would appear advantageous[25], this concept has recently been challenged.[26] Momotani et al. have shown that fetal thyroid hormone levels are similar in mothers taking either PTU or MMI[27] implying that if a difference in placental transfer exists, it may not be clinically relevant. Although MMI has been used in pregnancy without sequelae[28]; in North America, PTU is preferred due to its ability to block T4 to T3 conversion[29] and the lack of association with MMI-induced embryopathy.[30] In the studies noted, PTU was utilized at a total daily dose ranging from 100-300 mg with the majority starting on PTU 100-150 mg daily in divided doses. For most patients, this dose was maintained throughout pregnancy.

(iii) Fetal Monitoring

The optimal clinical monitoring of ATD during pregnancy in these patients is unclear. Although it is recommended that fetal heart rate (FHR) and intrauterine growth (IUG) parameters be monitored and ATD adjusted accordingly, stillbirth secondary to FH in the absence of increased FHR or IUG retardation has been reported.[31] In one patient, adjustment of PTU based on fetal tachycardia resulted in apparent over-treatment[11] Although a recent report has out-

Table 1

Pregnant euthyroid Graves' mothers treated with ATD to prevent FH/NH

MOTHER					INFANT				
Reference	**Age (yr) Parity**	**TRAb (method; fold X)**	**ATD Total starting dose (Weeks gestation)**	**Fetal Tachycardia**	**Sex/Weight (Gestation)**	**NH**	**Goiter**	**Development (Months)**	**Comments**
17 1979	27 G2P1	LATS ↑	PTU 300 mg (26 wk)	+	? /2500 g N/A	10d	--	NA	Previous NH
18 1982	34 G2P1	TSI 4.8	PTU 150 mg (NA)	+	? /2530 g (36 wk)	4d	+	NA	Previous infant death
19 1984	28 G3P2	TBII 5.0 TSI 13.4	PTU 100 mg (26 wk)	NA	? /3400 g (41 wk)	7d	--	Normal-24 m	Previous stillborn
20 1984	18 G1P1	TBII 4.3 TSI 13.7	CMI 20 mg (33 wk)	+	? /2110 g (36 wk)	1d	+	Hemiplegia-6 m	Previous stillborn
7 1985	28 G3P1	LATS 4.0 LATSP 42.4	CMI 40 mg (25 wk)	+	? /2750 g (36 wk)	4d	--	Normal-24 m	Previous stillborn
	30 G4P2	LATS 1.3 LATSP 6.7	CMI 15 mg (23 wk)	+	? /2999 g (36 wk)	4d	--	Normal-10 m	Previous NH (poor growth, dysmorphic, CS)
11 1995	27 G1P0	TSI 8.5	PTU 100 mg (34 wk)	+	? /3210 g (37 wk)	12d	--	NA	FHR unreliable guide to ATD dosage
21 1996	32 G8P0	TBII 54	PTU 150 mg (25 wk)	+	? /2693 g (36 wk)	5d	--	NA	7 previous pregnancy losses due to hydrops
22 1997	38 G3P1	TBII 19.8	PTU 150 mg (24 wk)	--	? /3740 g (37.5 wk)	NA	--	NA	Previous miscarriage Previous NH with CS
	40 G4P2	TBII 31.3	PTU 150 MG (21 wk)	--	? /3000 g (37.5 wk)	NA	+	NA	ATD adjusted based on PUBS
23 1999	31 G2P0	TBII 5.7	MMI 15 mg (30 wk)	+	? 2220 g (35 wk)	NA	--	NA	Previous stillborn with goiter, cardiomegaly
16 1999	28 G3P1	TBII 37.4 TSI 6.3	PTU 100 mg (22 wk)	+	? /3050 g (38 wk)	2d	+	Normal-2 m	Fetal goiter resolved with increased PTU
24 2001	32 G2P1	TSI 80	PTU 300 mg (20 wk)	+	? /4270 g (39 wk)	4d	+	NA	Fetal goiter with vascular flare responded to PTU

Index: ATD=Antithyroid drugs
CMI=Carbimazole
CS=Craniosynostosis
LATS=Long-acting thyroid stimulator
NH=Neonatal hyperthyroidism
PTU=Propylthiouracil
PUBS=Periumbilical blood sampling
TBII=Thyrotropin-binding inhibiting immunoglobulin
LATSP=Long-acting thyroid stimulator protector
MMI=Methimazole
NA=Not available
TRAb=TSH receptor antibody
TSI=Thyroid-stimulating immunoglobulin

lined the normal range for amniotic fluid thyroid hormone levels during pregnancy, the authors acknowledge that such measurements have not been determined for use in hyperthyroidism.[32] Others indicate that amniotic fluid thyroid hormone levels are inadequate to determine the thyroid status of the fetus and should not be considered for this purpose.[16] Cordocentesis or periumbilical blood sampling (PUBS) is the only accurate method to determine fetal thyroid status and obtain fetal TRAb levels.[33,34] In 6 of the 13 pregnancies previously reported, it was utilized for this purpose and successfully diagnosed fetal hyperthyroidism due to Graves' disease in all.[16,21-24] In some women, subsequent fetal thyroid indices obtained via PUBS showed improvement after ATDs were commenced and used to guide therapy.22 However, the procedure is associated with potential fetal loss or spontaneous abortion in up to 5% of cases and should only be used judiciously in experienced centers.[35]

(iv) Timing of ATD Introduction

The timing of the introduction of ATD is uncertain. In the patients reported, ATDs were introduced from 20-34 wk gestation. The fetal thyroid gland is capable of iodine concentration and iodothyronine synthesis as early as 10 wk.[36] However, thyroid hormone production may be limited until 18-20 weeks.[36] Undetectable fetal TSH concentrations in the presence of a markedly elevated FT4, suggests pituitary negative feedback as early as 20 wk gestation.[16] It would appear that treatment earlier than 20 weeks gestation is not justified.

(v) Fetal Goitre

Goitre formation occurs in both hyperthyroid and hypothyroid fetuses.[37] ATDs via transplacental transfer have the potential to produce fetal goitre and/or hypothyroidism.[38] Although unlikely at total doses of PTU less than 150 mg daily, there is no obvious dose dependency.[19] However, the presence of a "vascular flare" on colour flow Doppler is an indication of ongoing FH[24,39] and ATDs should be continued and potentially increased. In the reports reviewed, when fetal goitre was observed, ATDs were maintained without sequelae[16,24] and in one patient, fetal goitre was reduced on ultrasound with increased PTU therapy.[24]

(vi) Conclusion

Our literature review indicates that TRAb levels >5-fold above normal are predictive of NH and that elevated TRAb require ongoing monitoring during pregnancy. Confirmation of elevated TRAb levels with TSI measurement is strongly advised. In this circumstance, PUBS may give accurate assessment of fetal thyroid hormone levels and should be considered but confined to experienced centres in view of the associated obstetrical risk. If FH is confirmed or suspected, then providing ATDs to the mother should be considered. Although it is prudent to monitor FHR and IUG, such parameters may be misleading. Fetal goiter may be due to active FH as well as hypothyroidism. Increased vascularity on colour flow Doppler is consistent with FH and may guide continued ATD therapy. Maternal ATDs prevent the serious consequences of FH and should be considered when appropriate.

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Address correspondence to:
Dr. Jody Ginsberg
362 HMRC
University of Alberta
Edmonton, AB CANADA T6G 2S2
E mail: jody.ginsberg@ualberta.ca

SYSTEMATIC REVIEW

S-Adenosylmethionine (SAMe) as Treatment for Depression: A Systematic Review

Anna-leila Williams PA-C MPH
Christine Girard ND
Danny Jui ND
Alyse Sabina MPH
David L. Katz MD MPH

Yale Prevention Research Center, Derby, CT 06418
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Abstract

Purpose: To assess the evidence evaluating S-adenosylmethionine (SAMe) supplementation as treatment for depression.

Methods: Searches of Medline, Psychinfo, AMED, and Cochrane Controlled Trials Register were conducted from database inception through September 2001.
Randomized controlled trials, controlled clinical trials, intervention studies, case-control studies, reviews, and case reports examining the evidence behind S-adenosylmethionine (SAMe) supplementation in depression among humans were selected. No limits were placed on study populations for demographics or co-morbidities. Only English language papers were abstracted and assessed for trial quality. Two abstractors independently evaluated each study, and then reconciled findings. As data were available, between group treatment effect size was noted or, as needed, calculated.

Results: Eleven articles met initial inclusion criteria; five intervention trials, two RCTs, two reviews, one controlled clinical trial, and one meta-analysis. Using the one common outcome measure among all the intervention studies and RCTs, the Hamilton Rating Scale for Depression, direct comparison of effect sizes was made. A favourable and significant between group effect was seen.

Conclusion: All of the studies reviewed were short term, making translation to the clinical setting difficult. However, there appears to be a role for SAMe in the treatment of major depression in adults. Questions remain about mechanism of action, bioavailability, and absorption of oral SAMe. Further study of SAMe as independent and adjuvant therapy for major depression in adults is indicated.

Conflict of Interest: None of the authors experienced conflict of interest in the development, implementation, or dissemination of this project.

Approximately 3 to 7% of the North American population will suffer from a "clinically significant" episode of major depression in any given year.[1,2] The lifetime risk for at least one major depressive episode among this population is approximately 16 to 24%.[1,3] The World Health Organisation predicts that major depression will be the second leading cause of disability worldwide by 2020, closely following ischemic heart disease.[4] Individuals with major depression, as well as those with dysthymic disorder are high users of medical services and are as functionally impaired as patients with severe chronic medical disorders.[5,6] The economic impact of depression is high, with estimates approaching $43 bn/yr in the US;[7] 30% of costs are attributed to direct medical care and 70% is due to lost productivity.[8] One recent American study noted the annual per capita health and disability costs of depression are considerably higher than the cost for hypertension and comparable to the cost for heart disease, diabetes, and back problems.[9] In Canada, the overall cost associated with general mental health problems, including depression, is estimated to be over $14 bn, accounting for one of the highest costs in Canadian healthcare. This trend is observed globally in Australia, New Zealand, and the Member States of the European Union.[10]

Conventional treatment for major depression includes the use of pharmacotherapy, psychotherapy, pharmacotherapy combined with psychotherapy, and electroconvulsive therapy.[11] Each therapeutic option has advantages and disadvantages. Pharmacotherapy is typically easy to administer and is effective for mild, moderate, and severe depression. It requires patient compliance to the medication schedule, frequent visits to adjust the dose properly, and may produce unwanted side effects including dry mouth, weight gain, and decreased libido.[11] Psychotherapy lacks the unwanted side effects commonly associated with pharmacotherapy, and can be effective for some patients for whom pharmacotherapy is not. Psychotherapy may address underlying emotional issues and teach skills to help patients cope and/or avoid factors that contribute to the recurrence of major depressive disorder.[12] Psychotherapy alone, however, is not effective for patients with severe depression.[13] The combination of pharmacotherapy and psychotherapy is commonly used and perceived to have added therapeutic benefit as well as increased compliance.[11] Electroconvulsive therapy is appropriate for patients with severe or psychotic major depressive disorder who have failed more than one trial of pharmacotherapy.[11]

Complementary and alternative medicine (CAM) spans a wide spectrum of interventions considered outside the purview of conventional clinical care, including such diverse therapeutic modalities as nutriceuticals, meditation, massage, homeopathy, ayurveda, acupuncture, reiki, and chiropractic.[14] CAM has a high prevalence of use among patients with psychiatric disorder,[15-17] with depression being one of the most common reasons reported for using CAM.[17] CAM treatment of depression encompasses an expansive array of modalities with varying scientific validation of efficacy.[17] Nutriceuticals such as s-adenosylmethionine (SAMe), St. John's wort, fish oil, and folate, have all received a great deal of scientific attention for their potential efficacy in treating depression.[18]

Reported here is the result of a systematic review examining the therapeutic efficacy of s-adenosylmethionine (SAMe) as treatment for depression that was included in a more expansive examination of the evidence base for complementary and alternative medicine (CAM) practices in general.

Methods

With funding from the Centers for Disease Control and Prevention, the Yale Prevention Research Center undertook a "systematic review" of the evidence underlying CAM. Collaborating with a multi-disciplinary group of CAM practitioners and NIH-funded CAM researchers, the investigative team designed a systematic and replicable 9-step process termed evidence mapping.[19] A previous publication[19] describes the strict criteria used to pair more than 200 medical conditions with CAM interventions. When evidence existed, the pairs were then prioritised by public health importance for systematic review; when evidence did not exist, pairs were prioritised for pilot study development.

Electronic searches were conducted from December 2000 to September 2001 using Medline, Psychinfo, AMED (Allied and Complementary Medicine), and Cochrane Controlled Trials Register. Searches were limited to those engines with the widest accessibility and use among diverse practitioners to assure literature captures would be pertinent to the broadest possible readership. The databases were investigated from the earliest date available to the time of the search (Medline 1966; Psychinfo 1987; AMED 1983; Cochrane Controlled Trials Register 1995) and included articles in all languages. Each condition-intervention pair was searched with the following study design terms: randomized controlled trial (RCT), meta-analysis, review article, case-control study, controlled clinical trial (CCT), intervention study, case report, and pilot study.

Inclusion and Exclusion Criteria

All RCTs, CCTs, intervention studies, case-control studies, reviews, case reports and pilot projects that examined the evidence behind SAMe in depression among human subjects were selected. There were no limits placed on study populations for demographics or co-morbidities. Although papers in all languages were culled, only English language papers were abstracted and assessed for trial quality.

Abstraction Process

The investigative team, adapting methods applied by the Centers for Disease Control and Prevention (CDC) to the Guide to Community Preventive Services, and incorporating criteria from The Cochrane Reviewers' Handbook 4.0,20 developed an on-line data abstraction form. The Principal Investigator trained two masters-level research associates to abstract the accepted articles using a standardized process. The abstractors evaluated each study independently, and then reconciled their findings. Using explicit criteria to identify threats to internal and external validity as a means of

Table 1. Summary of SAMe and Depression Literature

Author/Year	Population (n)	Study Type	Outcome Measure	Duration of Study	Dose of SAMe	Effect Size	Quality Assessment
Rosenbaum JF 1990	Adults with major depressive illness (23)	Intervention study (open label)	Hamiliton Rating Scale for Depression	6 weeks	Increased incrementally from 400mg/d day 1 to 1600mg/dday 19-42 (po)	12.5 point decline (favourable)	Accept
Fava M 1990	Adults with major depression (17)	Intervention study (open label)	Hamiliton Rating Scale for Depression	6 weeks	Increased incrementally from 400mg/d day 1 to 1600mg/d day 19-42 (po)	9.4 point decline (favourable)	Accept
Rosenbaum JF 1988	Adults with major depression (10)	Intervention study (open label)	Hamiliton Rating Scale for Depression	6 weeks	Increased incrementally from 400mg/d day 1 to 1600mg/dday 19-42 (po)	13.6 point decline (favourable)	Accept
Fava M 1995	Adults with unipolar depressive disorder	Intervention study (open label)	Hamiliton Rating Scale for Depression	15 days	400mg/d (im)	6.6 point decline (favourable)	Accept
Carney MW 1986	Adults with major depression (51)	Intervention study (open label)	Hamiliton Rating Scale for Depression	14 days	200mg/d (iv)	6-10 point decline (favourable)	Accept
Janicak PG 1989	Adults with major depression (15)	RCT	Hamiliton Rating Scale for Depression	14 days	400mg/d (iv)	Favourable	Accept
Berlanga C 1992	Adults with major depression (40)	RCT	Hamiliton Rating Scale for Depression	8 weeks	200mg/d (im)	Favourable	Accept
Agnoli A 1978	Adults with major depression (30)	Controlled clinical trial	Hamiliton Rating Scale for Depression	22 days	45mg/d (im)	N/A	Reject*
Bottiglieri T 1994	N/A	Review	N/A	N/A	N/A	N/A	N/A
Baldessarini RJ 1987	N/A	Review	N/A	N/A	N/A	N/A	N/A
Bressa GM 1994	N/A	Meta-analysis	N/A	N/A	N/A	Favourable	N/A

N/A = non-applicable.
* Paper was rejected because there was no sampling frame for the study population, the study population was inadequately described, and there was no sample size or power calculation provided.

quality assessment, studies were "accepted," "rejected," or "audited". "Rejected" articles contained at least one major flaw in reporting that compromised validity. These included: failure to report on comparability of the units of analysis prior to exposure to the intervention, neglecting to specify the sampling frame for the study population, omitting a description of the intervention, failing to correct for controllable variables, or neglecting to institute procedures to limit bias. Articles were "audited" when minor flaws in reporting were identified, such as omission of study power analysis, description of the study population, or screening crite-

ria for study eligibility; or deficiencies such as not conducting statistical testing, not reporting which statistical tests were used, or not conducting intention to treat analysis. The project coordinator and members of the investigative team reviewed the audited papers and determined accept or reject status by consensus. "Accepted" papers were those that did not have any flaws identified during abstraction, or were judged acceptable after audit.

Determination of effect sizes

Studies that involved an intervention and that ultimately met criteria for "accept" were further analyzed to facilitate comparison. Whenever possible, plots were generated of outcome effect based on a common measure. As data were available, a between group treatment effect size was noted or, if need be, calculated by determining the difference between groups of the change from baseline to final outcome. Whenever possible, point estimates were surrounded by 95% confidence intervals but when sufficient data to generate confidence bounds were lacking in the original papers, a point estimate alone was plotted. In such cases where confidence intervals were lacking, P-values reported in the papers were used to indicate on plots whether or not the results observed were statistically significant. Within-group effects were plotted separately from between-group effects.

Results

Eleven articles were found that assessed SAMe as an intervention for depression. Five of the articles are intervention trials,[21-25] two are randomized clinical trials,[26, 27] two are reviews[28, 29] one is a controlled clinical trial,[30] and one is a meta-analysis.[31] (Table 1).

Quality assessment

After the data abstraction and reconciliation phases, the one controlled clinical trial[30] was rejected because of major design flaws that compromised external validity. All of the RCTs and intervention studies had at least one audit, indicating minor flaws in study design,[21, 24-26] data analysis,[21-24, 26] and/or description of the population;[21, 24-27] they were ultimately accepted.

Of the five intervention studies, Carney et al[23] conducted an open trial (n=22) including patients with primary depressive illness as defined by Feighner criteria and a Hamilton Depression Score >20. Patients were given 200 mg of SAMe by intra-muscular injection for 14 injections. Outcome measures included the Hamilton Rating Scale and the Beck Depression Scale. Rosenbaum et al[21] included 20 subjects with major depressive disorder (DSM-III) and a Hamilton Psychiatric Rating Scale for Depression (HAM-D-21) score of >18. Ten treatment resistant subjects were compared to 10 non-treatment resistant subjects. Each group received 200 mg oral SAMe twice daily

Legend	Author/Year	N	Type	Measure	Duration	Contact Hours	Effect Size	Effect**	SD	*P*
1	Janicak et al.1989	20/15 analyzed	RCT	HDRS	14 days	N/A	10	1	For SAMe:19 For placebo: 9.5	< .02 between groups
2	Carney et al. 1986	54	Interim results of intervention	HDRS	14 days	N/A	3.4	1	NR	< .05 within group
3	Fava 1995	195/163 analyzed	Intervention	HDRS	15 days	N/A	6.6	1	.48	< . 01 within group
4	Rosenbaum et al. 1988	8	Intervention	HDRS	6 weeks	N/A	13.6	1	NR	= .0014 within group
5	Rosenbaum et al. 1990	20	Intervention	HDRS	6 weeks	N/A	12.5	1	NR	< .001
6	Fava et al. 1990	17	Intervention	HDRS	6 weeks	N/A	9.4	1	NR	< .0005 within group
7	Berlanga et al. 1992	40	RCT	HDRS	8 weeks	N/A	5	1	NR	< .05 between groups

** Effect (1= favorable, 0=none, -1 = unfavorable)

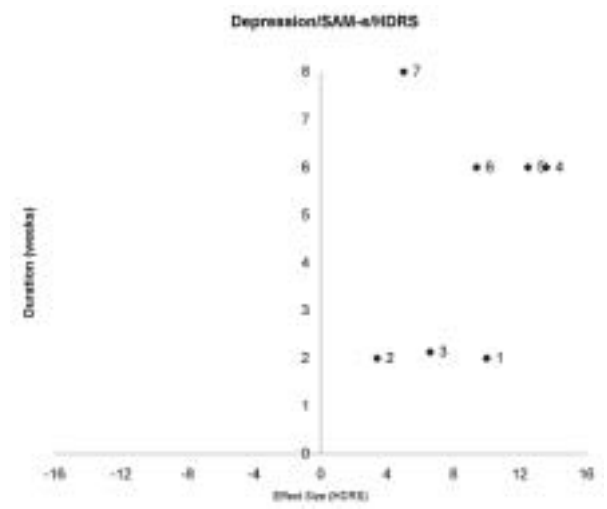

FIGURE A. Distribution of effect of SAMe for depression, with HRSD as common outcome measure.

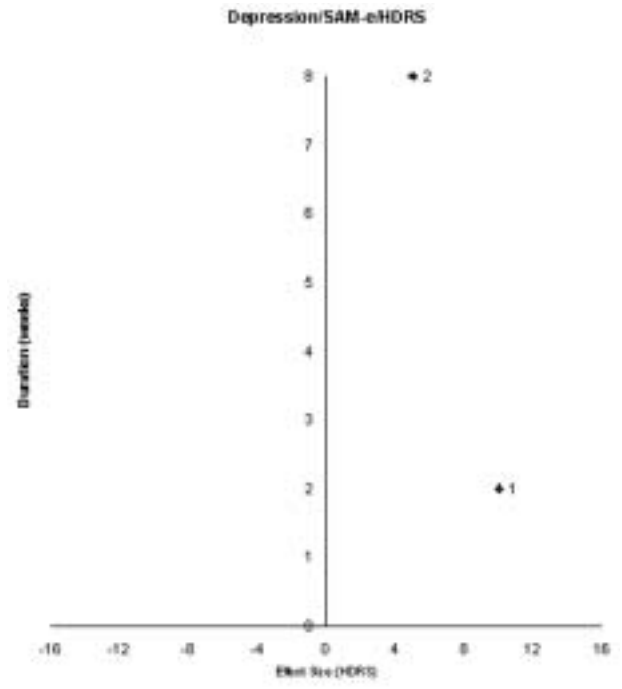

FIGURE B. Distribution of effect of SAMe for depression, between group differences with HRSD as common outcome measure.

and increased to 1600 mg by day 19. This dose was continued for the duration of the 6-week study. Outcome measures included HAM-D-21, 24-item HAM-D, Clinical Global Impressions Severity of Illness and Improvement Scale, Kupfer-Detre Scale, and the Beck Depression Inventory. Similarly, in 1990, Rosenbaum included 20 patients with major depressive disorder per DSM-III criteria and a Hamilton Psychiatric Rating Scale for Depression (HAM-D-21) score of >18. Nine of these subjects were considered treatment resistant. The other eleven subjects were considered non-treatment resistant. Each group received 200 mg oral SAMe twice daily and increased to 1600 mg by day 19. This dose was continued throughout the 6-week study. Outcomes measures used were HRSD-21, CGI-S, and the Kupfer-Detre Scale for side effects.

Fava et al[25] included 17 subjects with a diagnosis of major depressive disorder per DSM III-R criteria and a Ham-D score of >18. Patients were divided by sex into two groups. Each group received 200 mg oral SAMe twice daily and increased to 1600 mg by day 19. This dose was continued for the 6-week study. Outcome measures included prolactin and TSH levels as evaluated by TRH challenge, HAM-D-21, CGI, BDI, and Kupfer-Detre Scale. In 1995, Fava's group enrolled 195 patients with unipolar depressive disorders per DSM-III-R criteria.[22] Patients received 400 mg SAMe by intramuscular injection for 15 days. Outcome measures included HRSD, Clinical Global Impression Improvement Scale, and the Patient Global Impression Severity Scale.

Among the 2 RCTs, Janicak et al[26] included 15 subjects with major depression with or without melancholia or bipolar disorder, depressed phase (DSM-III) with an extended Hamilton Depression Rating Scale (HDRS-E) of >23. Subjects were randomly assigned to receive 400 mg IV SAMe, 150 mg IV imipramine, or IV saline as placebo. The primary outcome measure was HDRS-E. Berlanga et al[27] included 63 subjects who each met the criteria for a major depressive episode (DSM-III) and who scored >18 on the Hamilton Rating Scale for Depression. Twenty-three placebo responders were dropped from the study. The remaining 40 were randomly assigned to receive either 200 mg SAMe dissolved in 5ml sodium hypophosphate and administered via intramuscular injection or placebo injection in 5 cc sodium hypophosphate. The subjects also received concurrent administration of 150 mg

imipramine. Outcome measures included the HRSD.

There was only one common outcome measure among all the intervention studies and RCTs: The Hamilton Rating Scale for Depression (HRSD) (Table 1). This allowed direct comparison of effect sizes (Figure A). The 2 RCTs[26, 27] had comparably small sample sizes, and assessed between group differences comparing oral SAMe to placebo control. A favourable and significant between group effect was seen in both studies (Figure B); however, the effect realized in the Berlenga et al[26, 27] study should be interpreted with caution since participants were administered SAMe and imiprimine concurrently. Of the 5fiveintervention studies, 3 used oral SAMe[21, 24, 25] and 2 used parenteral SAMe.[22, 23] All of the intervention studies showed a favourable and significant effect.

Discussion

Since its discovery in 1952, SAMe has been identified as a metabolite present in all biological systems which plays a central role in cellular biochemistry as a precursor to three important pathways: methylation, transsulfuration, and aminopropylation.32 It acts as a methyl donor in cellular methylation reactions, producing nucleic acids, proteins, phospholipids, and various neurotransmitters such as dopamine, serotonin, and norepinephrine.[32,33] The intermediate product from these methylation reactions is S-adenosylhomocysteine which is quickly converted to homocysteine. With the transsulfuration pathway, glutathione, a major cellular antioxidant is then produced from homocysteine. The aminopropylation pathway involves the recycling of methionine as well as the synthesis of polyamines such as spermidine and spermine, substances with demonstrated analgesic and anti-inflammatory properties.[32]

SAMe is derived from the essential amino acid L-methionine through pathways that require adequate concentrations of folate and vitamin B12. Notably, deficiencies in both folate and vitamin B12 have also been associated with depression.[33] The mechanism of the antidepressant effect of SAMe is still being elucidated in the literature. One theory focuses on SAMe's stimulatory effect on central neurochemicals such as norepinephrine and serotonin in rats and 5-hydroxyindole acetic acid in humans, a marker for serotonergic and therefore antidepressive activity.[32] Another theory focuses on the potential role of SAMe in restoring neuroreceptor densities such as beta and muscarinic (M1) receptors via phospholipid methylation which ultimately increases membrane fluidity thereby restoring the underlying G protein-receptor coupling-uncoupling dysfunction.[32] In addition, its potential role in reversing regional brain volume loss in depressive rat models via natural polyamines such as spermidine and spermine, and their diamine precursor putrescine, has also been postulated.[34]

While all of the intervention studies included in this systematic review using the common metric, Hamilton Depression Rating Scale (HAM-D), demonstrated a favourable effect; it should be noted that the utility of the HAM-D has recently been questioned.[35] A systematic review of 70 studies using the HAM-D[35] concluded that the scale is psychometrically and conceptually flawed. Consequently, the comparative outcomes among the 11 reports reviewed here must be interpreted with caution. However, the HAM-D has been the prevailing measure for decades, and thus regardless of potential limitations, it represents the industry standard.

Since our systematic review of this topic, one more study has been published in the literature. This double-blind, randomized, multicentre study of SAMe was conducted in patients with a diagnosis of major depressive episode with a baseline score on the Hamilton Psychiatric Rating Scale for Depression (HAM-D-21) of >18. In one centre (MC3), 1600 mg SAMe/day po was given to 143 patients for six weeks. In another centre (MC4), 400 mg SAMe/day im was given to 147 patients for four weeks. In both centres, the efficacy and safety of SAMe were compared with those of 150 mg imipramine/day po given to 138 patients for 6 weeks at MC3 and 148 patients for 4 weeks at MC4. The results of SAMe and imipramine treatment did not differ for any efficacy measure. However, fewer adverse reactions were observed in patients treated with SAMe. The authors concluded that the antidepressive efficacy of 1600 mg SAMe/day po or 400 mg SAMe/day im is comparable with that of 150 mg imipramine/day po, but SAMe is better tolerated.[36,37]

SAMe has some limitations, and its use should be considered within a clinical and economic context. For example, side effects from SAMe such as headaches, restlessness, insomnia, and diarrhoea have been reported;[18] and in some cases, the manic symptoms of bipolar patients have been exacerbated while receiving SAMe.[33] The current average cost of SAMe is about US$1 per 200mg tablet,[18] which can be prohibitively expensive. Despite these challenges, there appears to be a meaningful effect of SAMe for major depression in adults in the current literature. However, all of the

studies reviewed were short term, making translation to the clinical setting difficult. While both oral and parenteral SAMe appear to be effective in the small studies reviewed here, questions remain about the bioavailability and absorption of oral SAMe. Further study of SAMe as independent and adjuvant therapy for major depression in adults is indicated. Attention should be given to mode of administration of SAMe, dosing, sustainability of effect of SAMe, and effectiveness and special precautions in particular sub-populations such as children, pregnant women, nursing women, and people with elevated homocysteine levels.

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Address correspondence to:
Anna-leila Williams
Yale Prevention Research Center
130 Division Street
Derby, CT 06418

anna-leila.williams@yalegriffinprc.org