and carbon monoxide inhaled by people smoking low tar cigarettes can be higher than the yields measured by standard laboratory methods[13-15] as machines do not smoke cigarettes in the same manner as people.[13] Hence the real exposure to tar of the smokers of low tar cigarettes in this study may have been close to that of the smokers of medium tar cigarettes, which may account for the similar prevalence of symptoms between these two groups.

The decline in the yield of tar in cigarettes has contributed to the reduced risk of cancer of the lung and chronic obstructive pulmonary disease among smokers until recent years.[16] Unfortunately, we have to wait for many years before the effects on health of new low tar brands of cigarettes can be shown in epidemiological studies using these diseases as an outcome. Many important differences in physical properties of new low tar cigarettes exist compared with the older ones, and therefore the conclusion referred to above cannot be interpreted as evidence that health hazards will diminish as a function of a yield of tar below 10 mg. On the contrary, our data and some other recent epidemiological[7-9] and experimental[17] studies have not shown any significant differences in the effects on health of low tar brands compared with the medium tar brands. Although we cannot draw conclusions on the risk of cancer of the lung or obstructive pulmonary diseases on the basis of these studies, the medical evidence available does not support the hypothesis that cigarettes yielding less than 10 mg tar are safer than those yielding 10-18 mg.

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References

1 Hammond EC, Garfinkel L, Seidman H, Lew EA. Some recent findings concerning cigarette smoking. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of human cancer. Book A. Incidence of cancer in humans. Cold Spring Harbor conferences on cell proliferation.* Vol 4. New York: Cold Spring Harbor Laboratory, 1977:101-12.
2 Auerbach O, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking, 1955-1960 vs 1970-1977. *N Engl J Med* 1979; **300**:381-6.
3 Higenbottam T, Clark TJH, Shipley MJ, Rose G. Lung function and symptoms of cigarette smokers related to tar yield and number of cigarettes smoked. *Lancet* 1980;i:409-12.
4 US Department of Health and Human Services, Public Health Service. *The health consequences of smoking. The changing cigarette. A report of the surgeon general.* Washington: United States Government Printing Office, 1981.
5 Lee P, Garfinkel L. Mortality and type of cigarette smoked. *J Epidemiol Community Health* 1981;**35**:16-22.
6 Schenker M, Samet J, Speizer F. Effect of cigarette tar content and smoking habits on respiratory symptoms in women. *Am Rev Respir Dis* 1982;**125**: 684-90.
7 Petitti D, Friedman G, Kahn W. Peptic ulcer disease and the tar and nicotine yield of currently smoked cigarettes. *J Chronic Dis* 1982;**35**:503-7.
8 Kaufman D, Helmrich S, Rosenberg L, Miettinen O, Shapiro S. Nicotine and carbon monoxide content of cigarette smoke and the risk of myocardial infarction in young men. *N Engl J Med* 1983;**308**:409-13.
9 Sparrow D, Stefos T, Bosse R, Weiss S. The relationship of tar content to decline in pulmonary function in cigarette smokers. *Am Rev Respir Dis* 1983; **127**:56-8.
10 Rimpelä A. Occurrence of respiratory diseases and symptoms among Finnish youth. A follow-up survey. *Acta Paediatr Scand* 1982;**297**(suppl):1-77.
11 Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of the National Cancer Institute* 1959;**22**: 719-48.
12 Armitage P. *Statistical methods in medical research.* Oxford: Blackwell Scientific Publications, 1971.
13 Kozlowski L, Rickert W, Pope M, Robinson JC, Frecker R. Estimating the yield to smokers of tar, nicotine, and carbon monoxide from the "lowest yield" ventilated filter-cigarettes. *Br J Addict* 1982;**77**:159-65.
14 Benowitz N, Hall S, Herning R, Jacob P, Jones R, Osman AL. Smokers of low-yield cigarettes do not consume less nicotine. *N Engl J Med* 1983;**309**:139-42.
15 Hoffmann D, Adams J, Haley N. Reported cigarette smoke values: a closer look. *Am J Public Health* 1973;**73**:1050-3.
16 Peto R, Doll R. The control of lung cancer. *New Scientist* 1985;**105**:26-30.
17 Sorsa M, Falk K, Heinonen T, Vainio H, Norppa H, Rimpelä M. Detection of exposure to mutagenic compounds in low-tar and medium-tar cigarette smokers. *Environ Res* 1984;**33**:312-21.

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Antibody guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor receptor and blood group A antigen

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Abstract

In a patient with recurrent grade IV glioma of the brain resistant to conventional treatment an antibody guided isotopic scan showed uptake by the tumour of a monoclonal antibody (9A) that was developed against epidermal growth factor receptor but cross reacted with blood group A antigen. As a therapeutic attempt antibody labelled with 1665 MBq (45·0 mCi) iodine-131 was delivered to the tumour area by infusion into the internal carotid artery. Computed tomography showed regression of the tumour after treatment, and an appreciable and sustained clinical improvement was noted without any toxicity.

Delivery of irradiation guided by monoclonal antibody delivered by arterial infusion of the tumour area may be of clinical value in the treatment of brain gliomas resistant to conventional forms of treatment.

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Introduction

Grade IV glioma is one of the most rapidly fatal neoplasms, with a median survival of 14 weeks after biopsy.[1] Postoperative radiotherapy extends survival and improves quality of life in most cases.[2] The remission, however, is usually short lived and the long term survival rate negligible. Some evidence exists that the length of survival can be increased by giving higher doses of radiation. Salazar *et al* reported a small series of cases in which doses of 7500 cGy (7·5 rads) were given to the tumour with apparent improvement in survival.[3] High biological doses

using fast neutrons have been shown to achieve tumour sterilisation but at the expense of unacceptable brain damage.[4] It is theoretically possible that antibody guided irradiation could deliver a tumoricidal dose with a minimal dose to normal brain tissue. Antibody guided irradiation of malignant lesions has been attempted in the past with only limited success,[5 6] but we have recently reported that the regional rather than systemic infusion of radioactively labelled tumour associated monoclonal antibodies can produce encouraging results in the treatment of some malignant diseases such as ovarian cancer.[7] This is probably because regional administration can produce higher uptake by the tumour and lower uptake by normal tissue of a radiolabelled antibody than can systemic administration.

We chose antibody 9A, which was originally produced against epidermal growth factor receptor but was subsequently found to cross react with blood group A antigen. On previous immunochemical tests we had found positive staining of antibody 9A on brain gliomas, but the morphological distribution of staining was patchy, indicating a heterogeneous population of cells (unpublished data). We chose iodine-131 (^{131}I) as the cytotoxic moiety in an attempt to deliver cytotoxic amounts of radiation to all tumour cells despite the expected patchy distribution of labelled antibody in vivo.

Patient and methods

CASE REPORT

A 26 year old man of blood group O presented in August 1983 with a two week history of headache, vomiting, and diplopia. Examination showed bilateral papilloedema and a left sixth nerve palsy. The computed tomogram showed a frontal mass with peripheral enhancement with intravenous contrast, suggestive of a glioma. Craniotomy was performed, to show a tumour on the inferior surface of the frontal lobe; this was partially removed. Astrocytoma Kernahan's grade IV was diagnosed histologically.

Postoperatively he made a full recovery, and oral steroids were reduced to dexamethasone 2 mg thrice daily. He then underwent a course of postoperative radiotherapy, receiving a maximum tumour dose of 7000 cGy (7 rads) in 35 fractions over seven weeks by a three phase shrinking field technique. At the completion of treatment he suffered an epileptic fit and was noted to have a right homonymous hemianopia. Repeat computed tomography in December 1983, however, showed regression of the residual cerebral mass. He remained well and returned to work.

In February 1984 his condition deteriorated: he suffered repeated epileptic fits and headaches and became uncoordinated and drowsy. Computed tomography showed increased size of the cerebral tumour and of cerebral oedema (fig 1).

In March 1984 monoclonal antibody labelled with ^{131}I was infused intra-arterially. This was followed by a dramatic improvement in his clinical state. Ten days later he was fully alert and coordinated; headaches had resolved, and the dexamethasone dosage was halved again to 6 mg daily.

Subsequently he remained well taking prednisolone 5 mg daily.

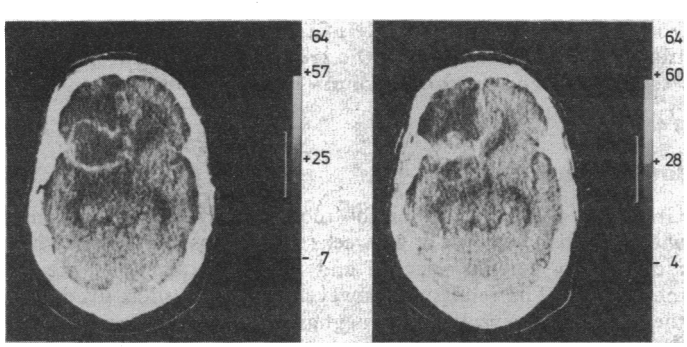

FIG 1—Computed tomograms taken at the same level (left) before antibody guided treatment, showing area of tumour and surrounding oedema, and (right) four months after antibody guided treatment, showing area of tumour. Note reduction in size of tumour but persistence of cerebral oedema.

He had a residual right homonymous hemianopia but no other neurological deficit. Repeat computed tomography showed reduction in tumour size, as judged by the region of contrast enhancement (fig 1).

METHODS

Monoclonal antibody

Antibody 9A is a mouse IgG_3. It was generated by immunising a balb/C mouse with A431 cells.[8] The A431 human epidermoid carcinoma cell line expresses high concentrations of epidermal growth factor receptor (2×10^6 receptors per cell). 9A recognises an oligosaccharide component of the epidermal growth factor receptor on A431 cells.[9] Immunochemical examination of a wide range of normal and neoplastic tissues (work in progress) using antibody 9A showed positive reactivity with a wide range of neoplastic tissues, including brain gliomas. In haemagglutination tests antibody 9A agglutinated blood group A and AB erythrocytes but not blood group B or O erythrocytes, indicating that this antibody reacts with blood group A antigen.

Radiolabelling

Iodine-123 (AERE Harwell) or iodine-131 (Amersham International IBS30) was added to immunoglobulin 5-10 mg/ml and the iodination procedure carried out in iodogen coated tubes.[10] Iodination proceeded for five minutes at room temperature, and the radiolabelled IgG was separated from free radioiodine with gel filtration on Sephadex-G50 in a 20 ml sterile syringe and phosphate buffered saline pH 7·4 as elution buffer.

Immunoreactivity of antibody

This was treated in an enzyme linked immunosorbent assay[11] with a solid phase antigen (purified epidermal growth factor receptors fixed on plastic plates with 96 wells). A comparison of antibody titres before and after radiolabelling and a direct radioimmunoassay, including competition with unlabelled antibody, were performed.

Immunoperoxidase reaction

Previously biopsied material was examined in an indirect immunoperoxidase reaction[12] against a panel of monoclonal antibodies, including 9A, as well as negative and positive controls.

Quality control

All reagents used were produced sterile, and monoclonal antibody was tested for sterility and pyrogenicity by an independent laboratory (Safepharm Laboratories).

Antibody guided studies

The patient's written informed consent was obtained. He was skin tested for allergy to mouse immunoglobulins and given potassium iodide 120 mg daily by mouth starting one day before the procedure and continuing for seven days during phase 1 and for 28 days after phase 2.

Phase 1

The purpose of phase 1 was to determine the amount of uptake of antibody in the tumour area and to perform dosimetry calculations to deduce the proportion of the administered activity going to the target and to other relevant organs. For this study antibody 9A labelled with 74 MBq (2 mCi) ^{123}I was administered intravenously.

Phase 2

This refers to the therapeutic delivery of radioactively labelled antibody by internal carotid artery infusion. Scans of head and total body were also taken after administration of labelled antibody to calculate the delivered dosage of radiation.

Dosimetry

For treatment to be effective the dose of radiation to the target should be high relative to the whole body or other sensitive organs. To calculate these doses it is necessary to know the proportion of the administered activity going to the target and to other relevant organs, the time course of the activity in the target and through the body, and the volume of distribution of the activity in the target. Volumes for the major organs of the body have been tabulated for "reference man" and may be assumed to be sufficiently accurate for most calculations of dosage.

Each of these factors can be found only approximately, and errors in the final dosimetry calculations are to be expected. Uptakes in the various parts of the body are assessed by quantitative imaging aided by blood activity disappearance curves. Conventional imaging of the body does not usually take into account the varying attenuation of the emitted radiation and the varying response of the detector over different sites, so that the picture of count rates that is obtained reflects only the distribution of activity. Scintillation cameras and rectilinear scanners can, however, be calibrated using phantoms and deriving attenuation factors to give results in millicuries. Sequential conventional imaging under the same conditions with recording of the results using a computer system allows a time activity curve to be obtained from any region of interest in the body. When the source is sufficiently well localised the disappearance of activity can be monitored with a collimated probe counter viewing the same site over several successive days.

Assessing the volume over which the activity in the tumour is distributed is difficult. Tomographic imaging is necessary for a full three dimensional description of the volume, and, although x ray computed tomography has been employed, the anatomy described may not correspond to the functioning tissues taking up the activity. Isotope tomography often has too low a resolution for the small volumes encountered. Thus often only an approximate estimate of volume based on a projected area combined with an assumed thickness is available. In the case of, say, a pleural cavity the unknown thickness may vary from millimetres to centimetres and the final dosimetry estimates will reflect this inaccuracy.

The mean dose absorbed to the target and other affected organs is obtained using the method of the Medical Internal Radiation Committee.[13] This dose is the product of the cumulated activity (in μCi hours) in the region and the summed products of all the equilibrium dose constants (in g rad/μCi hours) and specific absorbed fractions (in g^{-1}) for the emissions of the radionuclides used.

For this work a conventional camera, a rotating tomographic camera interfaced to a nuclear medicine computing system, and a quantitative whole body scanning camera with off line computing facilities have all been used at various stages to monitor and image the distributions of activity. A probe counter was also used to obtain time over activity curves for localised sources.

Results

Antibody 9A was radiolabelled to a specific activity of 185 MBq (5 mCi)/mg. Immunoassays did not show any loss of antibody reactivity after radiolabelling, and the patient did not show any allergic reactions to the injected antibody. Quality control tests showed the antibody to be sterile and apyrogenic.

Dosimetry calculations

A phase 1 study (fig 2) (intravenous route) showed that a dosage of 1665 MBq (45·0 mCi) ^{131}I labelled antibody could deliver to the tumour (30 g mass as estimated by computed tomography) 55 Gy (5500 rads) and 130, 600, and 600 mGy (13, 60, 60 rads) to the whole body, liver, and kidneys, respectively. It is recognised, however, that these results represent only a first order approximation to accurate microdosimetry. We calculated, however, that an arterial infusion of labelled antibody would deliver a higher tumour dosage than that obtained via the venous route and that this advantage would depend on factors influencing the time integrals of the arterial antibody concentrations obtained by arterial versus venous infusion respectively.

The regional advantage would depend on the total body clearance of radiolabelled antibody, on the extraction ratio of antibody by the target, and, inversely, on the rate of antibody infusion through the regional artery.[14] Thus significantly—that is, greater than twofold—increased exposure with carotid arterial infusion could be obtained.

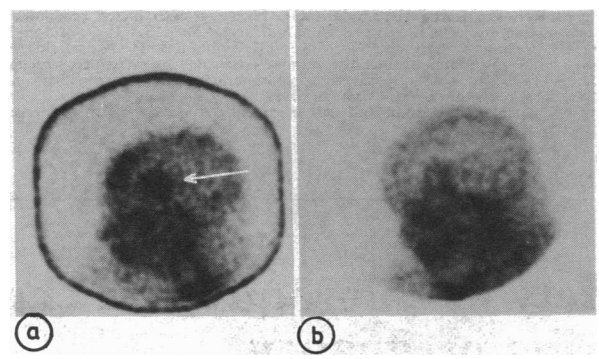

FIG 2—(*a*) Antibody scan 45 hours after intravenous administration of 9A antibody labelled with ^{123}I. The region of glioma is clearly seen (arrow). (*b*) Scan taken after injection of albumin labelled with technetium. This outlines the blood pool of the tumour region and differs from (*a*), showing that the specific antibody-antigen interaction accounts for the antibody scan compared with non-specific protein accumulation in (*b*).

Radiology

Computed tomography was performed before and after antibody guided treatment. Computed tomograms at four months after treatment showed reduction in tumour size (fig 2).

Discussion

This case report shows that a radiolabelled, tumour associated antibody given by an internal carotid arterial infusion resulted in tumour regression and was of value in relieving the symptoms and improving the quality of life in a patient with a recurrent grade IV glioma resistant to conventional forms of treatment. We used a monoclonal antibody that was originally thought to be against epidermal growth factor receptor but was subsequently found to react with blood group A antigen.

Epidermal growth factor receptors on A431 cells express the oligosaccharide detected by antibody A9 but it is not yet clear (work in progress) whether in other neoplasms such as gliomas this blood group A epitope is expressed as part of a truncated epidermal growth factor receptor or is expressed independently. Gliomas and other brain tumours of non-neuronal origin express epidermal growth factor receptors,[15] and this expression may accompany the malignant transformation.[16]

Blood group A and other blood group antigens have been shown to be expressed by some cancers.[17] In a patient such as this, with blood group O, who has a tumour expressing blood group A antigen, the use of an antibody to blood group A offers the chance of a "tumour specific" antibody guided approach. It is encouraging that this approach reduced the size of the tumour and benefited the patient. A further study is now needed to examine the reproducibility of this report and estimate the long term benefit, if any, to patients with brain tumours resistant to conventional forms of treatment.

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References

1 Walker MD, Alexander E, Hunt WE, *et al.* Evaluation of BCNU or radiotherapy in the treatment of anaplastic gliomas. *J Neurosurg* 1978;**49**:333-43.
2 Bloom HJG. Combined modality therapy for intracranial tumours. *Cancer* 1975;**35**:111-20.
3 Salazar OM, Rubin P, Feldstein ML, Pryzktiello R. High dose radiation therapy in the treatment of malignant gliomas. Final report. *Int J Radiat Oncol Biol Phys* 1979;**5**:1733-40.
4 Catterall M, Bloom JG, Ash DV. Fast neutrons compared with megavoltage x rays in the treatment of patients with supratentorial glioblastoma: a controlled pilot study. *Int J Radiat Oncol Biol Phys* 1980;**6**:261-6.
5 Order SE. The history and progress of serologic immunotherapy and radiodiagnosis. *Radiology* 1976;**118**:219-23.
6 Carrasquillo JA, Krohn KA, Beaumier P, *et al.* Diagnosis of and therapy for solid tumors with radiolabelled antibodies and immune fragments. *Cancer Treat Rep* 1984;**68**:317-28.
7 Hammersmith Oncology Group and Imperial Cancer Research Fund. Antibody-guided irradiation of malignant lesions: three cases illustrating a new method of treatment. *Lancet* 1984;i:1441-4.
8 Fabricant RN, De Larco JE, Todaro GJ. Nerve growth factor receptors on human melanoma cells in culture. *Proc Natl Acad Sci USA* 1977;**74**:565-70.
9 Parker PJ, Young S, Gullick WJ, Mayes CLV, Bennett P, Waterfield MD. Monoclonal antibodies against the human epidermal growth factor receptor from A431 cells: isolation, characterisation and use in the purification of active EGF receptor. *J Biol Chem* (in press).
10 Fraker PJ, Speck JC. Protein and cell membrane iodination with a sparingly soluble chloramide,1,3,4,5-tetrachloro-5,6-diphenylglycouril. *Biochem Biophys Res Commun* 1978;**80**:849-57.
11 Lansdorp PM, Astaldi GCB, Oosterhof F, Janssen MC, Zeijlemaker WP. Immunoperoxidase procedures to detect monoclonal antibodies against cell surface antigens. Quantitation of binding and staining of individual cells. *J Immunol Methods* 1980;**39**:393-405.
12 Epenetos AA, Canti G, Taylor-Papadimitriou J, Curbing M, Bodmer WF. Use of two epithelium-specific monoclonal antibodies for diagnosis of malignancy in serous effusions. *Lancet* 1982;ii:1004-6.
13 Snyder WS, Ford MR, Warner GG. *Estimates of specific absorbed fractions for photon sources uniformly distributed in various organs of a heterogeneous phantom.* New York: Society of Nuclear Medicine, 1978:5-67. (Medical Internal Radiation Dose (MIRD) pamphlet 5.)
14 Chen H-SG, Gross JF. Intra-arterial infusion of anticancer drugs: theoretical aspects of drug delivery and review of responses. *Cancer Treat Rep* 1980; **64**:31-40.
15 Libermann TA, Razon N, Bartal AD, Yarden Y, Schlessinger J, Soreg H. Expression of epidermal growth factor receptors in human brain tumours. *Cancer Res* 1984;**44**:753-60.
16 Downward DJ, Yarden Y, Mayers E, *et al.* Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* 1984;**307**: 521-7.
17 Hakomori S, Koscielau J, Bloch H, Jeanloch RW. Immunologic relationship between blood group substances and a fucose containing glycolipid of human adenocarcinoma. *J Immunol* 1967;**93**:31-3.

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Safety of chloroquine in chemosuppression of malaria during pregnancy

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Abstract

A cohort of 169 births to women who were exposed throughout pregnancy to chloroquine 300 mg base once a week for chemosuppression of malaria was studied. The birth defects in this cohort were compared with those in a control group of 454 births to women who were not exposed to chloroquine, most of whom lived in non-malarious areas. The proportion of birth defects in the exposed group was not significantly different from that in the control group. This observation must be considered within the limitations of the study, which could detect only a strong teratogenic effect. It could not exclude risks lower than a 5·7-fold increase in the incidence of birth defects when chloroquine was used.

Women using chloroquine during pregnancy for chemosuppression of malaria can be reassured that it is not a strong teratogen, but if it is to be used the risk of developing malaria should be balanced against the lack of data to determine whether it carries a low teratogenic risk.

Introduction

Chloroquine is the antimalarial chemosuppressive drug of choice for non-immune travellers to and residents in malarious areas free of chloroquine resistance.[1-3] In areas where *Plasmodium falciparum* is resistant to chloroquine the Centers for Disease Control recommend that chloroquine with pyrimethamine and sulfadoxine (Fansidar) should be used.[2] Concern about the potential teratogenic effects of chloroquine exists because of a few case reports.[4-6] This has led some physicians to advise pregnant women not to take chloroquine or indeed any antimalarial drug even in areas where chemosuppression of malaria is highly recommended. The World Health Organisation and the Centers for Disease Control have reported that chloroquine has not been found to affect the fetus adversely when used in the recommended doses for malarial prophylaxis.[1,2] This conclusion was based on case reports and the absence of studies showing teratogenicity.

We report the experiences of a cohort of women who used chloroquine for chemosuppression of malaria during pregnancy. No previous study such as this one has been reported.

Subjects and methods

From 1969 medical officers at the health units of United States embassies and consulates were requested to report births to staff of the foreign service and their dependants to the Office of Medical Services of the United States Department of State. The standard form used included date and place of birth, birth weight, illnesses of the mother during pregnancy, and a list of drugs taken and dates of administration during pregnancy. Birth defects, perinatal complications, and deaths during the neonatal period were also reported. Fetal wastage was not usually reported and was not considered in this study. Paternal and maternal ages were not reported. Minor anomalies were defined as features that did not have any clinical, surgical, or cosmetic importance—for example, isolated partial syndactyly of the second and third toes. Heart murmurs without evidence of congenital heart disease were not considered to be birth defects.

A total of 643 live births were reported among these subjects from 1969 to 1978. In 189 instances an antimalarial drug was used during pregnancy. In 169 cases the women used chloroquine in the recommended dose of 300 mg base once a week throughout pregnancy. The 20 other women used pyrimethamine, proguanil, or an unspecified antimalarial drug and were excluded from the analysis. The

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