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CHEMOTHERAPY OF MALARIA

ANNUAL SUMMARY REPORT

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P. berghei blood-induced malaria infection in mice - based on mortality.

P. gallinaceum sporozoite-induced malaria infection - Aedes aegypti - chicks - based on mortality.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Primary screen - quantitative evaluation of potential antimalarial activity.

Primary screen - to provide quantitative assessments of prophylactic values.

Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

Some 22,376 compounds were screened for antimalarial activity in the period from June 1, 1969 through May 31, 1970.

During the second year of occupancy of our new quarters several inhibitory and/or time-consuming adjustments or corrections still had to be made. Our operation might have been seriously affected by these incidents if our basic test systems had been less simple and flexible.

Tables 1, 2, 3, 4, 5 and 6 list, month by month, the number of compounds tested and the number of mice used from June 1, 1964 through May 31, 1970.

Table 7 is a summary of the total number of compounds screened from the inception of this program to date. p. 10

All compounds tested were obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research and included: (1) compounds structurally related to chemicals of known value as antimalarial agents; (2) compounds structurally unrelated to compounds known to have antimalarial activity; (3) structural analogues of compounds found active in our test system and representing several novel chemical groups.

Our own breeding colony of ICR/Ha Swiss mice supplied the large number of animals needed in our tests.

We have continued to use the original test system which was designed specifically to give relatively fast but reliable evaluations from standpoints of antimalarial effect and host toxicity.

This test is based on the responses to candidate compounds by Plasmodium berghei malaria in mice as expressed in comparisons of the maximum survival time of treated malaria-infected animals and the survival time of untreated malaria-infected controls.

TABLE 1.

MONTHLY SCREENING LEVELS

JUNE 1, 1964 - MAY 31, 1965

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	763	15,111
JULY,	758	12,810
AUGUST,	593	10,306
SEPTEMBER,	521	8,543
OCTOBER,	558	9,146
NOVEMBER,	612	9,788
DECEMBER,	1,279	20,249
JANUARY,	1,634	25,013
FEBRUARY,	1,399	21,228
MARCH,	1,999	30,831
APRIL,	1,378	23,188
MAY,	1,620	29,502
TOTAL FOR YEAR	13,114	215,715

TABLE 2.

MONTHLY SCREENING LEVELS

JUNE 1, 1965 - MAY 31, 1966

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	1,545	25,633
JULY,	1,297	19,873
AUGUST,	1,349	20,645
SEPTEMBER,	1,192	18,208
OCTOBER,	1,539	23,515
NOVEMBER,	1,667	25,525
DECEMBER,	1,740	26,650
JANUARY,	2,384	36,503
FEBRUARY,	2,197	33,015
MARCH,	2,613	39,987
APRIL,	2,241	34,395
MAY,	2,967	46,500
TOTAL FOR YEAR	22,731	350,449

TABLE 3.

MONTHLY SCREENING LEVELS

JUNE 1, 1966 - MAY 31, 1967

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	2,314	36,220
JULY,	2,686	41,175
AUGUST,	2,871	44,825
SEPTEMBER,	2,216	34,420
OCTOBER,	2,644	41,325
NOVEMBER,	2,670	42,285
DECEMBER,	2,712	42,055
JANUARY,	3,048	47,325
FEBRUARY,	3,838	59,970
MARCH,	3,215	49,545
APRIL,	2,886	45,510
MAY,	2,993	46,545
TOTAL FOR YEAR	34,093	531,200

TABLE 4.

MONTHLY SCREENING LEVELS

JUNE 1, 1967 - MAY 31, 1968

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	3,360	52,485
JULY,	2,629	42,690
AUGUST,	3,222	51,510
SEPTEMBER,	4,174	65,085
OCTOBER,	3,769	58,275
NOVEMBER,	4,255	66,690
DECEMBER,	4,772	73,125
JANUARY,	2,807	43,800
FEBRUARY,	1,679	27,195
MARCH,	3,403	53,460
APRIL,	2,953	47,475
MAY,	3,442	54,735
TOTAL FOR YEAR	40,465	636,525

TABLE 5.

MONTHLY SCREENING LEVELS

JUNE 1, 1968 - MAY 31, 1969

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	2,697	42,915
JULY,	1,206	19,080
AUGUST,	4,547	71,625
SEPTEMBER,	3,660	56,190
OCTOBER,	4,116	64,575
NOVEMBER,	3,746	44,250
DECEMBER,	2,561	45,225
JANUARY,	4,249	66,975
FEBRUARY,	3,667	57,435
MARCH,	2,903	45,360
APRIL,	2,001	32,115
MAY,	2,797	57,480
TOTAL FOR YEAR	38,150	603,225

TABLE 6.

MONTHLY SCREENING LEVELS

JUNE 1, 1969 - MAY 31, 1970

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE,	1,836	38,220
JULY,	2,054	35,625
AUGUST,	2,756	47,865
SEPTEMBER,	2,626	43,110
OCTOBER,	3,006	51,375
NOVEMBER,	1,651	33,465
DECEMBER,	1,056	25,155
JANUARY,	1,647	29,925
FEBRUARY,	990	19,155
MARCH	1,729	31,095
APRIL	1,535	27,540
MAY	1,490	28,740
TOTAL FOR YEAR	22,376	411,270

TABLE 7.

SUMMARY OF SCREENING LEVELS

DECEMBER, 1961 - MAY, 1970

DECEMBER, 1961 - NOVEMBER, 1962	250
DECEMBER, 1962 - MAY, 1964	6,665
JUNE, 1964 - MAY, 1965	13,114
JUNE, 1965 - MAY, 1966	22,731
JUNE, 1966 - MAY, 1967	34,093
JUNE, 1967 - MAY, 1968	40,465
JUNE, 1968 - MAY, 1969	38,150
JUNE, 1969 - MAY 31, 1970	22,376
TOTAL	177,844

Using young ICR/Ha Swiss mice and a standard inoculum of Plasmodium berghei, it has been possible to produce a consistently uniform disease that is fatal to 100% of untreated animals within 6 to 8 days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a high degree of parasitemia has become evident.

Test compounds were administered parenterally in a single dose on the third day post-infection by which time a 10-15% parasitemia has developed.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life-span of the treated animals over that of the untreated controls.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD

ANIMAL HOSTS The total supply of animals needed to screen candidate compounds was obtained from our own breeding colony of ICR/Ha Swiss mice. Test animals weigh from 15 to 18 grams, weight variations in any given experimental or control group being carefully limited to 2-3 grams. In any given test all animals are of a single sex and approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib.

TEST PROCEDURE Test animals receive an intraperitoneal injection of 0.5 ml. of a 1:100 dilution of heparinized heart's blood with a minimum of 90% parasitized cells, drawn from donor mice infected one week earlier with Plasmodium berghei. The donor strain is maintained by weekly passages in separate groups of mice inoculated with 0.5 ml. of a 1:500 dilution of heparinized heart's blood.

In order to check factors such as changes in the infectivity of our Plasmodium berghei strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at dose levels known to produce definite increases in survival time is included in every experiment as a positive control.

DRUG ADMINISTRATION Test compounds are dissolved or suspended in peanut oil before they are administered.

Treatment consists of a single dose given subcutaneously 3 days post-infection. At the time of treatment, a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

In each experiment the compound on test is dministered in graded doses. Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice.

If an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 60 days are considered as cured.

DRUG ACTIVITY Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. The minimum effective dose is defined as the minimum dose increasing the life-span of treated animals by 100% over the life-span of untreated controls.

An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are subjected to a dose-response curve so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

p 1

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM BERGHEI IN MICE. Of the 22,376 compounds tested from June 1, 1969 through May 31, 1970 over 1066 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

Supplementary procedures, using different hosts and parasites and performing reliably either as confirmatory tests or as other primary screens, are desirable adjuncts to any screening program.

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~~We have developed~~ a simple but dependable supplementary test with Plasmodium gallinaceum malaria in chicks. has been developed.

26,049 compounds were screened for antimalarial activity in the period from June 1, 1969 through May 31, 1970.

Tables 8, 9, 10*, 11, 12, and 13 list, month by month, the number of compounds tested and the number of chicks used from January, 1965 through May 31, 1970.

Table 14 summarizes the number of compounds tested and the number of chicks used from the inception of this assay system in January, 1965 to date. p. 20

Using 9-12 days old chicks and a standard inoculum of Plasmodium gallinaceum, we have been able to produce a consistently uniform disease that is fatal to 100% of untreated controls within 72-96 hours.

In this test, as in our mouse test, the antimalarial activity of candidate compounds is assessed by comparing the maximum survival time of treated malaria-infected chicks and the survival time of untreated malaria-infected controls.

As in the mouse test, a compound has been considered active if it has produced increases of at least 100% in the survival time of treated chicks over the survival time of untreated controls.

Again as in the mouse test, acceptance of a test compound's antimalarial activity was further predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED).

A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. A minimum effective dose is defined as the minimum dose increasing the life-span of treated animals 100% over the life-span of untreated controls.

*An outbreak of an avian infectious disease involving entire flocks made it impossible to get the healthy birds that we required, and the chick test was temporarily dropped.

TABLE 8.

MONTHLY SCREENING LEVELS

JANUARY 1, 1965 - MAY 31, 1965

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JANUARY, ...	41	260
FEBRUARY, ...	94	885
MARCH, ...	82	1,470
APRIL, ...	72	1,450
MAY, ...	86	1,650
TOTAL FOR YEAR	375	5,715

TABLE 9.

MONTHLY SCREENING LEVELS

JUNE 1, 1965 - MAY 31, 1966

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE,	94	1,620
JULY,	120	2,020
AUGUST,	166	1,580
SEPTEMBER,	246	1,365
OCTOBER,	464	3,195
NOVEMBER,	179	3,295
DECEMBER,	249	3,465
JANUARY,	197	3,455
FEBRUARY,	163	2,800
MARCH,	202	3,495
APRIL,	264	4,450
MAY,	56	1,195
TOTAL FOR YEAR	2,400	31,935

TABLE 10.

MONTHLY SCREENING LEVELS

JUNE 1, 1966 - SEPTEMBER 30, 1966*

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE, ...	352	5,865
JULY, ...	334	5,565
AUGUST, ...	105	2,250
SEPTEMBER, ...	211	3,540
TOTAL FOR YEAR	1,002	17,220

*An outbreak of an avian infectious disease involving entire flocks made it impossible to get the healthy birds that we required, and the chick test was temporarily dropped.

TABLE 11.

MONTHLY SCREENING LEVELS

SEPTEMBER, 1967 - MAY, 1968

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
SEPTEMBER,	90	1,410
OCTOBER,	349	3,330
NOVEMBER,	352	3,150
DECEMBER,	282	2,700
JANUARY,	231	2,400
FEBRUARY,	58	450
MARCH,	367	3,030
APRIL,	698	4,095
MAY,	555	4,290
TOTAL FOR YEAR	2,982	24,855

TABLE 12.

MONTHLY SCREENING LEVELS

JUNE 1, 1968 - MAY 31, 1969

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE,	418	3,900
JULY,	186	2,490
AUGUST,	472	5,295
SEPTEMBER,	657	5,700
OCTOBER,	549	6,270
NOVEMBER,	643	5,415
DECEMBER,	623	5,700
JANUARY,	844	6,200
FEBRUARY,	582	5,700
MARCH,	616	5,985
APRIL,	542	5,985
MAY,	590	5,985
TOTAL FOR YEAR	6,722	64,625

TABLE 13.

MONTHLY SCREENING LEVELS

JUNE 1, 1969 - MAY 31, 1970

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE,	597	5,875
JULY,	436	4,095
AUGUST,	482	6,300
SEPTEMBER,	529	6,615
OCTOBER,	794	11,750
NOVEMBER,	1,369	10,680
DECEMBER,	2,690	14,880
JANUARY,	3,771	20,675
FEBRUARY,	3,748	21,280
MARCH,	3,959	21,390
APRIL,	4,246	22,825
MAY,	3,428	20,400
TOTAL FOR YEAR	26,049	166,765

TABLE 14.

SUMMARY OF SCREENING LEVELS

JANUARY, 1965 - MAY 31, 1970

JANUARY, 1965 - MAY, 1965	375
JUNE, 1965 - MAY, 1966	2,400
JUNE, 1966 - SEPTEMBER, 1966	1,002
SEPTEMBER, 1967 - MAY, 1968	2,982
JUNE, 1968 - MAY, 1969	6,722
JUNE, 1969 - MAY 31, 1970	26,049
TOTAL	39,530

METHOD

TEST ANIMALS This test is done with 9-12 day old white leghorn cockerels.

Birds of fairly uniform stock, purchased from local hatcheries, are delivered to the laboratory when 1 day old and then maintained under standard conditions, including a non-medicated diet, until they are ready for testing.

TEST PROCEDURE Chicks on test are given an intrajugular injection of 0.2 ml. of heparinized heart's blood infected with *Plasmodium gallinaceum* and having a minimum of 80-90% parasitized red blood cells.

The parasitized blood is drawn by cardiac puncture from donor birds that had been infected 72 hours earlier with *Plasmodium gallinaceum*.

Donor strains are maintained in separate groups of chicks, 14-16 days old, that also receive inoculations of heparinized infected heart's blood.

In every experiment 100% of the untreated controls have died within 72-96 hours post-infection.

In order to check factors such as changes in the infectivity of our *Plasmodium gallinaceum* strain or in the susceptibility of the host or to detect technical errors, a group of infected birds treated with pyrimethamine at dose levels known to produce definite increases in survival time has been included in every experiment as a positive control.

DRUG ADMINISTRATION Candidate compounds are dissolved or suspended in peanut oil before they are administered.

In this supplementary test treatment consists of a single dose that is administered either subcutaneously or *per os* immediately after infection.

Each experiment is done with graded doses of the compound on test, and increases in the dose levels of highly active compounds were generally followed by increases in the survival time of the treated chicks.

If an active drug was toxic for the host, its toxicity became a limiting factor to changes in dosages.

Deaths that occurred within 48 hours after infection and treatment were considered as deaths due to the toxic effects of a test compound, not as the result of the infection introduced by the *Plasmodium gallinaceum* parasite.

Chicks with survival periods of 30 days are recorded as cured.

DRUG ACTIVITY In the chick test, as in the mouse test, an increase of 100% in survival time has been considered as the minimum significantly effective response to the antimalarial activity of a compound.

p.11

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM GALLINACEUM MALARIA IN CHICKS Of the 26,049 compounds tested in chicks from June 1, 1969 to May 31, 1970, over 262 demonstrated a degree of antimalarial activity that produced a minimum of 100% increase in the survival time of Plasmodium gallinaceum infected chicks.

A SUPPLEMENTARY SCREENING PROCEDURE WITH SPOROZOITE-INDUCED PLASMODIUM GALLINACEUM MALARIA IN CHICKS

Although this screening procedure may be useful as a confirmatory test and/or another primary screen of antimalarial activity, its basic purpose is the assessment of prophylactic values of candidate compounds.

Since we did not have an insectary and therefore could not rear the Aedes aegypti mosquitoes needed for a sporozoite-induced test in a system using Plasmodium gallinaceum in chicks, the initial phase of our study was completely dependent upon weekly shipments of frozen infected material from Insect Control and Research, Inc., Baltimore, Maryland.

Within a period of about twelve months studies involving approximately 12,000 chicks and 200 compounds indicated that this method of obtaining infected material would not provide us with a sporozoite-induced avian test approaching the degree of uniformity and reproducibility of our blood-induced mouse screen and our blood-induced avian screen.

It has become evident that the establishment of a mosquito-rearing facility on our premises is essential if we are to attempt to develop a satisfactory sporozoite-induced test system.

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