LASER PHOTOSENSITIZATION AND METABOLIC INHIBITION OF TISSUE CULTURE CELLS
TREATED WITH QUINACRINE HYDROCHLORIDE

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In a preliminary report (1) it was suggested that nucleolar function could

be probed by treatment of tissue culture cells with quinacrine hydrochloride

followed by laser microirradiation. Apparently the quinacrine associates selec-

tively with the nucleolus, thus making this organelle susceptile to selective

damage by laser microirradiation. If the laser technique is to be useful for

studying nucleolar function, the metabolic effects of the photosensitizing agent

(quinacrine) must be elucidated. In addition, since previous studies have sug-

gested shifts in absorption characteristics of other amino acridines (2) when

bound in vitro, it is important to determine if such shifts occur with quinacrine.

This is particularly important in selecting the appropriate wavelengths for

microirradiation.

In this manuscript we will present data: (a) indicating the quinacrine

hydrochloride has only a temporary effect on nucleolar function; (b) on the in

vitro absorption spectrum of quinacrine hydrochloride.

Materials and Methods

Cells utilized were the CMP line of human adenocarcinoma cells (3). For

metabolic studies all cells were seeded into Leighton culture tubes, 100,000

cells per tube, and incubated for 48 hours at 37°C in Eagle's medium supplemented

with 10% fetal calf serum, penicillin (100,000 IU/cc), and streptomycin sulfate

(0.25 mg/1). After 48 hours of growth, the medium was removed and quinacrine

hydrochloride in Eagle's medium in concentrations from 50 µg/ml - 0.005 µg/ml

was added for 30 minutes. Following quinacrine treatment the cells were rinsed

twice in balanced salt solution, and fresh Eagle's medium added for periods of

0, 30, 60 minutes followed by incubation in tritiated uridine (5 µg/ml, sp. act.,

8.0 c/mmole) for 30 minutes. Experiments were also conducted in which tritiated

uridine was added at the same time as the quinacrine solution. In all experi-

ments, the cells were rinsed twice with balanced salt solution following uridine

incubation, fixed for 10 minutes in Carnoy's (3:1 absolute ethanol: acetic acid),

and then passed through 70% alcohol, distilled water, 5% trichloroacetic acid at

4°C for 15 minutes, distilled water, 50% alcohol to absolute alcohol, and mounted

cell side up with permount. The slides were then subjected to standard emulsion

radioautography (NTB emulsion) for 1 week. The radioautographs were developed by

standard procedures, the slides were stained with Giemsa, dehydrated through

absolute alcohol and xylene, and mounted in permount. Grain counts were made by

two individuals who were trained together to give similar counts(±5 grains/cell).

All slides were blind coded so that each individual had no idea of the particular

treatment. At least 4 slides for each treatment were tabulated. All nuclear

grains were counted for 30-50 cells per slide. Cells were chosen by random move-

ment of the microscope mechanical stage. A background grain count was made of

each slide and subtracted from each cell grain count. The mean and standard

deviation of grain counts for each treatment were tabulated and the means between

groups were compared by the standard "t" test, for significance. All treatments

were compared to control cells incubated in uridine, but not receiving the

quinacrine treatment.

The determination of the in vitro absorption characteristics of quinacrine

was conducted, using CMP cells established in Rose multipurpose chambers, as

described in an earlier publication (1). Fifty thousand cells were injected into

the Rose chamber in Eagle's medium, as prepared above, and used for experimenta-

tion 48 hours later. A solution of quinacrine, 50 µg/ml of Eagle's medium, was

injected into the Rose chamber and left for 10 minutes before experimentation.

Cells were only irradiated during a period of 10-30 minutes of quinacrine expo-
sure. Irradiation was performed, using a modified version of the original
microbeam system described by us in earlier studies (4,5,6). In the current
studies, a Hughes 3044H argon ion laser was complexed with a Zeiss photomicro-
scope and a closed-circuit television. The peak power output of this laser
system was 15 watts, with a half pulse width of 10 μsec. A prism wavelength
selector was used to choose any of the argon laser wavelengths. Laser output
was recorded with a calibrated photodiode, and read on an oscilloscope. The
Rose chamber was placed under the photomicroscope, a desired cell located in the
field, and a particular nucleolus located under the cross-hair on the television
screen. The laser was then fired for 10 pulses, and the alteration in the nucle-
olus noted. The purpose of these experiments was to determine at what power for
each argon wavelength a visible lesion could no longer be produced. Lesions
could be produced of the two major types we have described previously: a "severe"
lesion consisting of a phase-light area surrounded by a dark margin; and a
"slight" lesion consisting of a coagulation or "phase darkening" of the irradi-
ated portion of the nucleolus. If this type of lesion was not produced within 5
minutes after irradiation, the nucleolus was classified as having "no lesion".
At each argon wavelength, the powers at which the "slight" lesions could and
could not be produced were determined. At least 10 cells were irradiated at each
power level. From these data the threshold laser output energy necessary to pro-
duce a structural alteration in the nucleolus could be calculated at each
wavelength and compared with the known absorption curve for quinacrine.

Results

Radioautographic Data

The effects of the highest concentration of quinacrine, 50 μg/ml, are illus-
trated in Tables I and II. Simultaneous incubation of cells in quinacrine and
[3]H-uridine resulted in a complete inhibition of isotope uptake (Table II).
Washing the cells with balanced salt solution, and then adding ^{3}H-uridine, or

adding normal Eagle's medium for 30 minutes followed by ^{3}H-uridine, resulted in

a marked incorporation of isotope. Incubation in Eagle's medium for 60 minutes

prior to isotope incubation, resulted in normal levels of isotope incorporation.

The effect of various concentrations of quinacrine on isotope uptake when

the quinacrine and the isotope are applied simultaneously, is illustrated in

Table II. In both of these replicate experiments, isotope incorporation was

entirely inhibited at 50 μg/ml (same as preceding experiment), and significantly

inhibited ("t" = 3.8 and 12.0), but not completely at 5 μg/ml. It was not

inhibited ("t" = 1.78) at 0.5 μg/ml and less.

TABLE I

RECOVERY FROM QUINACRINE HYDROCHLORIDE

Cells were incubated in quinacrine hydrochloride, 50 μg/ml, for 30 minutes;
washed twice with balanced salt solution, and either placed in ^{3}H-uridine,
5 μg/ml for 30 minutes directly, or incubated for 0, 30, and 60 minutes in
Eagle's medium prior to addition of ^{3}H-uridine. Each mean grain count was
derived from at least 60 cell counts.

Time in Recovery Medium	Grain $\bar{x}$ Counts Exp. 1	Grain $\bar{x}$ Counts Exp. 2
0 minutes	42	30
0 minutes	50	37
Control (no quinacrine)	88	74
30 minutes	45	48
30 minutes	42	54
Control (no quinacrine)	85	85
60 minutes	72	94
60 minutes	72	78
Control (no quinacrine)	77	86

TABLE II

^{3}H-URIDINE INCORPORATION SIMULTANEOUSLY WITH QUINACRINE
EXPOSURE

Quinacrine Concentration	Mean Total* Grains Exp. 1	"t" Values	Mean Grain Exp. 2	"t" Values
50 µg/ml	0	0	0	0
5 µg/ml	76.4	12.0	100.6	3.8
0.5 µg/ml	162.5	1.4	113	1.5
0.05 µg/ml	159.4	1.78	124.9	0.3
Control (no quinacrine)	177.4	-	122.9	-

*   Each mean value was derived from 60 individual cell counts.
** A "t" value greater than 1.8 indicates a mean significantly different
     from the control.

Absorbancy Data

A normal absorption curve for quinacrine hydrochloride in balanced salt solu-
tion is presented in Figure 1. The wavelengths of the argon laser are denoted at
points along the curve. The results of irradiating nucleoli with the different
argon lines during quinacrine treatment are illustrated in Table III. At each
wavelength the laser output power and energy above and below the level necessary
to create a threshold lesion is indicated. It should be noted that at the lowest
wavelength (4579 Å) the smallest amount of laser output is needed to produce a
lesion, and at longer wavelengths (4765, 4880 Å) progressively more laser output
is required to produce the same structural response. At the longest wavelengths
(4965 and 5145 Å) lesions cannot be produced even though the output at 5145 Å is
13-20 times as great as the output at 4579 Å.

TABLE III

THRESHOLD LESIONS WITH DIFFERENT ARGON WAVELENGTHS

Wavelength Angstra		Power Watts	Energy μ Joules	Lesion* Production (+)
4579	(Blue)	0.075	0.75	-
		0.10	1.0	+
4765	(Blue)	0.128	1.28	-
		0.136	1.36	+
4880	(Blue)	0.50	5.0	-
		0.55	5.5	+
4965	(Green)	0.53	5.3	-
5145	(Green)	1.3	13.0	-

* + = Dark coagulation at site of irradiation.
  - = No visible change in nucleolus for 5 minutes following irradiation.
** All power and energy measurements were made on the beam prior to its passing through the microscope. Measurements indicated that 43% of the output energy passed through the microscope. The measurement for 4965 and 5145 Å were at maximum output.

Figure 1

Quinacrine Absorption Curve with Argon Lines
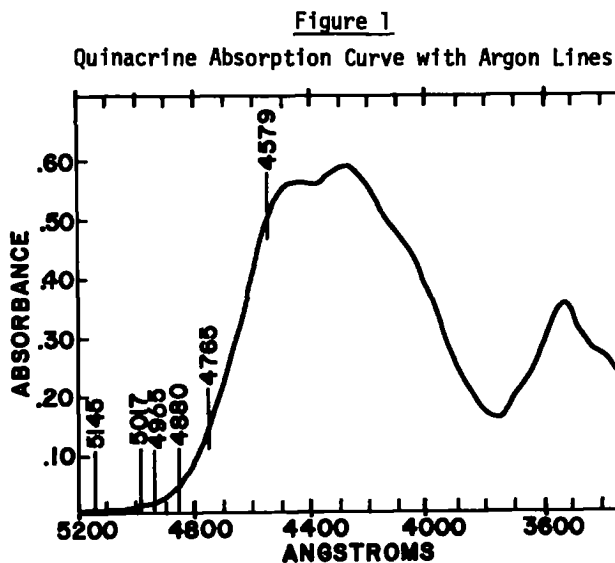
Experiments were also conducted to determine at what quinacrine concentra-
tion threshold lesions could no longer be produced when the laser was operating
at maximum output. In these experiments, cells were left in various concentra-
tions of quinacrine for 15 minutes, and then their nucleoli were irradiated with
10 pulses of the mixed laser beam (all 6 wavelengths together). At least 10
cells were irradiated at the following quinacrine concentrations: 5 µg/ml;
0.5 µg/ml; 0.4 µg/ml; 0.2 µg/ml; 0.1 µg/ml; 0.08 µg/ml; 0.05 µg/ml. It was
found that threshold lesions were produced with a quinacrine concentration as
low as 0.1 µg/ml.

Discussion

The radioautographic data indicate that cells can functionally recover from
quinacrine concentrations as high as 50 µg/ml if incubated in normal medium for
60 minutes after exposure. Furthermore, quinacrine concentrations below 0.5
µg/ml do not inhibit uridine incorporation when the uridine is applied simulta-
neously with the quinacrine.

Since uridine incorporation is an index of RNA metabolism, it appears that
the effect of quinacrine on RNA metabolism is only temporary at higher concentra-
tions of the drug. With 50 µg/ml of quinacrine, laser alteration of nucleoli can
be used to study RNA metabolism if one waits 60 minutes following treatment
before applying the isotope. However, it is interesting to note that uridine
uptake is not affected when 0.5 µg/ml or less of quinacrine is used, but a nucle-
olar lesion can be produced with quinacrine concentrations as low as 0.1 µg/ml.
For studies where incubation in isotope immediately following laser irradiation
is necessary, quinacrine concentration below 0.5 µg/ml should be used. It is
perhaps interesting that the intense laser beam is a more sensitive detector of
quinacrine than is the functional radioautographic technique.

It does not appear that there is a shift in the quinacrine absorption spec-
trum when the drug is bound in vitro. The results of the individual wavelength
experiments clearly reflect the absorption curve of quinacrine solutions in a
non-cellular environment. For those portions of the quinacrine absorption

spectrum that argon wavelengths are available, the action spectrum, as measured
by lesion-producing capacity, is what one would predict from the absorption
curve. These data further demonstrate a feature often not fully appreciated:
because of the high intensity of laser light, only a small amount of chromophore
or only a small amount of absorption by a chromophore is necessary for an effect.
This fact is evidenced by the ability to produce lesions with the 4880 A wave-
length.

    We can conclude from the radioautography and the absorption data, that
quinacrine hydrochloride is a useful agent for selective microirradiation of
nucleoli and subsequent functional analysis. Furthermore, the ability of cells
to recover from quinacrine treatment and the fact that quinacrine does inhibit
RNA synthesis, suggests that this drug could be useful in studying RNA metabo-
lism. Earlier studies have indicated that quinacrine acts like actinomycin-D,
by binding to DNA and inhibiting DNA-dependent RNA synthesis (7, 8, 9). If this
is really the mechanism of quinacrine action, the ability of cells to recover so
readily would be unique. Studies are currently underway to determine if
quinacrine is selective for a specific specie of RNA, and if certain RNAs recover
sequentially from quinacrine treatment.

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