INTRODUCTION

Hematoporphyrin derivative (HPD) photodynamic therapy (PDT) has proved effective in the treatment of selected neoplasms (1). The effectiveness of this form of therapy rests on the retention of the systemically administered HPD in neoplastic tissue and its photoactivation with visible light resulting in 'photodynamic' tumor destruction. Although it is generally agreed that singlet oxygen liberated during HPD-photodynamic therapy is responsible for the biologic damage created by PDT, the mechanisms of cell death have not been clearly defined (2). Previous studies in our laboratory have demonstrated a rapid and sustained decrease in tumor blood flow after HPD-photodynamic therapy (3,4). The present study was undertaken to correlate changes in tumor blood flow with tumor regression after HPD-PDT.

MATERIALS AND METHODS

Animals

Four week-old Fischer 344 rats (Charles River Breeding Laboratories, Boston MA) were housed 3 to a cage and provided with laboratory chow and water ad libitum.

Hematoporphyrin Derivative

One gram of HPD (Porphyrin Products, Logan UT) was dissolved in 50
ml of 0.1 N NaOH. The solutions was stirred for 1 hr at room tempera-
ture, neutralized to pH 7.1 with 0.1 N HCl and adjusted to a final volume
of 200 ml with 0.9% NaCl. The solution was then made isotonic with solid
NaCl, and sterilized by passage through a Swinnex 25 filter unit, 0.45 μm
(Millipore Corp., Bedford MA).

Tumor

The FANFT {N[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide} induced
urothelial tumor line (AY-27) was used for this investigation. This tumor
line was kindly provided by Dr. Samuel M. Cohen, University of Nebraska,
Omaha. The tumor line has been maintained in vivo since 1981 by periodi-
cally propagating small pieces into syngeneic animals.

For implantation, tumors were harvested from donor animals, gently
minced in 5 ml of Hanks balanced salts solution (GIBCO, Grand Island NY),
washed, placed in 5 ml of RPMI 1640 medium (GIBCO) to which 0.01 ml of
antimycotic antibiotic solution (penicillin, 10,000 units/ml; Fungizone,
25 μg/ml; Streptomycin, 10,000 μg/ml) had been added. This solution was
then passed through a series of #21 gauge needles, and 0.2 ml injected
subcutaneously. Tumors were palpable within 1 week of implantation and
had grown to approx. 1 cm diameter by 3 weeks. At this size, such tumors
are not necrotic.

In the present experiments, 2 tumors were grown in each animal, 1
below the xiphoid, the other above the pubis. After injection of HPD, 1
tumor was treated with light while the other was shielded; the latter
served as an internal control. Prior to phototherapy, all animals were
anesthetized with sodium pentothal administered intraperitoneally (65 mg/
kg). Anesthesia was continued during the subsequent blood flow measure-
ments.

Phototherapy Unit

A 500 watt Quartzline lamp (GE-CBA) in a Kodak slide projector
fitted with a red filter (Corning 2418) which excludes light <590 nm was
employed. The light beam was deflected 90 degrees with a mirror and
focused on the intact skin over the tumor with a convex lens. The light
intensity at the surface of the phototreated tumor was measured with a
radiometer (UDT #351). The tumor temperature was monitored with a #24
gauge hypodermic transistor probe (YSI #524X) placed percutaneously
beneath the surface of the tumor. The body-core temperature of the rat
was measured with an intrarectal thermistor probe (YSI #401). Tumor
temperature was maintained within 2°C of body-core temperature by direct-
ing a jet of cool air over the tumor during PDT.

Regional Blood Flow Determination

Regional blood flow determinations were made using a modification of
the reference sample method described by Malik (5). A papered PE 50
catheter (Clay-Adams) filled with heparinized saline (10 U/ml) was ad-
vanced into the left ventricle via the right common carotid artery. Con-
tinuous monitoring of the arterial pulse pressure via a Statham
transducer connected to a polygraph (Beckman Dynograph R511A) confirmed
proper placement of the left ventricular catheter as determined by a
change in pulse width. This was subsequently verified at necropsy. Both
femoral arteries were exposed and cannulated with PE 10 catheters. The
right was connected to a Statham transducer for continuous blood pressure
monitoring while the left catheter was connected to a Harvard syringe-
driven withdrawal pump. A total of 3.6 x 10^5 microspheres (15 μm
diameter) labeled with either 103Ru or 141Ce (New England Nuclear, Boston