Mitochondrial respiration depressed by camphor: A possible aid in radiotherapy[1]

D. Guilland-Cumming and G.J. Smith

Physics and Engineering Laboratory, Department of Scientific & Industrial Research, Lower Hutt (New Zealand), 27 December 1978

Summary. Camphor has been found to decrease the rate of oxygen consumption by rat kidney mitochondria. The rate of oxygen consumption is nearly halved by the addition of 8×10^{-3} M camphor. It is suggested that camphor may be of use in oxygenating tumours prior to radiotherapy.

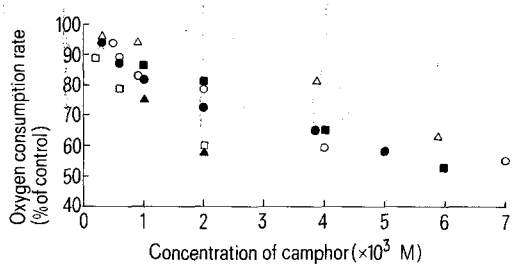
Well oxygenated cells are about 3 times more sensitive to ionizing radiation than anoxic cells[2]. Tumours are frequently poorly vasculated and problems arise in radiotherapy with the hypoxic regions which exist in tumours as a result of this inadequate blood supply. It has been suggested that a non-toxic substance capable of reducing cellular oxygen consumption would allow oxygen to diffuse further from the blood vessels/capillaries into the tumour[3].

Camphor is relatively non-toxic[4] and it has been observed to reduce respiration in plant mitochondria[5], *E. coli*[5] and isolated bull frog heart[7]. These observations suggested that a quantitative study of the effect of camphor on the oxygen consumption by the mitochondria of mammalian cells should be undertaken to see if it could be useful in oxygenating tumours prior to radiotherapy.

Experimental. Male Wistar rats were raised under controlled conditions to a b.wt of 170±10 g. Kidney mitochondria were prepared according to the method of Schneider[8] with the protein concentration of the preparation between 15 and 17 mg/ml. Protein was determined by the biuret reaction. The mitochondria were resuspended (0.80–0.90 mg protein · ml^{-1}) in a medium generally employed for the determination of respiratory control indices. The respiratory control of each preparation was routinely checked. The medium contained: 50 mM KCl; 25 mM Tris-Hepes pH 7.4; 10 mM KH_2PO_4/HPO_4 buffer pH 7.4; 8 mM $MgCl_2$; 5 mM Na-succinate; 70 mM sucrose; 0.05% bovine serum albumin. The stock solutions of D,L camphor (BDH) were prepared in 30% ethanol/water solvent to overcome the low aqueous solubility of the substance. The camphor solution was injected in aliquots of 25–50 µl and oxygen consumption was monitored with a Clark type electrode[9] after each injection. A run where equivalent amounts of 30% ethanol/water alone were injected was done as a blank.

Results and discussion. The effect of various concentrations of camphor on the rate of oxygen uptake by rat kidney mitochondria expressed as a percentage of the rate in the mitochondrial suspension without camphor (the control) is shown in the figure. No detectable change in the rate of oxygen consumption was observed when the 30% ethanol/water solvent was added to the mitochondrial suspension.

A number of additional runs at low camphor concentrations were done to determine the start of a measurable suppression of the rate of oxygen consumption (< 95% of control). This occurs at 3×10^{-4} M camphor. Oxygen consumption continues to decrease with higher concentrations of camphor reaching a minimum at $7\text{–}8 \times 10^{-3}$ M camphor. At higher concentrations of camphor, a dramatic increase in the rate of oxygen consumption occurs which possibly arises from an uncoupling effect.



The effect of camphor on the rate of oxygen consumption by rat kidney mitochondria. Each set of symbols corresponds to an experimental run.

Thomlinson and Gray[10] considered diffusion of oxygen into a cylinder of tissue from blood vessels at the periphery and deduced that the radius of the cylinder R_0 where the oxygen concentration just falls to zero at its centre is given by:

$$R_0 = \sqrt{4Dp_0/kQ} \qquad (1)$$

where k is a constant, D is the diffusion coefficient of oxygen, Q is the rate of oxygen consumption which is assumed to be independent of oxygen concentration, and p_o is the oxygen concentration in the blood.

The concentration of camphor present in a saturated aqueous solution is about 5×10^{-3} M and at this concentration the rate of oxygen consumption is reduced to about 60% of the rate in the absence of camphor. From equation (1) it is apparent that in the presence of 5×10^{-3} M camphor, the radius of the cylinder of tissue where the oxygen concentration just falls to zero increases by about 30%. In an effort to enhance the radiosensitivity of tumours other workers have decreased cellular oxygen consumption by introducing high levels of glucose[11–13] or electron transfer blocking chemicals[14] and by lowering the temperature[15]. The reduction of oxygen consumption caused by the presence of camphor compares very favourably with these other methods and it merits investigation as a possible means of oxygenating tumours prior to radiotherapy.

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