

## SHORT COMMUNICATION

### EFFECT OF A QUINAZOLINONE DERIVATIVE ON THE METABOLISM OF *STREPTOCOCCUS FAECALIS* - R (ATCC-8043)

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**Summary:** 2-methyl-3-(3'-methyl-2'-pyridyl)-4 (3H) quinazolinone (SRC-820) and methaqualone inhibited the growth of *Streptococcus faecalis*-R (ATCC-8043). Phosphoenolpyruvate formation from 3-phosphoglycerate by an enzyme extract of *S. faecalis* was inhibited by SRC-820. With edetate or fluoride, an additive inhibitory effect by SRC-820 was observed.

**Key words:** SRC-820            *S. faecalis*            respiration            phosphoenolpyruvate  
inhibition            EDTA            fluoride

#### INTRODUCTION

Quinazolinone derivatives substituted with alkyl and aryl radicals are known to exert a wide spectrum of pharmacological properties. Some, such as methaqualone, are well known as anticonvulsants, CNS depressants and tranquillizers (2,4). A derivative, 2-methyl-3-(3'-methyl-2'-pyridyl)4(3H) quinazolinone (SRC-820)\* exhibits pharmacological properties similar to those of methaqualone.† Some derivatives are well known as antimalarials, folate antagonists and bacteriostatic agents (1). The influence of SRC-820 on the growth and respiration of *Streptococcus faecalis*-R (ATCC-8043) as well as on a few enzymatic activities of the cell-extracts is presented in this communication.

#### MATERIALS AND METHODS

*Streptococcus faecalis* (ATCC-8043) was grown in Tryptone - Yeast extract medium. Growth was followed turbidimetrically (9), for several periods of time upto 25 hr. Respiration of washed cell suspensions was followed manometrically using glucose or fructose as the substrate (10) with air as the gas phase. Acetone powder and extracts were prepared as described by Gunsalus (5). Aldolase (EC.4.1.2b) activity was determined by the method of Sibley and Lehninger (7). Glyceraldehyde-3-phosphate (GAP) dehydrogenase (EC.1.2.1.12) activity was assayed by the method of Krebs (6). The combined activities of 3-phosphoglyceric acid (PGA) mutase (EC.2.7.5.3) and enolase (EC.4.2.1.11) was determined by the method of Grisolia (3) and the released phosphate from phosphoenolpyruvate (PEP) according to the method of Simonsen *et al.* (8).

\* Patented product of Sarabhai Research Centre, Barada.

† Personal communication from Dr. V. Srinivasan, Director, Sarabhai Research Centre, Barada.

## RESULTS

SRC-820 ( $1 \times 10^{-2}$  M of broth) inhibited the growth of *S. faecalia-R* at the early stages of incubation. At 3 to 4 hr of growth, inhibition was found to be  $59.0\% \pm 13.9$  ( $n=6$ ). In acid production,  $63.4\% \pm 10.0$  inhibition was observed at the same time period of growth ( $n=6$ ). With longer periods of incubation, inhibition of both growth and acid production progressively decreased. Methaqualone ( $1 \times 10^{-2}$  M) under the identical conditions was found to inhibit the growth and acid production by 85 to 90%. At 3 hr of growth period, with lower concentration ( $0.5 \times 10^{-2}$  M) of both growth and acid production was found to be inhibited by  $72.1\% \pm 3.8$  and  $75.3\% \pm 2.9$  respectively ( $n=3$  for both). The inhibitory effect was found to persist even at 6 hr of incubation.

Respiration of washed cell-suspensions with glucose as substrate was found to be inhibited by SRC-820 ( $1 \times 10^{-2}$  M) (inhibition  $19.4\% \pm 4.7$ , ( $n=5$ )). The effect of fluoride alone and with SRC-820 on the respiration was also studied. It was found that when both the inhibitors were present, cell respiration was completely abolished.

With acetone-dried cell extracts, it was found that SRC-820 did not inhibit the activities of aldolase and GAP dehydrogenase but inhibited the formation of phosphoenolpyruvate (PEP) from 3-phosphoglyceric acid. Under the experimental conditions of this study, ketopyruvate formation could not be demonstrated in the reaction mixture. For comparison, the effect of fluoride, edetate (EDTA), SRC-820 and combinations of EDTA and SRC-820 as well as fluoride and SRC-820 were studied. The results are presented in Table I.

## DISCUSSION

The inhibition of growth and acid production by SRC-820 at the early stages and subsequent fall in inhibition may indicate that alternate pathways of metabolism are established for providing energy for growth. It is also possible that adaptive mechanisms come into operation modifying the chemical nature of the inhibitor.

Oxygen uptake studies with washed cell suspension show that metabolism of glucose takes place by the Embden-Meyerhof pathway and is effected by fluoride. The influence of fluoride and SRC-820 is indicative of a single mechanism by which both produce their effect.

As can be seen from Table I, SRC-820 alone exerted an inhibitory effect (28.1%) on PEP formation while EDTA inhibited by 24.0%. When both these substances were present together, the inhibition was found to be not the sum but nearly the same as that exerted by either of these two substances. But very less inhibition due to SRC-820 could be demonstrated when magnesium concentration was increased to 10  $\mu$ moles per system. Fluoride alone under these conditions inhibited the activity by 76.1% and when both SRC-820 and fluoride were present together, here also the inhibition was found to be nearly the same (80.6%).

Our studies using slow-vacuum dried cell extracts show that oxidation of NADH is also inhibited in the presence of SRC-820 which will form the subject of our next communication. The results presented here indicate that SRC-820 may act as a metal binding agent, though not as strongly as EDTA.

TABLE I: Inhibition of formation of phosphoenolpyruvate (PEP) from 3-phosphoglycerate (3-PGA).

Additions	PEP formed @ μmoles/3 ml reaction mixture	No. of experi- ments	Inhibition (%)
<i>System A</i>			
No inhibitor	1.19 ± 0.04	(3)	—
+ Edetate (1.17 × 10 <sup>-3</sup> M)	0.90 ± 0.07	(4)	24.0
+ SRC-820 (1.0 × 10 <sup>-2</sup> M)	0.85 ± 0.08	(4)	28.1
+ Edetate (1.17 × 10 <sup>-3</sup> M) and SRC-820 (1.0 × 10 <sup>-2</sup> M)	0.82 ± 0.02	(4)	31.1
<i>System B</i>			
No inhibitor	1.13 ± 0.01	(5)	—
+ Sodium fluoride (2.5 × 10 <sup>-3</sup> M)	0.27 ± 0.03	(4)	76.1
+ SRC-820 (1.0 × 10 <sup>-2</sup> M)	1.01 ± 0.03	(4)	10.6
+ Sodium fluoride (2.5 × 10 <sup>-3</sup> M) and SRC-820 (1.0 × 10 <sup>-2</sup> M)	0.22 ± 0.02	(3)	80.6

@PEP was treated with alkaline hypiodite and the released phosphate estimated by Simonsen method (8), since SRC-820 interfered in Fisk-Subba Row's method.

*System A* contained, in a volume of 3.0 ml; Tris buffer (pH 7.0), 60 μmoles; MgCl<sub>2</sub>, 5.5 μmoles; 3-PGA (Sigma) 10 μmoles; enzyme, 0.2 ml supernatant equivalent to 2.0 mg of the acetone powder. Incubation for 2.5 min at 37°C.

*System B* contained, in a volume of 3.0 ml: Tris buffer (pH 7.0), 110 μmoles; MgCl<sub>2</sub>, 10 μmoles; 3-PGA, 10 μmoles; enzyme, 0.1 ml of supernatant, equivalent to 1.0 mg of the acetone powder. Incubation for 5 min at 37°C.

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