In vivo evidence for prostaglandin inhibitory activity in New Zealand green-lipped mussel extract

Thomas Miller PhD, Research Fellow; Henry Wu DVM, Research Associate, Department of Medicine, University of Auckland School of Medicine, Auckland

Abstract

Prostaglandin synthetase inhibitors (eg, aspirin, indomethacin and naproxen) interfere with ovulation and prolong the gestation period in the rat. These properties could provide a novel test bed for the assessment of agents with prostaglandin biosynthesis inhibitory activity.

Rats were fed green-lipped mussel extract and the effects on conception and the subsequent gestation period were determined. Parturition was delayed and fetal development was retarded in the Seatone-fed animals. The observations were consistent with the known effects of nonsteroidal anti-inflammatory drugs, suggesting that Seatone does contain pharmacologically active material inhibiting prostaglandin biosynthesis.

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Introduction

A commercial freeze-dried extract of the New Zealand green-lipped mussel Perna canaliculus (Seatone) has been claimed to provide symptomatic relief in some individuals with rheumatoid and osteoarthritis, but the claims have been questioned [1] and evidence from two clinical trials is conflicting [2,3].

Most laboratory evaluations of anti-inflammatory agents include tests using carrageenan-induced footpad oedema and extracts of the New Zealand green-lipped mussel have been shown to be active in this model [4,5]. Indomethacin, aspirin, naproxen and other similar drugs cause a similar reduction in carrageenan-induced oedema. These agents are believed to reduce inflammation principally through inhibition of prostaglandin biosynthesis and it is possible that Seatone might also act through the same pharmacological mechanism. Interestingly, these nonsteroidal anti-inflammatory drugs also interfere with ovulation [6,7] and prolong the gestation period through the inhibition of prostaglandin biosynthesis [8,9,10].

Rats were fed a normal diet supplemented with Seatone and the effects on conception and the subsequent gestation period were determined. Parturition was delayed and fetal development was retarded in the Seatone-fed animals. On examination, no teratogenic changes could be found and the results suggest that Seatone may indeed contain a pharmacologically active component inhibiting prostaglandin biosynthesis when adminstered orally.

Materials and methods

Animals: Equal numbers of young adult male and female rats from an inbred Dark Agouti strain and weighing 200-225 g were used. Two groups of 20 pairs each were established for each experiment and each pair was separately caged.

Mussel extract: Seatone, a freeze-dried commercial extract of the New Zealand green-lipped mussel (Perna canaliculus), was obtained from McFarlane Laboratories Ltd (Parnell, Auckland, NZ).

Diet: The control groups of 20 rat pairs were fed normal untreated diet 86. The test group of 20 rat pairs were fed diet 86 supplemented with Seatone at a ratio of 12.5 g extract to 1 kg standard diet. Pairing was performed after the animals had been fed these diets for 30 days. Food and water were allowed ad lib.

Time from pairing to parturition: In the first experiment, groups were established and fed as above, and this time was noted for each female rat in both groups.

Fetal development: In the second experiment, groups were established and fed as above. Pairing was allowed for 24 h only and pups were delivered by caesarean section 21 days later (ie, just prior to normal parturition). The weights of all the pups were measured and the first 80 pups from each group were then set aside for skeletal measurement. These 160 pups were processed to achieve transparency of the soft tissues and bone staining by Dawson's alizarin red technique [11]. An examination for skeletal defects was then made under a dissection microscope and the skeletal length was measured from the cranium to the first coccygeal vertebra.

Full term pup size: The progeny of all dams in the first experiment were weighed four days after birth (the shortest practical interval).

Statistical analysis: Parturition times were subjected to the Mann-Whitney U test for population differences. Skeletal lengths and weights of fetuses or pups were subjected to student's t test. All ranges in the text are the standard deviation of the mean.

Results

Time from pairing to parturition: Pairs of rats were established after feeding for 30 days with either normal diet 86 (control group) or diet 86 supplemented with Seatone (test group). All females in the control group produced litters and, in 16 out of the 20 dams, parturition occurred within 27 days of pairing. During the same period, 11 out of the 20 Seatone-fed dams produced litters. One Seatone-fed female failed to conceive and, in another, parturition occurred 71 days after pairing. Overall differences in the time from pairing to parturition for the two groups were compared and found to be significantly different ($p < 0.05$, Figure 1). There was no correlation between parturition times and litter sizes in either group.

Fetal weight at 21 days gestation: All the progeny from both groups in the second experiment were derived by caesarian section and then weighed. Both male and female fetuses from the Seatone-fed dams were lighter than control male and female fetuses, the differences being highly significant ($p < 0.001$): the means and SD for the male fetuses were (test versus control) 2.3 ± 0.8 g versus 3.3 ± 1.2 g and for the females 2.3 ± 0.8 g versus 2.9 ± 1.0 g (Figure 2).

Examination for teratogenic effects: All fetuses delivered by caesarean section were thoroughly examined for teratogenic defects using a dissecting microscope. No defects were found in any of the fetuses from either control or Seatone-fed dams.

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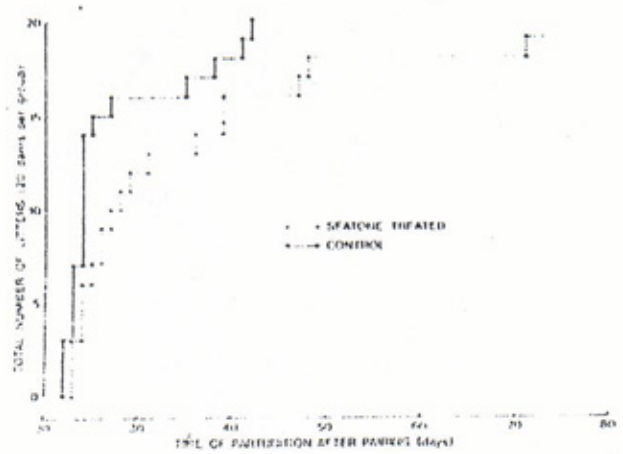

Figure 1.—Delay in parturition in Seatone-fed rats. The interval between pairing and parturition for each of 20 breeding pairs of Seatone-fed rats and 20 pairs of age-matched controls on a normal diet is plotted against the cumulative number of pairs delivering litters (significant difference: $p < 0.05$).

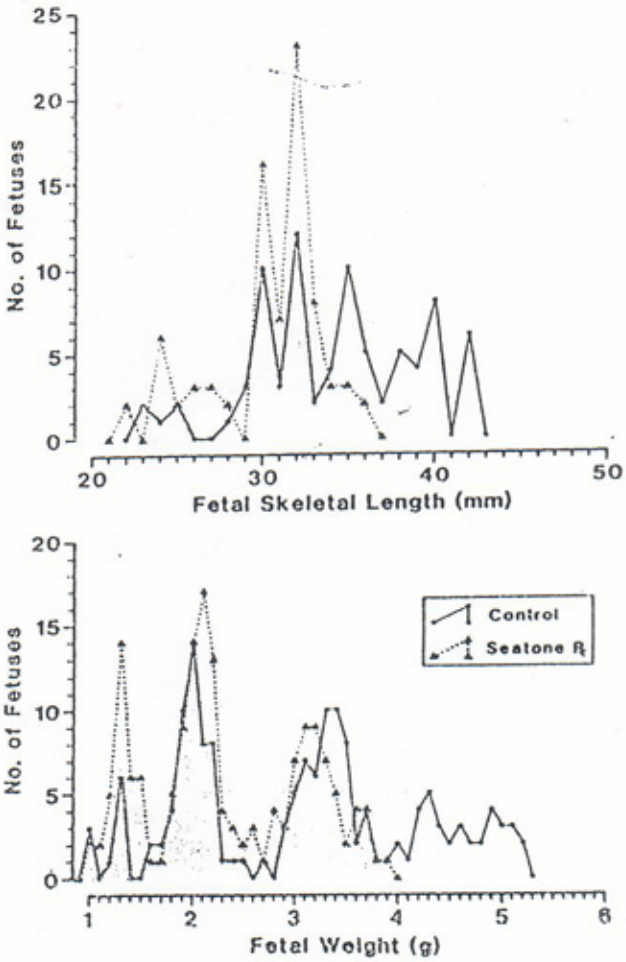

Figure 2.—Frequency polygons showing skeletal lengths and body weights of caesarean delivered 21-day fetuses. All progeny from 20 Seatone-fed dams and 20 control dams were delivered at 21 days gestation by caesarean section at necropsy. (Skeletal length n = 80 for each group; fetal weight n = 164 for Seatone-treated and 155 for controls. Significant difference of both weight and length comparisons: $p < 0.001$).

Skeletal length at 21 days gestation: The first 80 progeny derived by the above caesarean section from each group were measured. A highly significant difference in skeletal length was found when the two groups were compared ($p < 0.001$) in that the progeny of the Seatone-fed dams were smaller (mean of 30.4 ± 3.2 mm) than those of the control dams (mean 34.2 ± 4.7 mm) (Figure 2).

Pup weights at four days: The progeny of dams of both groups in the first experiment were weighed at four days after birth (the shortest practical interval). The mean weights were 8.2 ± 1.9 g and 7.4 ± 1.5 g for progeny from Seatone-fed and control dams respectively; they were not significantly different. These suggested that, although fetal development was delayed in the Seatone-fed group, prolongation of gestation ensured that pups born at term were fully developed and of normal weight.

Adult animals: Seatone administration did not have any observable effect on the condition or behaviour of the experimental groups. Consumption of food and water by the Seatone-fed animals was similar to the control animals; individuals in both groups consumed 15-22 g diet per day over the period of the experiments. No differences were found in weight gain, alertness or cage activity, but the coat condition appeared to be improved by Seatone. The fertility of male rats was not affected by Seatone administration.

Discussion

Our evidence that parturition was delayed and fetal development was retarded in animals fed Seatone provides firm evidence that the extract of the New Zealand green-lipped mussel (Perna canaliculus) is pharmacologically active when administered orally. On the evidence available, it is likely that the material contains an active component inhibiting prostaglandin biosynthesis or altering prostaglandin synthesis by the production of inactive or less active analogues. Previous studies, using carrageenan-induced footpad oedema, have demonstrated anti-inflammatory activity in extracts of the green-lipped mussel after parenteral but not oral administration. These findings were in conflict with the suggested clinical benefits that follow oral administration of the material and required further evaluation.

The experimental approach used in the present studies offered several advantages over standard methods used to demonstrate nonsteroidal anti-inflammatory activity. In the first instance, the test material was able to be taken orally as a food supplement, thus mimicking its use in practice. Secondly, the procedure allowed for the prolonged administration of the material, which is also similar to its recommened use. Thirdly, the linkage between an active pharmacological component (prostaglandin inhibition) and the biological end point (delayed parturition and fetal development) is direct and is supported by a considerable body of literature.

Many nonsteroidal anti-inflammatory drugs have been shown to inhibit prostaglandin biosynthesis [12,13] and it has been shown that prostaglandins play a significant role in the biology of reproduction [6,14]. Several reports have shown that prostaglandin synthetase inhibitors (such as indomethacin, aspirin and naproxen) delay ovulation [7,15,16], labour [17] and parturition [8-10,18], as well as extend the gestation period in rats [10]. These compounds are all well characterised pharmacological agents and are known for their ability to modify the local inflammatory response in rheumatoid and osteo-arthritis. Studies by Powell and Cochrane [10] have investigated the relationship between the structure and activity of nonsteroidal anti-inflammatory agents, and the blocking of parturition and the prolongation

of gestation in the rat. Their experiments have confirmed the procedure as a valid measure of potential anti-inflammatory activity. Recent reports have likened several stages of reproduction to the inflammatory process [19,20] and these observations add credibility to the use of the course of pregnancy in the rat as a model for evaluating agents capable of modifying prostaglandin biosynthesis.

The results obtained provide strong support for our belief that the extract of green-lipped mussel does indeed contain pharmacologically active material with the characteristics expected of an anti-inflammatory agent inhibiting prostaglandin biosynthesis. Careful examination of the fetuses failed to demonstrate any teratogenic effects which might have explained the results. A possible criticism of our experiments could be that we did not include a study of the effects of indomethacin or a related drug as a 'positive control'. These effects have already been observed [6,8] and, apart from posing logistic problems, the addition of such a group would not have contributed to the interpretation of the Seatone results. Indeed, similar studies have not employed such controls either [21]. The present work does conflict with our previous studies which showed that extracts of the green-lipped mussel were only active in suppressing inflammation when given parenterally [4,5]. Although the methodologies used in these studies were obviously different, the possibility that prolongation of gestation could be due to mechanisms other than prostaglandin inhibition, and therefore not imply anti-inflammatory activity, also needs consideration.

One outcome of these experiments has been the establishment of a pharmacological basis for the continued evaluation of the anti-inflammatory activity of the green-lipped mussel. This agent has attracted world-wide attention but has so far lacked laboratory evidence to support its claimed efficacy. Efforts are currently being made to formally identify the active component as a logical extension to the present observations.

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To view the practice of medicine as just another business undertaking like retailing or banking is to be blind to the role of agency in the work of a professional. To rely on the market to discipline money-grubbing professionals is to overestimate what the market should be asked to do or is capable of doing.

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We must also be reasonable in our own demands for recompense. Doctors deserve a good living but not an extravagant one. Greed and medical care are not compatible. We must take major responsibility ourselves for cost containment, not leaving it to government or to corporations, or we will lose our independence.

Davidson C. Are we physicians helpless? N Engl J Med 1984; 310: 1117-8.

It is particularly ironic that the work schedules followed by members of the health professions (particularly house officers, nurses, and technical personnel who must care for patients round the clock) are among the most disruptive as far as the circadian sleep-wake cycle is concerned. They are not easy to improve, however, partly because of the unique responsibilities of the physician and also because of the deeply entrenched traditions of the medical profesion. The weight of evidence both from the laboratory and from field studies suggests it is now time to address the problems of the disruption of the sleep-wake cycle in medical personnel . . . The ultimate benefit will be to the patient.

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. . . what matters socially and therefore historically in the field of 'race' is not science but beliefs.

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