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EXTRACTS OF RHINOCEROS HORN ARE NOT ANTIPYRETIC IN RABBITS

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ABSTRACT

We administered extracts of the horn of the African Black rhinoceros intragastrically to 7 rabbits, at the same time as injecting bacterial lipopolysaccharide (LPS) intravenously into the rabbits to produce fever. At a dose of horn (50 mg/kg) similar to that allegedly used to reduce human fever, and at ten times that dose, the fever response to LPS was not significantly different ($P > 0.05$, t-test) to the response to LPS injection when boiled water was administered instead of horn extract. The known antipyretic indomethacin, however, at a dose of 10 mg/kg significantly reduced the response to LPS.

KEY WORDS

fever, lipopolysaccharide, Chinese medicine, ethnopharmacology

INTRODUCTION

The horn of the African Black rhinoceros (*Diceros bicornis*) has been an important feature of the Chinese pharmacopoeia for at least 2000 years. As a result of the avid demand for horn products, primarily in Eastern communities, and the considerable financial rewards to those who procure horn for subsequent resale, the world's rhinoceros population, and that of the African Black rhinoceros in particular, is near extinction /1,2/. Rhinoceros horn is sought after for a variety of uses, but most often to relieve the signs and symptoms of infection, and particularly to reduce fever /1,3-5/. Only recently,

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however, have conventional scientific techniques been employed to test whether or not rhinoceros horn actually does reduce fever.

Some of the information on the properties of rhinoceros horn has been published in journals of Chinese medicine, and is effectively inaccessible to the scientific community at large. What emerges from reports available for scrutiny, however, is contradictory and inconclusive /5/. Some of the confusion arises because extracts of horn have been given to different experimental animals, by a variety of routes, in varying doses, and sometimes together with herbal or other accompaniments, as is the custom in Chinese medicine /3/. Also, the method of preparation of the horn, the dose, and the route of administration have not closely resembled the practices of Chinese medicine.

We have now studied the possible antipyretic properties of extracts of rhinoceros horn, using procedures which attempt to simulate the practices of Chinese human medicine, and incorporating standard international procedures for assaying antipyretic agents. We have used rabbits as experimental animals; because of known similarities in the mechanisms of fever production and antipyresis in rabbits and humans /6/, rabbits are the preferred animals in pyrogen assays. We administered horn extracts intragastrically, simulating the conventional human route. We made aqueous extracts of horn, following the Chinese recipe, without herbal accompaniments. We used two doses. The lower dose was that apparently used to reduce fever in patients according to Chinese custom (approximately 0.06 g/kg) /7/; the other dose was ten times higher. We induced fever by injecting a standard febrile dose of lipopolysaccharide, the pyrogenic component of Gram-negative bacterial cell walls, a pyrogen to which humans and rabbits are extremely sensitive /6/. The effects of the extracts of rhinoceros horn were compared first to the effects of horn extracts from another large African mammal, the reedbuck, and second to the effects of a well-established antipyretic, indomethacin. Our results lead us to believe that rhinoceros horn, in doses similar to the traditional human dose, is not antipyretic against bacterial pyrogen fever.

METHODS

Preparation of horn extracts

Horn from the African Black rhinoceros (*Diceros bicornis*) was donated by conservation authorities of the Natal Parks Board. We also obtained a sample of horn from the reedbuck (*Redunca arundum*), a non-endangered species of southern African antelope, the horn of which is not associated with any medicinal use, to our knowledge. Following traditional Chinese custom /1,7/, after washing the horns, we filed them to obtain fine shavings, over which we poured boiling water. The solutions were allowed to steep until they had cooled to approximately 38°C. The concentration of extracts of horn in the solutions was either 20 mg horn/ml H_2O or 200 mg horn/ml H_2O. Before we administered the extracts, we passed the solutions through a coarse sieve, to remove any large shavings of horn which might obstruct the intragastric catheters (see below).

Other agents

Fever was induced in the rabbits by an injection into an ear vein of 0.1 μg/kg of the purified lipopolysaccharide (LPS) of *Salmonella typhosa* (Difco) dissolved in sterile saline. As a positive control for antipyresis, we used the non-steroidal anti-inflammatory drug indomethacin. The contents of indomethacin capsules (Indocid, Logos, Johannesburg), 25 mg active ingredient, were suspended in sterile water immediately before intragastric administration at a dose of 10 mg/kg. Boiled water was used as the negative control for antipyresis.

Experimental animals and surgical techniques

New Zealand White rabbits of both sexes and of body weight approximately 3 kg were used in all experiments. The rabbits were housed individually, and fed standard rabbit pellets and water *ad libitum*.

At least 10 days before administering the agents, we inserted intragastric catheters in each of seven rabbits, under sterile operating theatre conditions. With the rabbits under alphaxolone/alphadolone general anaesthesia (Saffan, Glaxo, Johannesburg) a polyethylene catheter (1.8 mm OD and 1.0 mm ID), approximately 300 mm long,

was inserted into the animal's stomach, via a pharyngostomy, according to techniques described in detail previously /8/. The position of the catheter in the stomach was verified by aspiration of stomach contents. The catheter was exteriorized through the pinna of the ear, and the free end, protruding about 10 mm from the skin, was plugged using a Luer stub adaptor with a cap closure (Intramedic, Beeton Dickinson Company). The rabbits tolerated the intragastric catheters well for the several weeks of the study. Body masses of the animals were checked daily, to ensure that the intragastric catheters did not affect the animals' nutrition.

All administrations via the intragastric catheter were made in volumes of approximately 10 ml, with a flush of a further 10 ml of water. Immediately after administrations, the free, exteriorized end of the catheter was capped, to prevent any back-flow of fluid.

Temperature measurements

Body temperature of each rabbit was measured by inserting a copper-constantan thermocouple into the rectum, to a depth of about 100 mm. The thermocouples had been calibrated to an accuracy of 0.1°C against a standard mercury-in-glass thermometer. Thermocouple output was recorded on a data logger (Esterline Angus).

Experimental procedures

All experiments were carried out at room temperature (21-23°C). The rabbits were conscious and restrained in conventional rabbit stocks throughout the temperature measurements. Body temperature was monitored for forty minutes before, and for four hours after injections.

In the assays of antipyresis, the rabbits received an intravenous injection of LPS in a volume of 2 ml, and simultaneous administration, via the intragastric catheter, of either 10 ml boiled water, or a solution of horn extract made up to 10 ml, or of 10 mg/kg indomethacin in 10 ml water. In one series of experiments, rhinoceros or reedbuck horn was given in a dose equivalent to 50 mg of original horn solids per kg rabbit mass, and in another series at a dose equivalent to 500 mg/kg. In a further series of experiments, sterile saline was injected intravenously instead of the LPS, and simultaneous

intragastric administrations of rhinoceros or reedbuck horn were given in both doses.

Statistics

We used Student's paired and unpaired t-test, with Bonferroni correction, where appropriate, for multiple comparisons, to assess differences in body temperature responses to the substances injected. Values of $P < 0.05$ were considered to be significant.

Ethics

All procedures received prior approval from the Animal Ethics Screening Committee of the University of the Witwatersrand under protocols 88/154/5 and 91/128/4.

RESULTS

Figure 1A shows body temperature responses of seven rabbits to intravenous injection of LPS, with simultaneous intragastric administrations of either boiled water, or extract of rhinoceros horn or reedbuck horn in boiled water, at a dose of 50 mg/kg. In each case, after a latent period of about 20 minutes, body temperature rose to result in a biphasic fever. Neither the time-course of the fever nor the maximum temperature that ensued after LPS injection was affected by the administration of rhinoceros horn or reedbuck horn.

Figure 1B shows the 4-hour fever index (FI), the time integral of the fever response, calculated for the response of the rabbits to LPS with and without extracts of rhinoceros or reedbuck horn at the dose of 50 mg/kg. There was no significant difference between the FI when extracts of either horn were administered together with LPS, compared to when LPS was given together with boiled water. Also shown in Figure 1B are the FIs for administration of rhinoceros and reedbuck horn extracts together with an intravenous injection of sterile saline, instead of LPS. Neither horn extract had any significant effect on the body temperature of the afebrile rabbits.

Figure 2 shows that increasing the dose of rhinoceros or reedbuck horn to 500 mg/kg did not elicit any antipyretic effects on LPS fever. That the effects of the LPS could be antagonized by a known

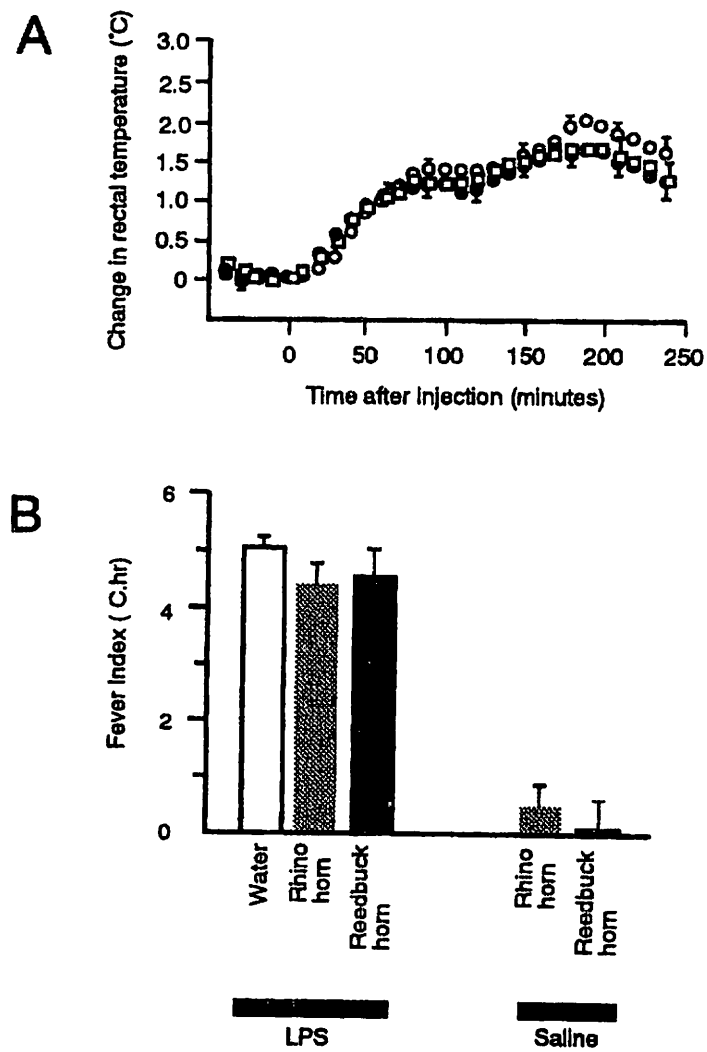

Fig. 1: **A:** Changes in rectal temperature of rabbits after injection intravenously at time 0 of bacterial lipopolysaccharide (LPS, 0.1 μg/kg) together with an intragastric administration of boiled water (O), 50 mg/kg rhinoceros horn extract (●) or 50 mg/kg reedbuck horn extract (□). Each point is the mean ± SEM for 7 rabbits. Ordinate, change in rectal temperature, in °C; abscissa, time after injection, in minutes. **B:** Four-hour fever indices (in °C.hr) of the responses shown in **A**, and when sterile saline was substituted for the LPS solution, showing the effects of horn extracts on afebrile animals. Fever indices following LPS injection did not differ significantly from each other ($P>0.05$, t-test with Bonferroni correction). Fever indices following saline injection did not differ significantly from zero.

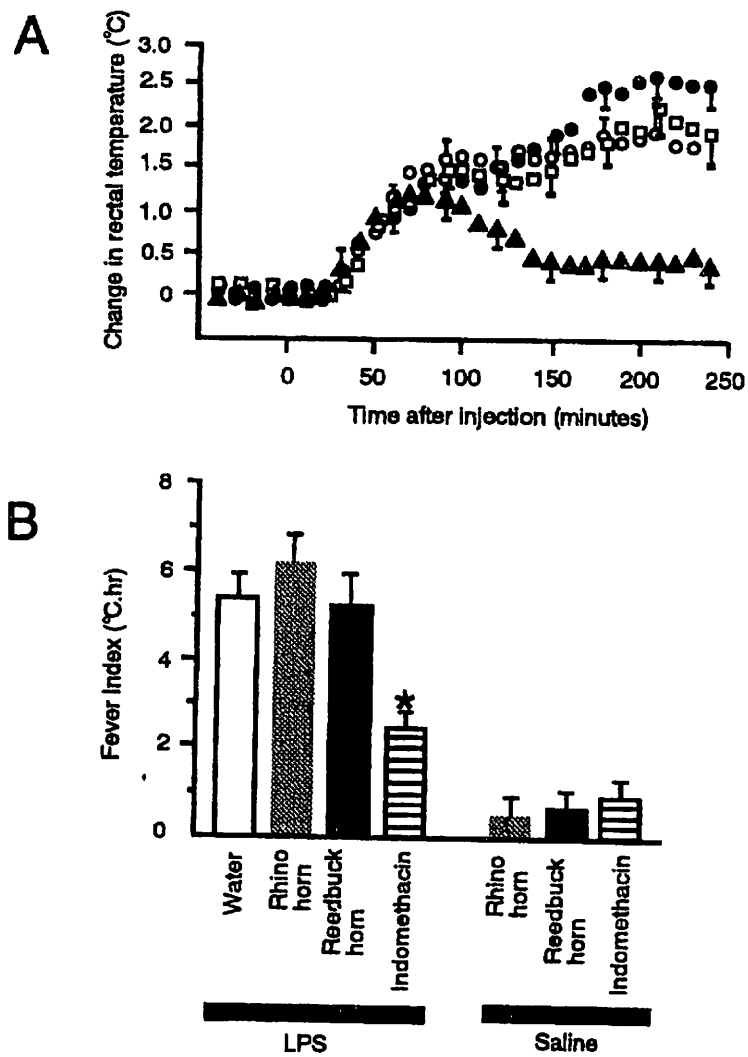

Fig. 2: **A:** Changes in rectal temperature of rabbits after intravenous injection of LPS (0.1 μg/kg) together with an intragastric administration of boiled water (O), 500 mg/kg rhinoceros horn extract (●), 500 mg/kg reedbuck horn extract (□) or 10 mg/kg indomethacin (▲). Other details as in Figure 1. **B:** Four-hour fever indices (in °C.hr) for the responses shown in **A**, and when sterile saline was substituted for the LPS solution, showing the effect of horn extracts on afebrile animals. The four-hour fever index following LPS injection and intragastric indomethacin administration was significantly lower than that which followed LPS injection and water administration ($P<0.05$, t-test). Fever indices for administration of horn extracts or indomethacin after saline injection did not differ significantly from zero.

antipyretic is evident from the temperature response to indomethacin administration. The effects of indomethacin administered intragastrically simultaneously with the pyrogen were not evident until two hours post-administration (Fig. 2A), at which time dramatic antipyresis occurred. Again, neither rhinoceros nor reedbuck horn extracts had any significant effect on the time-course or magnitude of the febrile response. As shown in Figure 2B, only in the case of indomethacin administration was the FI significantly different to that calculated for the control LPS injections.

DISCUSSION

We have assessed the putative antipyretic effects of rhinoceros horn in a manner which, we believe, simulates its administration to febrile patients more closely than does any previous study. Four features, in combination, set our study apart from those previously published. Firstly, we studied the effects of rhinoceros horn fever induced by a bacterial pyrogen, a common cause of febrile illness in humans /6/. Secondly, we administered the rhinoceros horn extract via an intragastric catheter, thus delivering the extract to the same site as febrile patients who might take horn extract orally. Thirdly, we administered doses of horn extract in the same range that would be consumed by a febrile patient. Finally, we used rabbit fever as a model for human fever. Clearly it would have been more convincing to use human subjects for the study, but we did not wish to risk repeated pyrogen injections in humans. We chose rabbits as the best alternative, because of the known similarities in febrile response between rabbits and humans, among them a consistent rise in body temperature during infection which is not ambient temperature dependent, and close similarities in the thermoregulatory strategies and biochemical pathways used to generate fever /6/. Our results show unequivocally that, in rabbits, rhinoceros horn extract, when given intragastrically and at a dose similar to that apparently used by febrile patients, has no antipyretic effect on bacterial pyrogen fever.

In other studies which have also attempted to simulate the oral ingestion of rhinoceros horn by patients, rhinoceros horn had no effect against the hyperthermia induced by the intracerebral injection of adrenaline in rabbits, or against *E. coli* fever /1/.

To our knowledge, the only studies in which rhinoceros horn appeared to be effective against hyperthermia were those in which rats, known to have variable responses to simulated infection, were used as experimental animals; rhinoceros horn was administered not orally, but intraperitoneally; the hyperthermia was induced by a subcutaneous injection of turpentine oil; and, by the authors' own admission, at doses of rhinoceros horn that were up to 100 times that likely to be used by humans /1,3/. In one of the studies /1/, rhinoceros horn, in the highest dose, affected the hyperthermia no better than did horn of the saiga antelope, or of cattle. In the other, a lower dose (but about 20 times that of the mass-specific traditional dose for humans) of rhinoceros horn extract reduced the hyperthermia, but only when in the form of a decoction with eight herbs. However, the herbal extracts alone reduced the hyperthermia. In other hands too /9/, combinations of Chinese plant material apparently are antipyretic.

Can one speculate why very high doses of horn, whether from rhinoceros, cattle or saiga antelope /1/, administered intraperitoneally, might attenuate the hyperthermia which follows administration of turpentine oil to rats? The horns consist primarily of keratin, other proteins, amino acids, peptides, sterols, and guanidine derivatives /1,5,10,11/. Traditional antipyretic agents, such as the non-steroidal anti-inflammatory agents, of which indomethacin is an example, act by inhibiting the synthesis of prostanoids /12/ induced by cytokine mediators of the response to bacterial infection. Though some peptides are antipyretic, we know of no reports that any of the constituents of horn, either individually or in combination, has any effect on prostaglandin synthesis. No possible mechanism of antipyretic action of high doses of horn in rats was suggested by But *et al.* /1,3/, nor do we know whether, injected in the absence of inflammation, at the apparent antipyretic doses, horn extracts affected rat body temperature. In our hands, neither the horn of the reedbuck nor of the rhinoceros had any effect on body temperature in afebrile rabbits.

Our results and those of others /1,5/ imply that rhinoceros horn extract, administered directly to the gastrointestinal tract in doses up to ten times those taken by febrile patients, is not antipyretic in rabbits. If rhinoceros horn's effect in febrile humans were to be significantly different to its effect in febrile rabbits, rhinoceros horn would be the first agent to be antipyretic in humans but not in rabbits /13/ and we do not expect it to be. Rhinoceros horn, administered

intraperitoneally and at doses two orders of magnitude higher than the normal human dose, appears to reduce high body temperature in rats after peripheral injection of turpentine oil, but is no more effective than at least three other types of horn /1/. As a possible anti-inflammatory agent, therefore, rhinoceros horn has no special role. We contend that there is no scientific evidence supporting a role for rhinoceros horn as an antipyretic. Certainly, no horn is as effective as the conventional synthetic antipyretic agents that are widely available. The near extinction of the African Black rhinoceros has been based largely on a myth.

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