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A Combined Regimen Study of WR 2721 and Sodium Nitrite for Protection against Cyanide Intoxication in Mice

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The antidotal action to cyanide poisoning by a combined regimen of sulphydryl compound WR 2721 and methemoglobin former, sodium nitrite was investigated in mice. The prophylaxis efficacy of the regimen was evaluated by administering candidate compounds in different doses to mice challenged by 2 × LD50 of sodium cyanide. A significant protection was observed against cyanide poisoning at a combined dose of WR 2721 400 mg/kg and $NaNO_2$ 100 mg/kg. The individual candidates when injected ip. in different doses did not show much potential against cyanide toxicity.

INTRODUCTION

Cyanide is listed as one of the most dangerous toxic substances which causes death rapidly on exposure, ingestion or inhalation. It would be desirable for persons who anticipate a possible exposure to cyanide to have a prophylaxis treatment. A survey of the literature for cyanide detoxification indicates that a number of antidotes are being used to antagonise the cyanide.[1–4] One of the methodologies is the use of compounds which directly bind the cyanide,. *e.g.* dicobalt edetate (Kelocyanar), Vit B12, hydroxocobalamine, cobaltous chloride, pyruvate and ketoglutaric acid etc.[4, 7] These compounds, though effective, are generally reported to cause severe anaphylactic reactions reducing the blood pressure, heart rate values, etc. and, therefore, their applications as antidote are limited. The other approach is of indirect binding of cyanide by use of potential methemoglobaemia inducers *e.g.* dimethylamino phenol, sodium nitrite, hydroxylamine hydrochloride and phenones.[8, 9] These compounds induce the hemoglobin to a cyanide reactive methemoglobin. Recently, Bhattacharya *et al.* [10–12] have shown that a co-administration of methemoglobin formers, (sodium nitrite, hydroxylamine or dimethylamino phenol) offers a sustained protection against cyanide poisoning. Because of the inherent danger of excessive levels of methemoglobin formation, large doses of these inducers cannot be employed. The third most commonly used approach is use of sulphane sulphur compounds.[1, 8]

Presently, a combined treatment of sodium nitrite and sodium thiosulphate is being used as a well accepted regimen antidote.[7, 13] However a shortcoming of this combination is the slow diffusion rate of sodium thiosulphate in the body. WR 2721 hydrolyses rapidily *in vivo* to a sulphur donor thiol. Therefore,

considering the basis for cyanide detoxification of combined regimen, it is logical to study sulphydryl compound WR 2721 or its disulphide derivative in combination with $NaNO_2$.

MATERIALS AND METHODS

Male albino mice were used during the experiments. The mice were fasted overnight while access water was allowed. Sodium nitrite, sodium cyanide, sodium thiosulphate used were of analytical grade. WR 2721 and its disulphide derivative were synthesized in the laboratory by the method reported.[14] The mice were divided into 6 experimental groups and totalling 140 in number. Each group was administered the candidate antidote and cyanide by i.p. in saline solution. Sodium cyanide was also given by i.p. twice of the lethal dose. The total volume of the injection was given 250 μL/20 gm of body weight. The positive control mice were administered sodium nitrite (100 mg/kg) and sodium thiosulphate (1000 mg/kg). WR 2721 in combination with sodium nitrite and WR 2721 alone were also given in different doses.

RESULTS AND DISCUSSION

The pretreatment control group was administered sodium nitrite and sodium thiosulphate 30 min before the cyanide was given. The combination of the proposed antidotes, *i.e.* $NaNO_2$ and WR 2721, was given simultaneously before the challenge of cyanide. The mortality was observed in each group, after 60 minutes of the cyanide injection. The effectiveness of the dose at 100, 400 and 500 mg/kg of WR 2721 alone and in combination with sodium nitrite at 100 mg/kg was examined. Similarly disulphide of WR 2721, alone and in combination withtion dose at 100, 200 and 300 mg/kg respectively were tested. All the administrations were carried out 30 min before the sodium cyanide challenge.

The behavioural change in all the mice was observed as per the criteria laid down by a newly developed scoring system of Dulaney *et al.*[6] A toxicity scale has been drawn in terms of the behaviour, depending on the kind of change in behaviour and severity signs of toxicity. Four types of score were given to the animals. A zero level score shows no toxicity, *i.e.* normal or grooming activity by the animal after the toxic dose. A sign of mild respiratory distress but still retaining use of intracostal as well as diaphragmatic muscles was given a score of one. The toxicity score of two was assigned to those animals which showed a distress in respiration and using intracostal muscles. A third grade of toxicity is the acute one which exhibits a heavy distress in respiration and convulsions of biting of the air.

The behaviour of all the mice in a cage was observed after every five minutes and a number was given to each animal for identification till it recovered for approximately one hour. The antidote efficacy and change in behaviour pattern of the animals are depicted in Figs. 1 to 6.

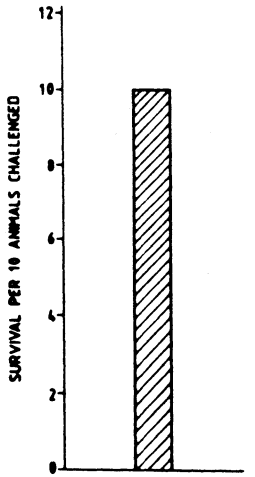

Fig. 1. Sodium thiosulphate and sodium nitrite at the dose of 1000, 100 mg/kg respectively.

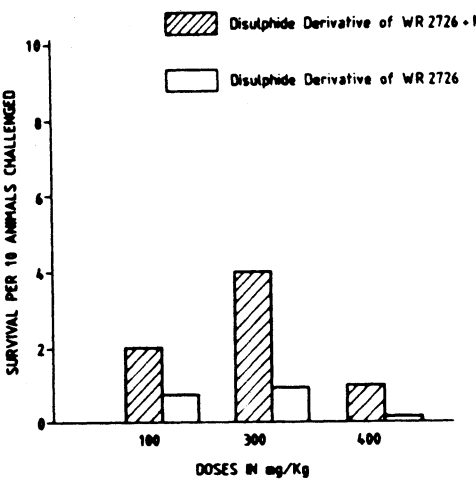

Fig. 2. Survival efficacy of the candidate pretreatment against 2 × LD50 × NaCN to mice.

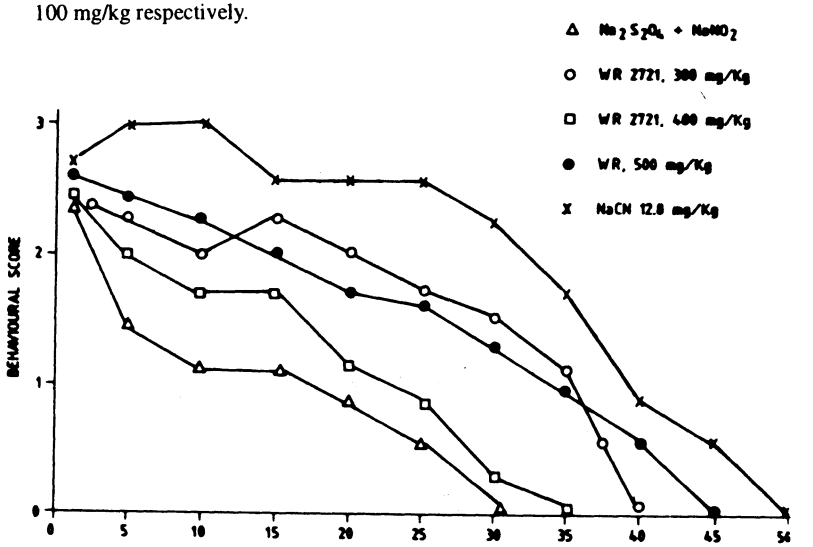

Fig. 3. Behavioural score of WR 2721 in combination with $NaNO_2$.

The behavioural curves (Figs. 1–4) indicate that the death in most instances was due to convulsion and loss of motor function after the 2 × LD50 dose of NaCN. The prophylactic administration of the proposed regimen (WR 2721 + $NaNO_2$) was found to keep the mice alive up to 80% against cyanide poisoning.

Different doses of WR 2721 with sodium nitrite were tried in mice i.p. It was found that a dose of WR 2721 (400 mg/kg) gives comparable protection to control. However, the lower doses *i.e.* 200 and 300 mg/kg showed less protection when WR 2721 was given to the animals. Only a combination study *i.e.* WR 2721 (400 mg/kg) and $NaNO_2$ (100 mg/kg) gives significant survivals to the animal at a 2 × LD 50 dose of sodium cyanide (Fig. 3). The injection of disulphide

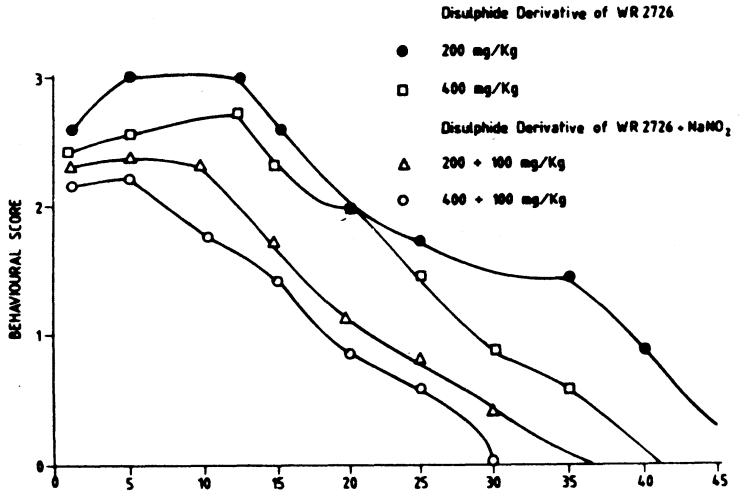

Fig. 4. Behavioural study of sori alone and in combination with $NaNO_2$.

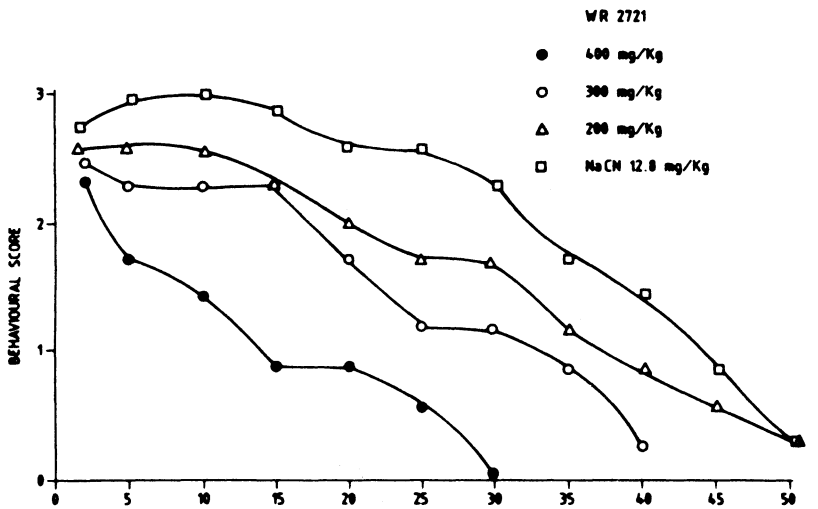

Fig. 5. Behavioural study of WR 2721 alone.

derivative of WR 2721 alone and in combination resulted in change of mortality rate from 60 to 100%. From the above data, it is clear that a regimen of WR 2721 and $NaNO_2$ shows antidotal effect and the candidate compounds alone do not have much efficacy against cyanide. The mechanism of protection, however, is not very clear.[15–17] $NaNO_2$ converts the hemoglobin to a methamoglobin, in various percentages depending upon the dose of $NaNO_2$.[18] It has been observed that sodium nitrite (100 mg/kg of body weight) is nontoxic to the animals. The methamoglobin obtained reacts with cyanide removing it from the blood stream. The cyanide ion at tissue or cellular level may be detoxified by the sulphur donor compounds. *In vivo* the enzyme rhodanase forms an intermediate persulphide with the sulphane sulphur and this neutralizes the cyanide ion to form a nontoxic thiocyanate.

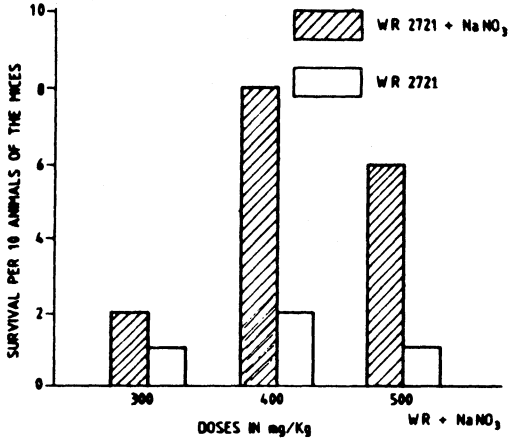

Fig. 6. Survival efficacy of WR 2721 alone and in conbination with $NaNO_3$ pretreatment against 2 × LD50 × NaCN to mice.

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