

## Short Communications

# Naloxone as an Adjuvant in Chemotherapy of an Experimental Tumor

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**Summary.** The antitumor effect of DAB (L-2,4 diaminobuturic acid) has been demonstrated in a previous study. Severe side-effects (especially weight loss and severe neurological symptoms) accompanying DAB treatment have raised the theory that DAB, through a direct effect on the hypothalamus, might cause a diabetes insipidus-like condition, which in turn would activate endogenous opiate systems.

This study has verified this state of dehydration and hemoconcentration in a group of 41 mice treated daily with 0.5 ml 0.1 M DAB solution IP. A rise in the serum albumin concentration to 28.8 g/l (SD 2.04) was demonstrated, as against 23.7 g/l (SD 2.3) in a control series. Furthermore, to prevent the neurological side-effects, an adjuvant treatment with an opiate antagonist (Nalone; naloxone) was tried in a group of 19 tumor-bearing mice receiving DAB. This group was compared with a group of 19 tumor-bearing mice receiving DAB only. The mortality rate was significantly reduced in the group receiving Nalone together with DAB (2 dead out of 19) compared with the other group (9 dead out of 19). The tumor weight reduction was about the same in the two groups, 40.5% and 46%, respectively.

Combined treatment with DAB and Nalone seems to indicate a possible way of reducing the severe side-effects hitherto accompanying DAB alone, making this unique amino acid a potentially useful antitumor agent.

**Key words:** Murine fibrosarcoma, L-2,4-DAB, incubation experiments and treatment with – Side-effects – Naloxone (Nalone), concomitant treatment with DAB

## Introduction

The antitumor effect of a nonphysiological amino acid, L-2,4 diaminobuturic acid (DAB), has been demonstrated in a previous investigation (Ronquist et al. 1980). This amino acid at a concentration of 10 mmol/l produced 100% cell death within the first 20 h when fibrosarcoma cells were incubated in an in vitro experimental system. The irreversible tumor cell damage was most probably due to an osmotically induced lytic effect on the cells as a result of an influx of this amino acid that did not obey the laws of saturation kinetics.

The amino acid also displayed an unequivocal activity against neoplastic growth in vivo. This was exhibited in mice in which fibrosarcoma cells had been transplanted and which received regular treatment with DAB by IP injections. There were, however, serious side-effects in the animals with elevated concentrations of DAB, and several deaths among the mice were apparently drug-related (Ronquist et al. 1980). Weight loss and neurological symptoms involving rigor and spasm and stiffness of the tail and extremities were prominent features. Since the weight loss took place at a rate exceeding that caused by simple starvation, other mechanisms had to be considered. An alternative mechanism behind the relatively sudden weight loss would therefore be plausible.

One such mechanism may be the induction during DAB therapy of a diabetes insipidus-like condition with dehydration and hemoconcentration. Such a condition may stimulate endogenous opiate systems, and opiate receptors have been recognized in both the central nervous system (Lord et al. 1976) and the peripheral tissues (Bloom et al. 1978). Opiate antagonists have recently been reported to play a role in the treatment of hypotension and hypovolemic shock (Faden and Holaday 1979; Janssen and Lutherer 1980).

The aim of the present investigation was to study the possible development of dehydration and he-

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Abbreviation: DAB, L-2,4 diaminobuturic acid

**Table 1.** Summary of treatment with DAB alone or with naloxone in tumor-bearing mice

Series <sup>a</sup>	Number of treated animals within each series	Number of days with treatment	Duration of tumor growth in surviving animals (days)	Total amounts of 0.1 M DAB (ml) given to surviving animals (mean $\pm$ SD)	Calculated mean amount of DAB given per day (ml)	Fatalities during treatment	Total amount given to animals not surviving to end of experiment (mean $\pm$ SD)
1	9	16	23	13.1 $\pm$ 1.0	0.80	5	6.7 $\pm$ 2.6
2	10	17	24	12.7 $\pm$ 1.9	0.75	4	3.5 $\pm$ 0.6
3	9	15	22	11.9 $\pm$ 1.8	0.80	2	3.3 $\pm$ 2.1
4	10	14	22	9.7 $\pm$ 0.9	0.70	0	0

<sup>a</sup> Series 1 and 2, no naloxone given; series 3 and 4 received naloxone

**Table 2.** Effects of treatment with DAB alone or with naloxone in tumor-bearing mice

Treatment	Treated animals	Dead during treatment	Controls	Dead controls	Tumor weight reduction
DAB (Group I)	19	9	10	0	46%
DAB + naloxone (Group II)	19	2	10	2	40.5%

moconcentration as monitored by changes in serum albumin concentration during DAB treatment in mice and the possible effect of an opiate antagonist in prevention of serious neurological side-effects during treatment.

## Material and Methods

### Chemicals

L-2,4-Diaminobuturic acid (dihydrochloride with molec. wt 191.1) was obtained from Serva Fine Biochemicals, Federal Republic of Germany. Naloxone was a generous gift from Dr T. Döberl, Sterlin-Winthrop, Stockholm, Sweden.

### Animals

Adult white male mice of the NMRI strain (Anticimex, Stockholm, Sweden) with an average weight of about 30 g and fed an ordinary diet (from Astra-Ewos AB, Sweden, type R3) were used for the experiments.

### In Vivo Experiments

A transplantable experimental fibrosarcoma was maintained in tissue culture. For the experiments  $5 \times 10^7$  cells were transplanted sc into the right flank to each of 58 animals (cf ref). The experiments were run in two different groups and each group contained two different series. In the first group 19 mice were treated with DAB alone and in the second group 19 animals were given DAB and naloxone 0.1–0.3 ml daily, either with DAB or, most often, 4–8 h after DAB administration. Ten mice receiving no DAB served as controls in each series. Treatment began on day 4 after transplantation and 0.60–0.90 ml isotonic 0.1 M DAB solution (60–90  $\mu$ mol) was given IP, generally as a single dose daily. The animals received treatment for 14–17 days. They were tumor-bearing for 22–24 days before termination of the experiment (Table 1). The solid tumors were removed surgically and weighed.

### Administration of DAB in Non-Tumor-Bearing Animals and Collection of Blood Samples

Forty-one mice (NMRI) received 0.5 ml 0.1 M DAB solution IP daily for 1–13 days. Blood samples were taken daily by heart puncture and the serum fraction after centrifugation was frozen until analysis of serum albumin. These results were compared with those from a control group of 11 animals which received 0.5 ml isotonic NaCl solution IP daily.

Two animals died soon after the DAB injection, most probably due to accidental injection into a blood vessel. Two mice showed severe neurological symptoms of rigor and spasm and stiffness of the tail, and also severe weight loss.

## Results

It is evident from Table 1 that the amount of DAB given to the animals in the four different series was comparable. The animals that did not receive naloxone protection were given a little more DAB than the others but the experiment was also somewhat extended (Table 1). Therefore, if the overall doses are recalculated on a per day basis, all animals received about the same amount of DAB (Table 1). Nevertheless, the number of fatalities was substantially higher in the absence of naloxone treatment (Table 1). Among the DAB-treated animals nine died during treatment and they all showed drowsiness, loss of appetite and pronounced rigor and spasm. In the surviving animals the weight of the tumors was reduced by 46% compared with controls (Table 2). In the two other series where naloxone was added to the DAB treatment only two animals died. One of them died suddenly and unexpectedly and the other one also had

none of the above-mentioned toxic symptoms. The tumor weight reduction in these two series was 40.5% (Table 2).

Animals receiving daily injections of 0.5 ml DAB displayed a high mean value for serum albumin, viz.  $28.8 \pm 2.04$  g/l (range 25–35 g/l). This value contrasted sharply with the significantly lower ( $P < 0.001$ ) value seen among mice receiving isotonic NaCl solution,  $23.7 \pm 2.3$  g/l (range 21–27 g/l).

## Discussion

This study shows, although comprising a limited number of mice, that the side-effects induced by DAB when given to mice IP can be substantially reduced by naloxone. Furthermore, this ameliorating effect of naloxone did not seem to influence the antitumor activity of DAB. The reduction of tumor growth was about the same with or without naloxone, and was consistent with previous results (Ronquist et al. 1980).

The serious side-effects of DAB were of two main types, possibly connected with each other, i.e., severe weight loss and particular neurological symptoms presenting as rigor and spasm and stiffness in the tail and extremities.

The substantial weight loss could most easily be explained by dehydration, which was probably due to a diabetes insipidus-like condition. This could not have been due to osmotic diuresis induced by DAB, because of the small amounts of this substance that were given. Instead we propose a mechanism that might be exerted in the neurohypophysis itself or in the renal tubules. The significant rise in the serum albumin concentration in DAB-treated animals compared with the controls also emphasizes a dehydration state.

The dehydration and the concomitant hypovolemia may stimulate endogenous endorphin production and release of endorphins (Faden and Holaday 1979). These events could be responsible for the neurological symptoms observed in some animals during treatment with DAB. Such a proposal would be in accordance with the finding of Lord et al. (1978), who reported the presence of multiple opiate receptors intracerebrally. The unequivocal effect of naloxone observed in the present study when it was given together with DAB was therefore most probably due to a direct action of this potent opiate antagonist at receptor level in the target cells. This hypothesis is corroborated by the observations of Faden and Holaday (1979), who found a direct effect of naloxone in the treatment of hypovolemic shock in rats.

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