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● Session A: Tissue Oxygenation Manipulation and Tumor Blood Flow

BIOCHEMICAL AND PHYSIOLOGICAL CHANGES INDUCED BY NICOTINAMIDE IN A C3H MOUSE MAMMARY CARCINOMA AND CDF1 MICE

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We have continued our investigation into the mechanism by which nicotinamide can enhance radiation damage in tumors, using a C3H mouse mammary carcinoma grown in CDF1 mice. Biochemical analysis of tumor extracts showed that nicotinamide (1000 mg/kg; i.p.) increased the ATP/Pi and ATP/ADP + AMP ratios. This change in metabolic activity was consistent with nicotinamide increasing tumor oxygenation. Moreover, the greatest effect occurred 0.5-2.5 hr after drug injection, a time at which radiosensitization by nicotinamide in this tumor had previously been shown to be maximal. These changes were observed without any apparent modification in tumor blood perfusion, measured using the 86-RbCl uptake procedure, and occurred despite nicotinamide producing a 50% decrease in mean arterial blood pressure, estimated directly by a carotid cannulation technique.

Nicotinamide, C3H mouse mammary carcinoma, CDF1 mice, Tumor metabolism, Oxygenation, Blood perfusion, Blood pressure.

INTRODUCTION

Numerous efforts have been made to overcome tumor radioresistance which results from the presence of hypoxic cells (for review see (5)). One agent in which interest appears to be increasing with regard to its ability to eliminate hypoxia in tumors is the compound nicotinamide. It is relatively non-toxic both in mice (7) and humans (18) and in general appears to enhance radiation damage in several tumor models in preference to normal tissues (7, 11).

The mechanism by which nicotinamide increases the radiation response of tumors is not clear. *In vivo*, nicotinamide is not cytotoxic to hypoxic cells, does not directly radiosensitize them, nor does it inhibit the repair of radiation-induced potentially lethal damage (8). It does, however, appear to increase tumor oxygenation status at the time of irradiation, apparently as a result of an increase in tumor blood perfusion (6, 8). In the current study we continue our investigation of the *in vivo* radiosensitization by nicotinamide in an attempt to understand the mechanism further.

METHODS AND MATERIALS

Animal and tumor model

All experiments were performed on 10- to 14-week-old female C3D2F1/Bom mice. The tumor model used was the C3H/Tif mammary carcinoma. Its derivation and maintenance have been previously described (14). Experimental tumors were grown either in the right flank or right hind foot of mice and treatments were carried out when the tumors had reached a volume of about 200 mm³.

Drug preparation

Nicotinamide* was dissolved at a concentration of 50 mg/ml in sterile saline (0.9% NaCl) immediately before each experiment. It was injected intraperitoneally (i.p.) into mice at an injection volume of 0.02 ml/g body weight.

Biochemical analysis

Preparation and analysis of tumor extracts have been described in detail elsewhere (12). Basically, mice with flank tumors were anesthetized with propomid (500 mg/

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Fig. 1. Metabolism in a C3H mouse mammary carcinoma as a function of time after administration of nicotinamide (1000 mg/kg; i.p.). The results were obtained from biochemical analysis of tumor extracts and represent the ratios of ATP to either (A) Pi or (B) ADP + AMP. Values show means (± 1 S.E.) from 3 to 6 mice per group.

kg; i.p.) and the skin opened. The tumors were then exposed and freeze clamped, using forceps pre-cooled in liquid nitrogen, prior to excision. Tumors were subsequently weighed, perchloric acid extracted, and then neutralized to pH7. ATP, AMP, and ADP were measured by enzymatic analysis (13) and Pi by a colorimetric assay (15).

Estimates of tumor perfusion

The effect of nicotinamide on tumor perfusion was assessed using the ^{86}Rb extraction technique (16). A volume of 0.1 ml $^{86}\text{RbCl}$ (specific activity 1–8 mCi/mg) was injected intravenously (i.v.) into each mouse. After 2 min the mice were sacrificed by cervical dislocation and the tumor-bearing leg clamped to prevent any further flow to the tumor. Tumor material was excised immediately following sacrifice and transferred to pre-weighed counting tubes. Care was taken to exclude normal tissue and to wipe off any excess blood. Tails of injected mice were also excised and counted to check on residual activity at the site of injection, and samples rejected if the tail counts were greater than 10% of the injected solution. Radioactive counts per minute were determined on a counter $\ddagger$ and expressed as percent injected/g of tumor.

Blood pressure measurements

Exact details of this procedure have been previously described (4). Mice with foot tumors were injected with pentobarbital anesthetic (60 mg/kg; i.p.). Under anesthesia the carotid artery was surgically exposed and a small fluid-filled tube, containing 100 IU/ml of heparin in saline, inserted about 1 cm into the artery and ligatured in position. The tube was then heat-sealed and passed under the skin and out through the nape of the neck. All incisions were then ligatured and the mice returned to individual cages with food and water and allowed to recover overnight. The following day they were transferred to jigs which lightly

Table 1. Metabolic affects of nicotinamide (1000 mg/kg; i.p.) in a C3H mouse mammary carcinoma

	Control*	Nicotinamide* $\ddagger$	Relative change $\ddagger$
ATP	0.54 [0.44–0.63]	0.71 [0.63–0.78]	1.31 [1.17–1.44]
ADP	0.32 [0.25–0.39]	0.20 [0.18–0.23]	0.62 [0.56–0.72]
AMP	0.14 [0.11–0.18]	0.06 [0.05–0.07]	0.41 [0.33–0.49]
Pi	5.20 [4.29–6.10]	3.50 [3.19–3.81]	0.67 [0.61–0.73]

*Values show means (± 1 S.E.) in mM.

$\ddagger$ Results represent the average value measured between 0.5 to 2.5 hours after nicotinamide injection.

$\ddagger$ Shows nicotinamide values relative to controls.

restrained them, yet allowed access to the small tube still inserted in the carotid artery. By breaking the sealed end and attaching the tube to a pressure transducer $\S$, repeated blood pressure measurements could be made on the same non-anesthetized mouse both before and after nicotinamide injection.

RESULTS

Figure 1 illustrates the effect of nicotinamide on the metabolic activity in this C3H mouse mammary carcinoma. The ratios of ATP/Pi and ATP/ADP + AMP were elevated after drug injection, reaching a maximum between 0.5 to 2.5 hr. While the adenine nucleotide sum (ATP + ADP + AMP) remained relatively constant over this time period (data not shown), ATP was observed to increase by an average of 31% (Table 1) and ADP, AMP and Pi all decreased by 38%, 59%, and 33%, respectively.

The effect of nicotinamide on physiological parameters of tumor blood flow and mouse blood pressure is shown in Figure 2. It appears that nicotinamide did not alter blood perfusion in this C3H mammary carcinoma. However, it did result in an almost 50% decrease in mean arterial blood pressure within 15–30 min of injection and this reduction was maintained for several hours. In fact, even 6 hr after injection blood pressure was still reduced by 35%, but had returned to normal within 24 hr (data not shown).

DISCUSSION

Our previous studies have shown that nicotinamide (1000 mg/kg; i.p.) could enhance the radiation response of this C3H mouse mammary carcinoma, with maximal radiosensitization observed when nicotinamide was injected between 0.5 and 2 hr prior to irradiation (9). The time course for this was identical to that for the nicotin-

$\ddagger$ Amersham International plc, Little Chalfont, England.

$\ddagger$ Packard Gamma model Auto-Gamma 5650.

$\S$ Siemens-Elema AB, Solna, Sweden.

Fig. 2. Physiological changes occurring in a C3H mouse mammary carcinoma and CDF1 mice as a function of time after administration of nicotinamide (1000 mg/kg; i.p.). (A) Change in tumor perfusion estimated by the RbCl-uptake procedure. (B) Change in mean arterial blood pressure (MABP) measured directly following cannulation of the carotid artery. Results show means ($\pm$ 1 S.E.) for 3 to 6 mice per group.

amide effect on tumor metabolism (Fig. 1). This alteration in metabolic activity after drug injection suggests an increase in the available oxygen in this tumor and further supports our earlier suggestions from data in other tumor models that the ability of nicotinamide to increase tumor radiation damage is the result of an improvement of tumor oxygenation (6, 8, 17).

There was no apparent change in blood perfusion in this C3H mammary carcinoma (Fig. 2), which was contrary to our findings in other tumors (6, 8). This would suggest that the oxygen supply to this tumor remained constant. If true, then the decrease in metabolic rate, evidenced by the drop in ADP and Pi, would lead to an increased oxygen tension

in the tissue as a result of a reduced oxygen utilization. This would make more oxygen available for use by hypoxic cells. In fact, we have additional data in this same tumor model clearly showing that nicotinamide can convert hypoxic cells into aerobic cells (9). More specifically, it does this in the C3H/Tif (9) and SCCVII tumors (3) by preventing the transient fluctuations in tumor blood flow, which are known to result in the development of acute hypoxia (1). This indicates that nicotinamide does increase oxygen delivery to the tumor, by at least improving blood perfusion at the microcirculatory level. Such a result is still consistent with the metabolic affects, but perhaps in this situation the perfusion changes are too small to be detected by the RbCl uptake procedure. Surprisingly, nicotinamide substantially decreased mean arterial blood pressure in CDF1 mice (Fig. 2). One might, therefore, have actually expected to see a decrease in tumor blood perfusion. Hydralazine, for example, also produces a 50% decrease in mean arterial blood pressure in CDF1 mice, and this results in a 90% decrease in perfusion in the C3H mouse mammary carcinoma (10).

Whatever the explanation of our findings, nicotinamide does enhance radiation response and appears to do so by increasing the oxygenation status of tumors, presumably by preventing the development of acute hypoxia. In the clinic, nicotinamide may therefore have a role to play in combination with agents that are used specifically to overcome radioresistant chronic hypoxia. Recent animal studies have indeed shown that the enhancement of radiation damage by two such agents, hyperthermia and a perfluorochemical emulsion, can be significantly improved by including nicotinamide in the treatment regime (2, 9).

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