Acta Oncologica Vol. 34, No. 3, pp. 391–395, 1995

EFFECT OF NICOTINAMIDE AND PENTOXIFYLLINE ON NORMAL TISSUE AND FSA TUMOR OXYGENATION

MATTHEW J. PRICE, LEON T. LI, JONATHAN D. TWARD, IRINA BUBLIK, WILLIAM H. MCBRIDE and ROBERT S. LAVEY

Nicotinamide (NA) and pentoxifylline (PTX) sensitize experimental murine tumors to radiation without sensitizing normal tissues. They are presumed to exert this effect by reducing hypoxia in tumors. The present study evaluated the individual and combined effects of NA and PTX on oxygen levels in subcutaneous normal tissue and subcutaneous FSa fibrosarcoma tumors in the hind foot dorsum of C3H mice. Oxygen measurements were made using a polarographic needle electrode inserted into the tissue immediately before and/or 15–60 min after intraperitoneal administration of 500 mg/kg of NA, 50 mg/kg of PTX, or saline. The median tumor pO_2 increased from a mean ± S.E.M. of 4.1 ± 1.1 mm Hg in saline-treated control mice to 6.8 ± 1.9 mm Hg 15 min after NA, 7.6 ± 1.4 mm Hg 60 min after PTX, and 6.7 ± 1.1 mm Hg after NA and PTX in combination. PTX raised the median tumor pO_2 level from 21% to 39% of the median subcutaneous normal tissue pO_2 ($p < 0.01$). PTX also significantly reduced the proportion of tumor pO_2 values ≤2 mm Hg from 41 ± 10% to 8 ± 7% ($p = 0.02$). Although NA did increase the proportion of tumor that was well oxygenated, it did not significantly reduce the proportion of tumor pO_2 values ≤2 mm Hg ($p = 0.34$). The combination of NA and PTX did not add to the tumor oxygenation enhancement achieved by PTX alone. NA increased the median subcutaneous normal tissue pO_2 by an average of 5.1 ± 2.2 mm Hg from a baseline of 17.1 ± 2.2 mm Hg ($p = 0.04$). PTX had no effect on the median normal tissue pO_2 ($p = 0.93$). PTX showed greater therapeutic potential in this model system than did NA.

Nicotinamide (NA) and pentoxifylline (PTX) have both been shown to significantly increase the radiation sensitivity of several experimental murine tumors without sensitizing normal tissues. It is uncertain how NA, the amide form of vitamin B_3 (1–4), and PTX, a methylxanthine derivative (5–7), preferentially radiosensitize tumors. NA has been proposed to decrease acute hypoxia in tumors by preventing the transient closure of tumor microvasculature (4). While NA increases blood flow through experimental mouse and rat tumors (8–11), most investigators have not detected an effect of NA on the oxygen level in these tumors (2, 10, 11). PTX is hypothesized to alleviate the chronic component of tumor hypoxia by increasing blood cell deformability and plasma 2,3-diphosphoglycerate levels, decreasing plasma viscosity, and inhibiting platelet aggregration (12–14). Unlike NA, PTX has been found to increase the mean partial pressure of oxygen (pO_2) in both mouse and rat tumors (5, 6, 15).

Both acute and chronic hypoxia probably play significant roles in increasing tumor resistance to radiation. It is therefore rational to combine NA and PTX in an attempt to diminish both these elements of tumor hypoxia. NA and PTX are each well tolerated by humans in the dose range necessary to achieve serum concentrations that radiosensitize tumors in vitro (12, 16, 17). The present study evalu-

Received 25 September 1994.
Accepted 21 December 1994.
From the Department of Radiation Oncology and Jonsson Comprehensive Cancer Center, University of California (UCLA), Los Angeles, California, U.S.A.
Correspondence to: Robert S. Lavey, M.D., M.P.H., 200 UCLA Medical Plaza, Suite B265, Los Angeles, CA, 90095-6951, U.S.A.
Paper presented at the Tumour Microenvironment Workshop in Granada, Spain, September 23–25, 1994.

 ISSN 0284-186X

ated the individual and combined effects of NA and PTX on oxygen levels in subcutaneous normal tissue and subcutaneous FSa fibrosarcoma tumors in the hind foot dorsum of the C3H mouse.

Material and Methods

Animals and tumors. Male C3H/Sed//Kam mice produced and maintained in our defined-flora mouse colony were used for all experiments. All mice were 8–10 weeks of age and 25–28 g in weight at the time of oxygen measurement. Food and water were available ad libitum until the time of measurement. Measurements of normal tissue pO_2 were made in non-tumor bearing stock mice. Measurements of tumor pO_2 were made in tumors derived from the FSa cell line, a methylcholanthrine-induced fibrosarcoma syngeneic to C3H mice. Experimental tumors grew in the right hind foot dorsum following subcutaneous injection of approximately 1×10^6 FSa cells prepared from a brei of 3–5 stock FSa tumors and suspended in Hanks solution. Oxygen measurements were taken when tumors reached 35–100 mm^3 in volume, which occurred 10–14 days after inoculation. Tumor volumes were calculated using the formula for an ellipse. All experimental procedures were approved by the institutional animal research committee.

Drugs. Nicotinamide (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% NaCl. Freshly prepared solution was administered intraperitoneally to mice at a dose of 500 mg/kg in a volume of 0.25 ml. Pentoxifylline (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% NaCl. Freshly prepared solution was administered intraperitoneally to mice at a dose of 50 mg/kg in a volume of 0.25 ml. When the two chemicals were used in combination, 0.125 ml of a freshly prepared solution of nicotinamide in saline was administered intraperitoneally at a dose of 500 mg/kg 45 min before intraperitoneal administration of 0.125 ml of a freshly prepared solution of pentoxfylline in saline at a dose of 50 mg/kg. An intraperitoneal injection of 0.25 ml of 0.9% NaCl was given to control mice.

Oxygen tension measurement. Tumor and normal subcutaneous tissue oxygen tension levels were determined using the KIMOC-6650 pO_2 Histograph (Eppendorf Corp., Hamburg, Germany). Details of the method of measurement using this polarographic needle microelectrode system are provided elsewhere (10, 15). During measurement, mice were confined in a jig designed for this procedure through which only their hind limbs protruded. They breathed room air at normal pressure throughout the experiment. No sedation or anesthesia was used. The Ag/AgCl-ECG reference electrode was taped to a shaved area of abdominal skin. The measurement electrode was inserted and advanced through three separate radial tracks in each measured tumor and normal foot. Between 10 and 15 pO_2 measurements were made along each track, producing 30–36 pO_2 measurements per site. Normal tissue pO_2 measurements were made in one hind foot dorsum immediately before drug administration and in the other hind foot dorsum of the same animal 15 min after intraperitoneal administration of pentoxifylline or 60 min after intraperitoneal administration of nicotinamide. Half of the initial pO_2 measurements in each group were made in the left foot and half in the right foot. Tumor pO_2 measurements were made 15 min after intraperitoneal administration of pentoxifylline and/or 60 min after intraperitoneal administration of nicotinamide or saline.

Data analysis. Results are expressed as either a mean $\pm$ standard error of the mean (S.E.M.) or as percentage of the total pO_2 measurements in the specified treatment group. The effects of nicotinamide and pentoxifylline on normal tissue were assessed using a two-tail, matched pair t-test for means. The mean tumor oxygen values of different treatment groups were compared using a two-tail, two sample t-test assuming unequal variances. Subgroup analysis of the percentage of tumor pO_2 measurements in each pO_2 range was performed using the two-sided χ^2-test with continuity correction.

Results

Tumor oxygenation. pO_2 levels were measured in 28 subcutaneous FSa tumors, 7 in each of the 4 treatment groups. The mean $\pm$ S.E.M. tumor volume was 62.2 ± 3.2 mm^3 (range 37 to 97 mm^3). The mean tumor volume did not differ significantly between treatment groups ($p > 0.40$). Between 30 and 36 pO_2 measurements were taken in each tumor. The results of those measurements are listed in Table 1. The mean $\pm$ S.E.M. of the median tumor pO_2 values was 4.1 ± 1.1 mm Hg in the saline group, 6.8 ± 1.9 in the NA group, 7.6 ± 1.4 mm Hg in the PTX group, and 6.7 ± 1.1 mm Hg in the combined NA & PTX group. The difference in median tumor pO_2 values approached statistical significance only between the saline and PTX alone groups ($p = 0.07$). The mean $\pm$ S.E.M. of the proportion of measurements ≤ 2 mm Hg was $41 \pm 10\%$ after saline compared to $8 \pm 7\%$ after PTX alone ($p = 0.02$), $10 \pm 6\%$ after PTX with NA ($p = 0.02$), and $31 \pm 9\%$ after NA alone ($p = 0.34$). The mean of the proportion of measurements ≤ 2 mm Hg after PTX with or without NA was significantly lower than after NA alone ($p = 0.05$).

The distribution of pO_2 values in each treatment group are shown in Fig. 1. The proportion of the tumor having a $pO_2 < 2$ mm Hg was markedly lower after PTX, with or without NA, than after either saline or NA alone. In contrast, above 2 mm Hg the PTX oxygen distribution curve is parallel to the saline curve. PTX thus produced a generalized 4 mm Hg increase in tumor oxygen levels. While PTX shifted the oxygen distribution curve to the right, NA had the effect of decreasing its slope. The proportion of pO_2 measurements reading 0 mm Hg was

Table 1

Treatment effect on tumor oxygenation

	Treatment group			
	Saline	NA	PTX	NA + PTX
Number of measurements	234	232	234	242
Mean tumor volume (mm^3) ± S.D.	59 ± 17	61 ± 22	63 ± 19	66 ± 12
Avg. median pO_2 (mm Hg) ± S.E.M.	4.1 ± 1.1	6.8 ± 1.9	7.6 ± 1.4	6.7 ± 1.1
Overall median pO_2 (mm Hg)	3.3	5.6	7.2	6.0
Distribution of pO_2 values (%)				
0–2 mm Hg	39.7	31.9	8.6***	9.5***
2–12 mm Hg	46.2	38.8	65.1***	71.5***
12–22 mm Hg	13.7	23.3**	22.4*	14.0
>22 mm Hg	0.4	6.0***	3.9*	5.0**

NA = Nicotinamide. PTX = Pentoxifylline.
χ^2-comparison with the saline group: *p = 0.02 **p ≤ 0.01 ***p < 0.001

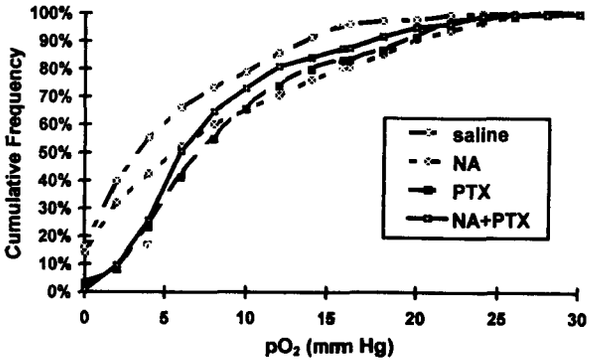

Fig. 1. Cumulative distributions of oxygen tension measurements in tumors after i.p. injection of normal saline, nicotinamide (NA), pentoxifylline (PTX), or both NA and PTX (NA + PTX).

14% in both the saline and NA groups, compared with 2% in the groups given PTX with or without NA ($p < 0.0001$). The NA group had a significantly lower proportion of pO_2 measurements in the 0–15 mm Hg range (64%) than either the saline (80%) or PTX with or without NA (81%) groups ($p < 0.0001$). The proportion of pO_2 measurements >15 mm was significantly higher in both the NA alone (22%) and the PTX ± NA (17%) groups than in the saline (6%) group ($p < 0.0001$). The joint administration of NA and PTX resulted in a tumor oxygenation profile similar to that obtained with PTX alone. PTX was at least as effective in improving every indicator of tumor oxygenation (median and proportion of pO_2 measurements <2, <5, and >15 mm Hg) when given alone as when used together with NA (one-tail p for benefit of adding NA to PTX >0.90).

Subcutaneous normal tissue. Subcutaneous pO_2 levels were measured in the hind foot dorsum of 20 mice immediately before and 15 or 60 min after intraperitoneal injection of PTX or NA respectively. Results of the normal tissue pO_2 measurements are listed in Table 2. The median baseline pO_2 levels in the normal tissue were approximately quadruple the median pO_2 in the untreated tumors and double the median pO_2 in the tumors treated with NA and/or PTX ($p < 0.0001$). The median baseline normal tissue pO_2 values of 17.1 ± 2.0 mm Hg in the NA group and 18.0 ± 2.2 mm Hg in the PTX group were not significantly different ($p = 0.78$). PTX had no effect on the median normal tissue pO_2 level ($p = 0.95$). NA administration significantly increased the median pO_2 value in the 10 measured feet by a mean ± S.E.M. of 5.1 ± 2.2 mm Hg ($p = 0.04$). NA affected subcutaneous normal tissue oxygen distribution in the same way as it affected FSa tumor oxygenation. The proportion of pO_2 measurements <2 mm Hg remained 6%, but NA decreased the slope of the curve between 0 and 16 mm Hg, as shown in Fig. 2. The proportion of pO_2 measurements >15 mm Hg increased

Table 2

NA and PTX effects on normal tissue oxygenation

	NA	PTX
Number of measures pre-Tx/Post-Tx	353/338	330/336
Median pretreatment pO_2 (mm Hg) ± S.E.M.	17.1 ± 2.0	18.0 ± 2.2
Median posttreatment pO_2 (mm Hg) ± S.E.M.	22.3 ± 1.3	18.1 ± 1.4
Avg. increase in median pO_2 (mm Hg) ± S.E.M.	5.1 ± 2.2*	0.2 ± 1.7
Avg. decrease in % $pO_2 \leq 2$ mm Hg ± S.E.M.	1.0 ± 4.5	2.2 ± 2.1

Tx = Treatment with either nicotinamide (NA) or pentoxifylline (PTX).
*Paired t-test p = 0.04

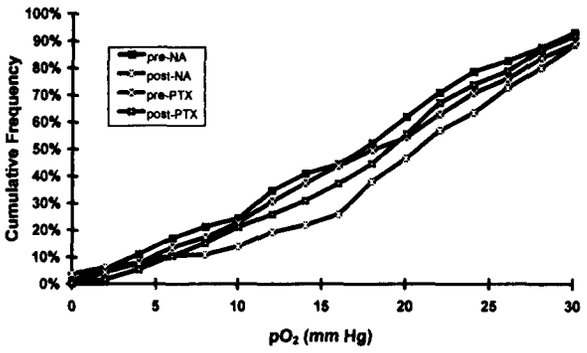

Fig. 2. Cumulative distributions of oxygen tension measurements in normal subcutaneous tissue before and after i.p. injection of nicotinamide (NA) or pentoxifylline (PTX).

from 58% before NA to 76% after NA ($p < 0.0001$). In contrast, PTX was associated with a decrease in the proportion of pO_2 measurements <2 mm Hg from 4.2% to 1.7%, but no significant change in the proportion of pO_2 measurements >15 mm Hg ($p = 0.20$).

Discussion

Both NA and PTX increased the median pO_2 in subcutaneous FSa tumors in this study. However, the drugs differed in their effect on the distribution of oxygen levels in both the subcutaneous tumor and normal tissue. PTX specifically reduced the proportion of sampled tissue having an oxygen tension <2 mm Hg, by 80% in the tumors and 60% in normal tissue. It had no detectable effect on better oxygenated areas of either tumor or normal tissue. These findings are consistent with previously published reports. Teicher et al. (15) tested the effect of PTX on mean tumor pO_2 in three rodent tumor systems under both air and carbogen breathing conditions. Under air breathing conditions, PTX significantly increased oxygen levels in poorly oxygenated tumors (mean pO_2 values of 0.4 and 7.4 mm Hg), but not in the better oxygenated tumor (mean pO_2 of 12.3 mm Hg). As in our system, the PTX effect was limited to decreasing the proportionate volume of the murine tumor that was severely hypoxic. While either carbogen breathing or administration of a perflubron emulsion increased the proportionate volume with a $pO_2 > 2.5$ mm Hg, PTX did not. The PTX had no effect on any of the tumor lines under carbogen breathing conditions, in which all three tumors had a mean $pO_2 > 9$ mm Hg (15). Song et al. (6) also found that PTX increased the measured pO_2 in two mouse tumor lines, but not in normal thigh muscle. Proposals to explain the mechanism of PTX action should account for the specificity of its effect in reducing severe hypoxia. PTX may permit red blood cells to flow through abnormal vessels that they could not otherwise traverse, while not improving supply to marginally perfused areas. PTX has been found to significantly increase blood flow through tumors without affecting normal tissue perfusion in both mouse (7) and rat (18).

NA did not change the proportionate volume of either normal tissue or FSa tumors that was severely hypoxic ($pO_2 \leq 2$ mm Hg) in our study, but increased the pO_2 levels in tumor and normal tissue areas that were mildly to moderately hypoxic. NA has also been reported to increase the mean and median pO_2 in human tumor xenografts of FSaII, a derivative of the FSa cell line (2). In other model systems, NA significantly increased tumor blood flow without altering the median tumor oxygen level (10, 11). The observed pattern of increase in tumor and subcutaneous tissue pO_2 suggests that NA and PTX differ in their effects on the microcirculation. It is generally supposed that PTX acts on the cellular elements in blood, whereas NA acts on blood vessels. General vasodilation is the dose-limiting toxicity of NA. Its conversion to nicotinamide adenine dinucleotide (NAD^+) may lead to additional local vasodilation (19). NA has been found to decrease transient blood vessel closure in a murine carcinoma (4), perhaps as a result of decreasing interstitial fluid pressure (9, 20).

PTX alone affected FSa tumor oxygenation in our model in a similar manner and to as great an extent as PTX combined with NA. The effect of adding NA to PTX was also studied in an FSaII mouse tumor model (5). PTX alone produced about as large an increase in mean tumor pO_2 as did PTX and NA in combination. Single agent PTX administered for 3 days prior to a single 10 Gy dose of radiation also delayed tumor growth for as long as combined treatment with PTX and NA before irradiation. Thus, there is no evidence that adding NA to PTX increases tumor oxygenation or radiosensitivity beyond that achieved with PTX alone.

In conclusion, we found that intraperitoneal administration of either 500 mg/kg of NA or 50 mg/kg of PTX increased the median pO_2 level in subcutaneous FSa tumors. Even after NA and/or PTX administration, though, tumor pO_2 levels remained significantly below those of normal subcutaneous tissue. NA and PTX differed in their effects on the tumor and normal tissue oxygen distribution profiles. PTX appeared to specifically decrease severe hypoxia ($pO_2 \leq 2$ mm Hg), whereas NA produced a generalized increase in oxygen levels. The combination of NA and PTX did not add to the tumor oxygenation enhancement achieved by PTX alone.

ACKNOWLEDGEMENTS

The authors are grateful to Colin McLean and Alex Betancourt for production and maintenance of defined-flora mice and to Aaron Tward for tumor preparation.

REFERENCES

1. Horsman MR, Chaplin DJ, Brown JM. Radiosensitization by nicotinamide in vivo: A greater enhancement of tumor dam-

age compared to that of normal tissues. Radiat Res 1987; 109: 479–89.
2. Lee I, Song CW. The oxygenation of murine tumor isografts and human tumor xenografts by nicotinamide. Radiat Res 1992; 130: 65–71.
3. Ono K, Masunaga S, Akuta K, Akaboshi M, Abe M. Radiosensitization of SCCVII tumours and normal tissues by nicotinamide and carbogen: analysis by micronucleus assay. Radiother Oncol 1993; 28: 162–7.
4. Chaplin DJ, Horsman MR, Trotter MJ. Effect of nicotinamide on the microregional heterogeneity of oxygen delivery within a murine tumor. J Natl Cancer Inst 1990; 82: 672–6.
5. Lee I, Kim JH, Levitt SH, Song CW. Increases in tumor response by pentoxifylline alone or in combination with nicotinamide. Int J Radiat Oncol Biol Phys 1992; 22: 425–9.
6. Song CW, Hasegawa T, Kwon HC, Lyons JC, Levitt SH. Increase in tumor oxygenation and radiosensitivity caused by pentoxifylline. Radiat Res 1992; 130: 205–10.
7. Honess DJ, Dennis IF, Bleehen NM. Pentoxifylline: its pharmacokinetics and ability to improve tumour perfusion and radiosensitivity in mice. Radiother Oncol 1993; 28: 208–18.
8. Honess DJ, Bleehen NM. Effects of the radiosensitising agent nicotinamide on relative tissue perfusion and kidney function in C3H mice. Radiother Oncol 1993; 27: 140–8.
9. Horsman MR, Brown JM, Hirst VK, et al. Mechanism of action of the selective tumor radiosensitizer nicotinamide. Int J Radiat Oncol Biol Phys 1988; 15: 685–90.
10. Kelleher DK, Vaupel PW. Nicotinamide exerts different acute effects on microcirculatory function and tissue oxygenation in rat tumors. Int J Radiat Oncol Biol Phys 1993; 26: 95–102.
11. Horsman MR, Nordsmark M, Khalil A, Chaplin DJ, Overgaard J. Tumour radiosensitization by nicotinamide: Is it the result of an improvement in tumour oxygenation? Adv Exp Med Biol 1994; 345: 403–9.
12. Ward A, Clissold, SP. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. Drugs 1987; 34: 50–97.
13. Novick WJ, Sullivan G, Mandell GL. Newer pharmacological effects of pentoxifylline. Biorheology 1990; 24: 449–54.
14. Currie MS, Simel DL, Christenson RH, et al. Anti-inflammatory effects of pentoxifylline in claudication. Am J Med Sci 1991; 301: 85–90.
15. Teicher BA, Sotomayor EA, Robinson MF, Dupuis NP, Schwartz GN, Frei E. Tumor oxygenation and radiosensitization by pentoxifylline and a perflubron emulsion/carbogen breathing. Int J Oncol 1993; 2: 13–21.
16. Horsman MR. Carbogen and nicotinamide: Expectations too high? (Response to J. Martin Brown). Radiother Oncol 1992; 24: 121–2.
17. Horsman MR, Hoyer M, Honess DJ, Dennis IF, Overgaard J. Nicotinamide pharmacokinetics in humans and mice: a comparative assessment and the implications for radiotherapy. Radiother Oncol 1993; 27: 131–9.
18. Song CW, Makepeace CM, Griffin RJ, et al. Increase in tumor blood flow by pentoxifylline. Int J Radiat Oncol Biol Phys 1994; 29: 433–7.
19. Kelleher DK, Vaupel PW. Possible mehcanisms involved in tumor radiosensitization following nicotinamide administration. Radiother Oncol 1994; 32: 47–53.
20. Lee I, Boucher Y, Jain RK. Nicotinamide can lower tumor interstitial fluid pressure: Mechanistic and therapeutic implications. Cancer Res 1992; 52: 3237–40.