CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20221/S012

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review No. 7

Keywords: tumor protection, 3 month toxicity study

NDA No.: 20-221

Serial No(s).: 012 **Type:** SE1 **Letter dated:** 12/23/98 **Received by CDR:** 12/24/98

Information to be Conveyed to Sponsor: Yes (X), No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Review Completion Date: 3/22/99

Sponsor: U.S. Bioscience, Inc.

Manufacturer (if different): Ben Venue Laboratories, Inc.

Drug Name:

Code Name: WR-2721
Generic Name: amifostine
Trade Name: ethyol
Chemical Name: ethanethiol, 2-[(3-aminopropyl)amino] dihydrogen phosphate (ester)
CAS Registry Number:
Molecular formula/weight: mw=214.22; $C_5H_{15}N_2O_3PS$
Secondary therapy(s): radiation
Structure: $H_2N(CH_2)_3NH(CH_2)_2S\text{-}PO_3H_2$

Related INDs/NDAs/DMFs: INDs

Drug Class: Chemoprotectant

Indication: new: to reduce the incidence and severity of radiation-induced xerostomia.
Previous indication: to reduce the cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer or NSCLC.

Clinical Formulation: sterile lyophilized powder

Route of Administration and dosage form: iv

Proposed dose and administration: 200 mg/m2 iv once daily as a 3 minute infusion 15-30 minutes prior to radiation.

Previous Review(s), Date(s) and Reviewer(s): W. Schmidt #1, 1/92; #2, 6/92; #3, 3/93; #4, 12/94; #5, 1/95; and #6, 12/95.

Studies Reviewed within this submission:

Pharmacology:

1. Menard, TW et al., 1984. Radioprotection by WR-2721 of gamma-irradiated rat parotid gland: effect on gland weight and secretion at 8-10 days post irradiation. Int. J. Radiat. Oncol. Biol. Phys. 10:1555-1559.
2. Sodicoff, M et al. 1978. Radioprotection by WR-2721 against long-term chronic damage to the rat parotid gland. Radiat. Res. 76: 172-179.
3. Sodicoff, M. et al. Short-term radioprotective effect of WR-2721 on the rat parotid glands. Radiat. Res. 75: 317-326.
4. Hubner RH et al. 1997. Radioprotection of salivary glands by amifostine. Radiol. Oncol. 31: 279-285.
5. Dendale, R. et al. 1997. Effect of systemic and topical administration of amifostine on radiation-induced mucositis in mice. Proc. Amer Soc Clin Oncol. 16:64a. Abstract.
6. Fichtner, I et al, 1997. Effects of amifostine (WR-2721, ethyol) on tumor growth and pharmacology of cytotoxic drugs in human xenotransplanted neuroblastomas. Anti-Cancer Drugs 8:174-181.

Toxicology:

1. 90 day intravenous toxicity study of amifostine in Sprague Dawley Rats.

Studies Not Reviewed within this submission:

1. Capizzi, RL. 1996. The preclinical basis for broad spectrum selective cytoprotection of normal tissues from cytotoxic therapies. Semin. Oncol. 23: 2-17.
2. Yuhas, JM. 1980. Active versus passive absorption kinetics as the basis for selective protection of normal tissues by WR2721. Cancer Res. 40:1519-1524.
3. Rasey, JS et al. 1986. Synthesis, biodistribution, and autoradiography of radiolabeled WR3689. Radiat. Res. 106: 366-379.
4. Calabro-Jones, PM et al.. 1985. Alkaline phosphatase promotes radioprotection and accumulation of WR-1065 in V79-171 cells incubated in medium containing WR-2721. Int. J Radiat Biol. 47: 23-27.
5. Utley, JF. Et al.. 1976. Distribution of 35 S-labeled WR-2721 in normal and malignant tissues of the mouse. Radiat. Res. 68: 284-291.
6. Utley, JF et al. 1981. Protection of normal tissue against late radiation injury by WR-2721. Radiat. Res. 85: 408-415.
7. Calabro Jones, PM et al. 1988. Uptake of WR-02721 derivatives by cells in culture: identification of the transported form of the drug. Cancer Res. 48: 3634-3640.
8. Smoluk, GD et al. 1988. Radioprotection of cells in culture by WR-2721 and derivatives: form of the drug responsible for protection. Cancer Res. 48: 3641-3647.
9. Fulda, S et al. 1997. Effects of WR-2721 and its metabolite WR-1064,on the antiproliferative activity of chemotherapeutic agents on neuroblastoma cells in vitro. Anticancer Drugs 8: 34-41.
10. Alberts, DS et al. 1996. WR-1065, the active metabolite of amifostine , does not inhibit the cytotoxic effects of a broad range of standard anticancer drugs against human ovarian and breast cancer cells. Eur. J. Cancer 32A, suppl. 4: S17-S20.
11. Clarke, SEM. 1994. Radioiodine therapy of the thyroid. In Murray, IPC, Ell PJ eds. Nuclear Medicine in Clinical Diagnosis and Treatment. 2nd ed. Edinburgh: Churchill Livingstone, 833-845.

Studies Previously Reviewed:

Review #1

1. Yuhas, J.M., and F. Culo. Selective inhibition of the nephrotoxicity of cDDP by WR2721 without altering its antitumor properties. Cancer Treat. Rep. 64: 57-64. 1980.
2. Yuhas, J.M., Spellman,J.M., Jordan, S.W., Pardini, M.C., Afzal, S.M.J., and F. Culo. Treatment of tumors with the combination of WR2721 and cDDP or cyclophosphamide. Br. J. Cancer 42: 574-585. 1980.
3. Carfagna P. F., Chaney, S.G., Chang, J. and D.J. Holbrook. Reduction of tetrachloro(d,l, trans) 1,2-diaminocyclohexaneplatinum (IV) (tetraplatin) toxicity by the administration of diethyldithiocarbamate (DDTC), WR2721, or sodium selenite in the Fischer 344 rat. Fundamental and Applied Toxicol. 14: 706- 719. 1990.
4. Wasserman, T.H., Phillips, T.L., Ross, G., and L.G. Kane. Differential protection against cytotoxic chemotherapeutic effect on bone marrow CFU's by WR2721. Cancer Clinical Trials 4: 3-6. 1981.
5. Green, D. and P. Schein. Evaluation of chemoprotection by i.p. WR2721 and oral WR151327 in mice. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991
6. Peters, G.J., van der Wilt, C.L., Gyergyay, F., van Laar, J.A.M., Treskes, M., van der Vijgh, W.J.F. and H.M. Pinedo. Protection of WR2721 of the toxicity induced by the combination of cisplatin and 5-FU. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991.
7. Mollman, J.E. Protection against cisplatin neurotoxicity in cultured dorsal root ganglion cells by WR2721. Seventh International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, FLA. 1991.
8. Millar, J.L., McElwain, J.J., Clutterbuck, R.D., and E.A. Wist. The modification of melphalan toxicity in tumor bearing mice by WR2721. Am. J. Clin. Oncol. 5: 321-328. 1982.
9. Valeriote, F. and S. Toten. Protection and potentiation of nitrogen mustard cytotoxicity by WR2721. Cancer Res. 42: 4330-4331. 1982.
10. Phillips, T.L., Yuhas, J.M., and T.M. Wasserman. Differential protection against alkylating agent injury in tumors and normal tissues. In Radioprotectors and Anticarcinogens, Academic Press, Inc., 735-748. 1983.
11. Allalunis-Turner, M.J. and D.W. Siemann. Modification of cyclophosphamide-induced pulmonary toxicity in normal mice. NCI Monographs 6: 51-53. 1988.
12. Nagy, B., Dale, P.J. and D.J. Grdina. Protection against cDDP cytotoxicity and mutagenicity in V79 cells by WR2721. Cancer Res. 46: 1132-1135. 1986.
13. Nagy, B., Dale, P.J. and D.J. Grdina. Protective effects of WR2721 against bleomycin and nitrogen mustard-induced mutagenicity in V79 cells. Int. J. Radiat. Oncol. Biol. Phys. 12: 1475-1478. 1986.
14. Treskes, M., Holwerda, U., Nijtmans, L., Fichtinger-Schepman, A.M.J., Pinedo, H.M., and W.J.F. van der Vijgh. Modulation of cisplatin and carboplatin with WR2721, molecular aspects. 7th international conference on chemical modifiers of cancer treatment. 1991.

Pharmacokinetics:

1. Plasma concentrations of radioactivity after a single intravenous dose of 14C-WR2721 to male and female rats. Sterling Research. 1990.
2. The excretion and metabolic profiles of 14C-WR2721 following a single intravenous dose to male and female rats. Sterling Research. 1990.
3. Measurement of WR2721 in plasma: preliminary pharmacokinetics in the beagle. Southwest Research. 1985.
4. The disposition of 14C-WR2721 following a single oral or i.v. dose to male dogs. Sterling Research. 1990.
5. Swynnerton, N.F., Huelle, B.K., Mangold, D., and T.M. Ludden. A method for the combined measurement of WR2721 and WR1065 in plasma: application to pharmacokinetic experiments with WR2721 and its metabolites. Int. H. Radiation Oncology Biol. Phys. 12: 1495-1499. 1986.

6. Washburn, L.C., Rafter, J.J., Hayes, R.L. and J.M. Yuhas. Prediction of the effective radioprotective dose of WR2721 in humans through an interspecies tissue distribution study. Radiation Research 66: 100-105. 1976.
7. Washburn, L.C., Carlton, J.E., Hayes, R.L., and J.M. Yuhas. Distribution of WR2721 in normal and malignant tissues of mice and rats bearing solid tumors: dependence on tumor types, drug dose, and species. Radiation Research 59: 475-483. 1974.
8. Utley, J.F., Seaver, N., Newton, G.L., and R.C. Fahey. Pharmacokinetics of WR1065 in mouse tissue following treatment with WR2721. Int. J. Radiation Oncol. Biol. Phys. 10: 1525-1528. 1984.
9. Mangold, D.J., Miller, M.A., Huell, B.K., Sanchez-Barona, D.O.T., Synnerton, N.F., Fleckenstein, L., and T.M. Ludden. Disposition of the radioprotector WR2721 in the rhesus monkey: influence of route of administration. Drug Metabolism and Disposition 17: 304-310. 1989.
Toxicology:
1. A dose ranging study to assess the toxicity of WR2721 following daily intravenous administration to rats for 7 days. Sterling Research Group. 1989.
2. An assessment of the toxicity of WR2721 in rats following daily intravenous administration for 28 days. Sterling Research Group. 1989.
3. Exploratory study of WR2721 administered intravenously to Beagle dogs for two weeks. Sterling Research Group. 1989.
4. One month subchronic safety evaluation and plasma concentration analysis study of WR2721 administered intravenously to Beagle dogs. Sterling Research Group. 1989.
5. Sodicoff et al. Effect of WR2721 on fetal development in the rat. Radiation Research 107: 49-57. 1986.
6. Effect of WR2721 in the Ames test. Walter Reed Army Institute of Research. 1983.
Review #2:
1. Bellnier, D.A. and T.J. Dougherty. 1989. Protection of murine foot tissue and transplantable tumor against Photofrin II mediated photodynamic sensitization with WR2721. J. Photochem. Photobiol. 4: 210-225.
2. Biscay, P., Lespinasse, F., Oiry, J., Huczkowski, J., Imbach, J., Malaise, E.P., and M. Guichard. 1986. Radiobiological evaluation of a newly synthesized cysteamine derivative. Int. J. Radiat. Oncol. Biol. Phys. 12: 1469-1473.
3. Brown. D.Q., Graham, W.J., MacKenzie, L.J., Pittock, J.W., and L.M. Shaw. 1988. Can WR2721 be improved upon? Pharmac. Ther. 39: 157-168.
4. Byfield, J.E. 1986. Combined use of drugs and radiation in the treatment of liver metastases. Recent Results in Cancer Research 100: 298-306.
5. Clement, J.J., and R.K. Johnson. 1981. Influence of WR2721 on the efficacy of radiotherapy and chemotherapy of murine tumors. Int. J. Radiat. Oncol. Biol. Phys. 8: 539-542.
6. Denekamp, J., Stewart, F.A., and A. Rojas. 1983. Is the outlook grey for WR2721 as a clinical radioprotector? Int. J. Radiat. Oncol. Biol. Phys. 9: 1247-1249.
7. Durand, R.E. 1983. Radioprotection by WR2721 in vitro at low oxygen tensions: implications for its mechanisms of action. Br.J. Cancer 47: 387-392.
8. Ikebuchi, M., Shinohara, S., Kimura, H., Morimoto, K., Shima, A., and T. Aoyama. 1981. Effects of daily treatment with a radioprotector WR2721 on Ehrlich's ascites tumor in mice: suppression of tumor cell growth and earlier death of tumor-bearing mice. J. Radiat. Res. 22: 258-264.
9. Kanclerz, A., and J.D. Chapman. 1988. Influence of misonidazole, SR2508, RSU-1069, and WR2721 on spontaneous metastases in C57BL mice. Int. J. Radiat. Oncol. Biol. Phys. 14: 309-316.

10. Lespinasse, F., Oiry, J., Fatome, M., Adrouin P., Imbach, J., Malaise, E.P., and M. Guichard. 1985. Radioprotection of EMT6 tumor by a new class of radioprotectors based on a pseudo-peptide cysteamine combination. Int. J. Radiat. Oncol. Biol. Phys. 11: 1035-1038.
11. Lunec, J., Cullen, B., Walker, H., and S. Hornsey. 1981. A cautionary note on the use of thiol compounds to protect normal tissues in radiotherapy. Br. J. Radiol. 54: 428-429.
12. McNally, N.J. 1982. The effect of misonidazole combined with WR2721 on tumor response and leucopenia due to cyclophosphamide or melphalan. Br. J. Cancer 46:670.
13. Milas, L., Ito, H., and N. Hunter. 1983. Effect of tumor size on S-2-(3-aminopropylamino)ethylphosphorothioic acid and misonidazole alteration of tumor response to cyclophosphamide. Cancer Res. 43: 3050-3056.
14. Milas, L., Hunter, N., Ito, H., and L.J. Peters. 1984. Effect of tumor type, size and endpoint on tumor radioprotection by WR2721. Int. J. Radiat. Oncol. Biol. Phys. 10: 41-48.
15. Milas, L., McBride, W.H., Hunter, N., and H. Ito. 1984. Protection by S-2-(3-aminopropylamino)ethylphosphorothioic acid against radiation and cyclophosphamide-induced attenuation in anti-tumor resistance. Cancer Res. 44: 2382-2386.
16. Milas, L., Murray, D., Brock, W.A., and R.E. Meyn. 1988. Radioprotectors in tumor radiotherapy: factors and settings determining therapeutic ratio. Pharmacol. Ther. 39: 179-187.
17. Milas, L., Hunter, N., and B.O. Reid. 1981. Protective effects of WR2721 against radiation-induced injury of murine gut, testes, lung, and lung tumor nodules. Int. J. Radiat. Oncol. Biol. Phys. 8: 535-538.
18. Milas, L., Hunter, N., Reid, B.O., and H.D. Thames. 1982. Protective effects of S-2-(3-aminopropylamino)ethylphosphorothioic acid against radiation damage of normal tissues and a fibrosarcoma in mice. Cancer Res. 42: 1888-1895.
19. Penhaligon, M. 1984. Radioprotection of mouse skin vasculature and the RIF-1 fibrosarcoma by WR2721. Int. J. Radiat. Oncol. Biol. Phys. 10: 1541-1544.
20. Rasey, J.S., Krohn, K.A., Menard, T.W., and A.M. Spence. 1986. Comparative biodistribution and radioprotection studies with three radioprotective drugs in mouse tumors. Int. J. Radiat. Oncol Biol. Phys. 12: 1487-1490.
21. Rasey, J.S., Grunbaum, Z., Krohn, K.A., Menard, T.W., and A.M. Spence. 1985. biodistribution of the radioprotective drug ^{35}S-labeled 3-amino-2-hydroxypropyl phosphothioate (WR77913). Rad. Res. 102: 130-137.
22. Rasey, J.S., Krohn, K.A., Magee, S., Nelson, N., and L. Chin. 1986. Comparison of the protective effects of three phosphorothioate radioprotectors in the RIF-1 tumor. Rad. Res. 108: 167-175.
23. Rojas, A., Stewart, F.A., and J. Denekamp. 1983. Interaction of misonidazole and WR2721—II. Modification of tumor radiosensitization. Br. J. Cancer 47: 65-72.
24. Stewart, F.A., Rojas, A. and J. Denekamp. 1982. Radioprotection of two mouse tumors by WR2721 in single and fractionated treatments. Int. J. Radiat. Oncol. Biol. Phys. 9: 507-513.
25. Tabachnik Schor, N.F. 1987. Adjunctive use of ethiofos (WR2721) with free radical generating chemotherapeutic agents in mice: new caveats for therapy. Cancer Res. 47: 5411-5414.
26. Travis, E.L. 1984. The oxygen dependence of protection by aminothiols: implications for normal tissues and solid tumors. Int. J. Radiat. Oncol. Biol. Phys. 10:1495-1501.
27. Twentyman, P.R. 1983. Modification by WR2721 of the response to chemotherapy of tumors and normal tissues in the mouse. Br. J. Cancer 47: 57-63.
28. Twentyman, P.R. 1981. Modification of tumor and host response to cyclophosphamide by misonidazole and by WR2721. Br. J. Cancer 43: 745.
29. Williams, M.V., Rojas, A., and J. Denekamp. 1984. Tumor sensitization and protection: influence of stromal injury on estimates of dose modification. Int. J. Radiat. Oncol. Biol. Phys. 10: 1545-1549.

30. Wist, E.A. 1985. Effect of the radioprotector WR2721 on the response of metastatic Lewis lung carcinoma colonies to alkylating agents. Acta Radiol. Oncol. 24: 259-306.

31. Yuhas, J.M. 1979. Differential protection or normal and malignant tissues against the cytotoxic effects of mechlorethamine. Cancer Treat. Rep. 63: 971-976.

Review #4

I. Pharmacodynamics:

1. RCC Group, Project 359065- General Pharmacology of WR2721: effect on isolated ileum of guinea pigs. NDA 20221, AM 7/12/94

2. RCC group, Project 359076- general pharmacology of WR2721: effect on isolated uterus of female rats. NDA 20221, AM 7/12/94

3. Rcc groups, project 359087-general pharmacology of WR2721: effects on isolated aorta of the rabbit. NDA 20221, AM 7/12/94

4. RCC group, project 359100-general pharmacology of WR2721: effects on inferior eyelid contractions, cardiovascular and respiratory systems of the rat. NDA 20221, AM 7/12/94

II. Reproductive Toxicology:

1. 621-001 Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to CRL:CDBR VAF/plus presumed pregnant rats. IND #162.

2. 621-002: Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of ethyol administered intravenously to New Zealand white rabbits. IND #162.

III. Genotoxicology

1. Study to determine the ability of WR1065 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan requiring strains of Escherichia Coli. (Report 1185/4) IND #173.

2. Study to determine the ability of WR2721 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan requiring strains of Escherichia Coli. (Report 1185/5) IND #173.

3. Study to evaluate the chromosome damaging potential of WR1065 by its effects on cultured human peripheral blood lymphocytes using an in vitro cytogenetics assay (report # 1185/2) IND #173.

4. Study to evaluate the potential of WR2721 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report #1185/3) IND #173.

5. Study to evaluate the potential of WR1065 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice (report # 1185/7) IND #173.

Study to determine the ability of WR1065 to induce mutations at the thymidine kinase (tk) locus in mouse lymphoma L5178Y cells using a fluctuation assay (report # 1185/1) IND #173.

6. Amifostine report to US Bioscience by Dr. David J. Grdina. Human lymphoblastoid tk test and CHO cell hprt test of WR2721 and WR1065. IND #173.

Review #6

1. In vitro studies of the effect of WR1065 on the antitumor activity of standard anticancer drugs against human MCF-7 breast cancer and human A2780 Ovarian cancer

2)Effect of ethyol and paclitaxel on survival of SCID mice bearing human ovarian cancer Xenografts:

3) Investigation of cisplatin cytotoxic activity in sensitive and resistant human embryonic carcinoma cell lines, in the presence and absence of amifostine

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION and DRUG HISTORY:

Ethyol has been previously approved "to reduce the cumulative renal toxicity associated with repeated administration of cisplatin in patients with advanced ovarian cancer or non small cell cancer....the clinical data do not suggest that the effectiveness of cisplatin based chemotherapy is altered by ethyol." One of the major issues during the approval process was the protection of tumor tissue from the effects of chemotherapy.

PHARMACOLOGY:

1. Menard, TW et al., 1984. Radioprotection by WR-2721 of gamma-irradiated rat parotid gland: effect on gland weight and secretion at 8-10 days post irradiation. Int. J. Radiat. Oncol. Biol. Phys. 10:1555-1559.

Male Sprague Dawley rats (175-200 g) were administered 400 mg/kg WR2721 ip 15-30 minutes prior to gamma irradiation (15.3 Gy at 0.63 Gy/min). At 8-10 days following irradiation, the parotid gland was cannulated and the saliva collected (rate volume measured) after pilocarpine stimulation; parotid glands were then dissected out and weighed. Body weights were measured daily.

The rats without WR-2721 lost weight consistently through the recovery period, while WR-2721 treated rats began gaining weight on day 2 and returned to baseline by day 7 (approximately 30% difference between the two groups). A similar weight loss pattern to that with the WR-2721 + irradiation was noted with WR2721 alone. Parotid gland weight in the WR-2721 treated rats was approximately double that in the irradiation alone group but approximately 33% lower than that of unirradiated rats. Flow rate and total volume of secretion was approximately 5-8 fold higher in the WR-2721 treated rats as compared to the irradiation alone rats, but still less than at least one of the control groups. The sponsor calculated the relative protection factors with WR-2721 for gland weights, reduction in parotid gland flow rate, total salivary gland volume to be between 2 and 3.

2. Sodicoff, M et al. 1978. Radioprotection by WR-2721 against long-term chronic damage to the rat parotid gland. Radiat. Res. 76: 172-179.

Female Sprague Dawley rats treated with 400 mg/kg WR-2721 ip 15-20 minutes prior to irradiation of the right side of the head (left side shielded) at 1.6, 2.4, 3.2, 4.8., or 6.4 kR (140 R/min) and the gland weight and amylase ratios measured at day 30, 60 or 90.

Weight reductions in parotid glands leveled off by day 60, so 60 day measurements were used for comparisons. In animals without WR-2721 at doses above 2.4 kR, dose dependent reductions of greater than 50% in parotid weight were seen (max 90% at 4.8 kR). In comparison, with WR-2721, reduction in parotid weight at 4.8 kR was approximately 45%. The sponsor calculated a dose modification factor (DMF) of 2.3. Amylase measurements were variable. In the acute phase (up to day 9), DMFs were between 1.7 and 2.5 for gland weight and amylase.

3. Sodicoff, M. et al. Short-term radioprotective effect of WR-2721 on the rat parotid glands. Radiat. Res. 75: 317-326.

Female Sprague Dawley rats treated with 400 mg/kg WR-2721 ip 15-20 minutes prior to irradiation of the right side of the head (left side shielded) at 1.6, 2.4, 3.2, 4.8., or 6.4 kR (140 R/min) and the gland weight and amylase ratios measured at days 1-9.

Parotid gland weights reached a nadir on day 4, then rose towards baseline. Amylase levels showed a similar time curve. The DMF (calculated from log plots of relative weight versus radiation dose) with WR-2721 for weight was 2.5; for amylase, 1.7.

4. Hubner RH et al. 1997. Radioprotection of salivary glands by amifostine. Radiol. Oncol. 31: 279-285.

Five 3 month old male New Zealand white rabbits were administered 1GBq I-131; 3/5 of these rabbits were also given 200 mg/kg WR-2721 (timing not given). Salivary gland scintigraphy (uptake of Tc-99mpertechnetate) was conducted prior to radio-ablation of the thyroid, and at 4, 8 and 12 weeks post-treatment. Rabbits were killed at 12 weeks and the salivary glands examined microscopically.

Thyroid ablation was complete in all rabbits at 4 weeks. In the group without WR2721, parotid and submandibular gland uptake of Tc was decreased by 63 and 46% respectively at 12 weeks while in the +Wr2721 group, uptake was decreased by approximately 15% in both tissues. Lipomatosis and inflammation were less pronounced in the glands of animals given ethyol.

5. Dendale, R. et al. 1997. Effect of systemic and topical administration of amifostine on radiation-induced mucositis in mice. Proc. Amer Soc Clin Oncol. 16:64a. Abstract.

Female C57B1/6 mice (6-10 weeks old, n=8) were given either 50 mg topical ethyol to the mouth, 200 or 400 mg/kg ethyol ip 30 minutes prior to irradiation with 24 Gy in 4 fractions every 8 hours to the tip of the mouth. Weight loss and mucosal reactions were scored.

Maximal mucositis was observed at day 11. Severity of mucositis and weight loss was diminished (magnitude not given) with amifostine.

6. Fichtner, I et al, 1997. Effects of amifostine (WR-2721, ethyol) on tumor growth and pharmacology of cytotoxic drugs in human xenotransplanted neuroblastomas. Anti-Cancer Drugs 8:174-181.

Human neuroblastoma xenografts IMR5-75 and Kelly were transplanted into nude mice. Mice were treated with either cyclophosphamide (ip, 150 mg/kg), ifosfamide (ip 400 mg/kg), cisplatin (ip 8 mg/kg), adriablastin (10 mg/kg iv), vincristine (1 mg/kg ip), or etoposide (40 mg/kg ip) with or without 200 mg/kg amifostine (ip, 30 minutes prior to cytotoxic). Tumor volume was measured twice weekly, body weight and blood counts were also monitored.

At weeks after the start of treatment, no meaningful differences in tumor response with amifostine was observed. Likewise, no meaningful changes in WBC parameters were noted with amifostine either.

SAFETY PHARMACOLOGY none submitted

PHARMACOKINETICS AND TOXICOKINETICS: none submitted

TOXICOLOGY:

Long Term

1. 90 day intravenous toxicity study of amifostine in Sprague Dawley Rats.

Study # and Study Title:

Conducting laboratory and location:

Date of study initiation: June 2, 1997 (in life completed October 23, 1997)

GLP compliance: Yes (X) No ( ) QA: Yes (X) No ( )

Key study findings: Lethality at HD resulted in dose reduction by day 34/35 to MD levels. No NOAEL was established. Target organs were leukocytes, male gonads, and sporadic effects on liver and kidney. Toxicokinetic timepoint was poorly chosen.

Species and strain: Sprague Dawley rats

#/sex/group or timepoint: 10/sex/dose for main study; 5/sex Control, HD for recovery; satellite groups used for TK or recovery: 10/sex MD and HD for TK.

Age: 8 weeks

Weight: not provided
drug, lot#, radiolabel, % purity: Lo2 95L10-17
formulation/vehicle: 0.9% NaCl, drug 50 mg/mL
dosage groups: 0, 25, 50, 75 mg/kg/day (0, 150, 300, 450 mg/m2/day, HD was decreased to 50 mg/kg/day at day 35 for males, 34 for females.
route, form, volume, infusion rate: iv at 0, 0.5, 1.0 or 1.5 mL/kg
Duration: daily X 90 with necropsies at day 90/91, day 119.

NOTE: The number of animals examined at termination did not match the initial proposed #/dose. There were 15 control (10 day 90, 5 day 119), 10 LD, (5 day 90 and 119), 15 MD (10 day 90, 5 day 119) and 20 HD (10 day 90, 119).

Observations:
Clinical signs (twice daily): Deaths are summarized in the following table. Mortality was increased in the males. No deaths were observed in the control and LD groups. With the exception of 1 animal with a urinary tract infection, no cause of death was attributable and no abnormal clinical signs heralded death in these animals.

	Males		Females	
	# dead/group	Days of death	# dead/group	Days of deaths
Control	0/15	—	0/15	—
LD	0/10	—	0/10	—
MD	9/15	19,29,35,42,45(2),46,66,76	1/15	45
HD	13/20	11,19,32,42,44,46,51,52,59, 60,66,73,112	8/20	16,22,29(2),45,55(3)

Clinical observations that were dose dependent included dried brown matting around mouth, salivation, rough coat, nasal discharge and urogenital wetness/staining.

Body weights (weekly): Body weight decrements are shown below.

% decrement in body weight on day 90 as compared to controls			
	LD	MD	HD
Males	↓10%	↓22%	↓21%
Females	—	↓8%	—

Hematology (week 7, 13, 17): WBC # in both males and females were decreased at all doses. Some recovery was seen after 4 weeks. Drops in WBC # were reflected primarily in lymphocyte decreases.

% change in WBC # as compared to controls				
Sex	Dose	D43	D90	D119
Males	LD	—	↓36%	↑11%
	MD	↓38%	↓62%	↓20%
	HD	↓52%	↓64%	↓15%
Females	LD	↓12%	↓14%	—
	MD	↓31%	↓57%	↑13%

	HD	↓47%	↓60%	↓32%

Serum chemistry (week 7, 13, 17): Changes are shown in the following table. All but the glucose in males resolved by the end of the recovery period.

% change as compared to controls				
Parameter	Sex	D43	D90	D119
BUN	M	↑19% M, H	↑20% H	—
Cholesterol	M	—	↑38%M	—
	F	↑20% L, ↑25% M, ↑40% H	↑30% L, ↑34% M, ↑40% H	—
Glucose	M	↓12% H	↑38% M, ↑19% H	↑21% L, ↓15% M
	F	—	↑22% M, ↑18% H	—
total protein	M	↓10% H	—	—
Ca++	M	—	↓14% H	—
K+	M	—	↓17% H	—
	F	–	↓11% H	—

Ophthalmoscopy (pretest, termination): No drug related changes were noted.

EKG: (pretest, weeks 13, 17): At week 13, one LD female, 1 HD male and 2 HD females had EKG changes. All were premature depolarizations with QRS of the right bundle branch block configuration. One rat had persistent depolarizations through the recovery phase. Only one rat had "striking" changes.

Organ weights: (week 13, 17): There were no consistent changes in organ weights between absolute and organ weight relative to body weight or between sexes at either the end of treatment or recovery. There was a hint of a trend in decreases in testes/prostate/epididymis weights at the end of the recovery period, but this was not statistically significant.

Gross pathology (week 13, 17): Few of the macroscopic lesions increased in incidence or severity with dose. Two HD males had small testes while one HD female showed uterine dilatation. One MD animal had urinary bladder dilatation, pale kidneys, red lung discoloration, and fluid in the thoracic cavity; all of which were attributed to a urinary tract infection.

Histopathology (week 13; 17 on control, HD): Histopathology was not conducted at day 119. The major finding was testicular degeneration and associated hypospermia in the MD and HD males. All relevant findings are summarized in the following table.

Observation	Males	Females
Adrenal—focal cortex hypertrophy	2/10C, 2/3M, 1/4H	2/7 H
Heart—myocardial degeneration	2/10 C, 1/4 H	—
Kidney—nephropathy	—	1/7 H
Liver—hepatodiaphragmatic nodule and focal fibrosis	—	1/7 H
Pancreas—atrophy	1/5 LD	—
Epididymis—hypospermia	1/10C, 1/3 M, 4/4 H	
Testes—degeneration	3/3 M, 4/4 H	
Testes—hyperplasia	1/10 C, 4/4 H	

Toxicokinetics (day 1, 90 at 6 hours post-dose): No measurable levels of drug were found.

Histopathology Inventory for NDA #20-221

Study	IV						
Species	Rat						
Study Duration	DX90						
Adrenals	X						
Aorta							
Bone Marrow smear	X						
Bone (femur)	X						
Brain	X						
Cecum	X						
Cervix							
Colon	X						
Duodenum	X						
Epididymis	X						
Esophagus	X						
Eye	X						
Fallopian tube							
Gall bladder							
Gross lesions	X						
Harderian gland							
Heart	X						
Hypophysis							
Ileum	X						
Injection site	X						
Jejunum	X						
Kidneys	X						
Lachrymal gland							
Larynx							
Liver	X						
Lungs	X						
Lymph nodes, cervical							
Lymph nodes mandibular	X						
Lymph nodes, mesenteric	X						
Mammary Gland	X						
Nasal cavity							
Optic nerves							
Ovaries	X						
Pancreas	X						
Parathyroid	X						
Peripheral nerve							
Pharynx							
Pituitary	X						
Prostate	X						
Rectum	X						
Salivary gland	X						
Sciatic nerve	X						
Seminal vesicles	X						
Skeletal muscle	X						
Skin	X						
Spinal cord	X						
Spleen	X						
Sternum							
Stomach	X						
Testes	X						
Thymus	X						
Thyroid	X						

Tongue							
Trachea	X						
Urinary bladder	X						
Uterus	X						
Vagina							
Zymbal gland							

Immunotoxicology: none submitted

Reproductive Toxicity: none submitted

Genetic Toxicity: none submitted

OVERALL SUMMARY AND EVALUATION:

Ethyol appears to diminish the acute and long term effects of radiation on the oral cavity in several models. Doses of 200-400 mg/kg/day in the rats (1200 –2400 mg/m2) are similar to those used in earlier chemoprotection studies both with chemotherapy and radiation, but are greater than the recommended human dose of 910 mg/m2. Minimal effects on the response of neuroblastoma xenografts to chemotherapy were noted with the addition of ethyol.

In previously reviewed articles, decrements in tumor response to radiation and chemotherapy have been demonstrated. A series of the protection factors are shown in the following table. Most of the models where tumor protection is seen are of well-oxygenated micro-metastases. The mechanism of ethyol's activity is believed to be preferential uptake of WR-2721 and its metabolite WR-1065 by normal tissue as compared to tumor tissue based on alkaline phosphatase levels, pH and other considerations. Thompson and Chaney (Cancer Res 55:2837-2846, 1995) in comparing metabolism of ormaplatin in fibrosarcoma bearing rats observed no difference in ethyol specificity between tumor and normal tissue (and, when both drugs are administered ip, showed significant inactivation of platinum complex by ethyol).

SUMMARY OF PROTECTION OF TUMORS (DMFs) IN SELECTED PAPERS

AUTHOR	TUMOR	TREATMENT	DMF
Clement, 1981	P388	500 mg/kg WR2721+irrad.	1.4
	Lewis lung	500 mg/kg WR2721+irrad	1.3
Milas	Fsa 5 mm tumor (leg)	400 mg/kg WR2721+irrad	1.11
	Fsa 8 mm tumor (leg)	400 mg/kg WR2721+irrad.	0.94
	MCa-4	400 mg/kg WR2721+irrad	1.2-1.3
	NFsa metastases	400 mg/kg WR2721+irrad.	1.22
Stewart, 1982	SA FA	400-600 mg/kg WR2721+irrad.	0.95-2.5
	CA MT	250 mg/kg WR2721+single irrad.	1.1
		250 mg/kg WR2721+fract.irrad.	1.2
Milas, 1982	Fsa (metastases)	400 mg/kg WR2721+irrad.	1.28
Rasey, 1985	RIF-1	400 mg/kg WR2721+irrad.	1.58

The preferred model for tumor protection studies is *in vivo*, not *in vitro*. No *in vitro* model to date has shown protection from the effects of chemotherapy with ethyol. Prior discussions within the division and with the sponsor have determined that the ultimate proof of lack of tumor protection will be clinical data. After discussion with Dr. I. Chico, no data has been submitted that supports the sponsor's deletion of the text on possible tumor protection in the package insert label.

The 90 day toxicology experiment conducted was not optimal. Both the MD (50 mg/kg) and HD (75 mg/kg) resulted in significant lethality (the HD was decreased to the same level as the MD after day 34/35). No NOAEL was established (<25 mg/kg). Target organs of toxicity included the leukocytes, male gonads, and sporadic effects on the heart and kidney. Given the short half-life of ethyol, it is not clear why a single time point of 6 hours was chosen for toxicokinetic measurements.

RECOMMENDATION: The pharmacology/toxicology data supports the approval of the new indication. However, the sponsor's request to alter the tumor protection data in the package insert is not acceptable.

a) **Comments for further studies:** none
b) **Points discussed with Medical Officer:** tumor protection data from literature
c) **Label comments:** The first paragraph in Clinical Pharmacology section should be replaced with the following.

Draft Letter to the Sponsor:

The request to delete the text on tumor protection in the package insert label is not acceptable.

The first paragraph in Clinical Pharmacology section should be replaced with the following.

/S/
Wendelyn J. Schmidt, Ph.D.
Pharmacologist/Toxicologist
5/12/99
Date

Concurrence: /S/
Paul A. Andrews, Ph.D.
Pharmacology Team Leader
5/12/99
Date

NDA # 20-221 Review 7
Original IND/NDA/DMF
c.c. /Division File NDA 20221
/WSchmidt
/PAndrews
/IChico
/MPelosi