Tirapazamine: A new drug producing tumor specific enhancement of platinum-based chemotherapy in non-small-cell lung cancer


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Summary

Background: Tirapazamine (TPZ), a new anti-cancer drug activated to a toxic free radical under hypoxic conditions, produces a tumor specific potentiation of cell kill by cisplatin. In the present study we discuss the mechanism and clinical potential of this effect, as well as investigate the influence of p53 mutations on the activity of TPZ.

Materials and methods: For in vitro experiments we have used mouse SCCVII tumor cells, minimally transformed mouse embryo fibroblasts (MEFs) from wild-type and p53 knockout mice, and several human NSCLC cell lines. For in vivo experiments we have used RIF-1 tumors implanted subcutaneously into C3H mice.

Results: Prior injection of TPZ into tumor-bearing mice markedly potentiated tumor cell kill by cisplatin, but produced no effect on systemic toxicity. The maximum potentiation occurred when TPZ was injected two to three hours prior to cisplatin administration. Experiments performed with cells in vitro showed a similar synergistic interaction between the two drugs when cells were exposed to TPZ under hypoxic conditions prior to exposure to cisplatin. Experiments with MEFs from either p53 wild-type or p53-knockout mice showed no influence of p53 on the sensitivity of cells to killing by TPZ under hypoxia. A similar lack of influence of p53 on the toxicity to TPZ was obtained for a panel of NSCLC cell lines.

Conclusions: TPZ is a novel anticancer drug that produces tumor selective potentiation of cisplatin and carboplatin in both pre-clinical and clinical studies. The fact that the drug produces no potentiation of the systemic side effects of these drugs, or of other anticancer drugs used in combination with platinum in NSCLC, suggests that TPZ could become a useful agent in the treatment of lung cancer.

Key words: carboplatin, cisplatin, hypoxia, NSCLC, p53, tirapazamine

Introduction

Tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide, SR 4233, TPZ) is a new anti-cancer drug with specific activity towards cells at low oxygen tensions [1, 2]. This preferential toxicity towards hypoxic cells has been demonstrated by a number of different laboratories with many different cell lines of rodent and human origin. The hypoxic cytotoxicity ratio (ratio of drug concentrations under aerobic to hypoxic conditions to give the same level of cell kill) is typically in the range of 50–150. This is much larger than that found for the prototypic bioreductive drug mitomycin C for which values of 1 (no differential toxicity) to 5 have been reported [3, 4]. Figure 1 shows typical data for the killing of cells under aerobic and hypoxic conditions exposed to TPZ for 1.5 hours.

The mechanism for the preferential toxicity of TPZ towards hypoxic cells is a result of an enzymatic reduction that adds an electron to the TPZ molecule forming a highly reactive radical [2]. This radical causes cell killing by producing DNA damage leading to chromosome aberrations [5]. However, in the presence of oxygen, the radical is 'back oxidized' to the nontoxic parent with a concomitant generation of superoxide radicals [6]. Because of the high reactivity of the TPZ radical (and hence its limited diffusion capability), as well as the similarity of the chromosome breaks produced by TPZ to those induced by densely ionizing radiation, we have suggested that the activating reductase(s) must be close to DNA, thereby producing high local concentrations of the radical [2]. Our laboratory has recently obtained support for this hypothesis by showing that DNA damage occurs only from TPZ metabolism within the nucleus [7]. A diagrammatical representation of TPZ metabolism close to DNA producing DNA damage is shown in Figure 2.

TPZ combined with platinum based chemotherapy: A tumor specific synergistic interaction

Because of the demonstration in pre-clinical studies with transplanted tumors that hypoxic cells in these tumors are resistant to killing by cisplatin [8], we reasoned that TPZ with cisplatin would be an effective combination based on the complementary cytotoxicity of the two agents. When this combination was tested in transplanted tumors, we indeed found that the combination was highly synergistic unless the two drugs were given at the same time. Figure 3 shows the data for cell
survival of the RIF 1 tumor treated in vivo with TPZ and cisplatin with different time periods between the two injections. These data demonstrate that, whereas the two agents given at the same time produce additive cytotoxicity, when TPZ is given before cisplatin, there is a highly synergistic interaction of the two agents. Despite this enhancement of tumor cell kill, we have shown that there is no change in the systemic toxicity of cisplatin when TPZ is combined with cisplatin [9]. Thus, the synergistic interaction of TPZ with cisplatin is tumor specific and therefore represents a therapeutic gain over cisplatin alone. We, and others, have obtained similar TPZ synergy with carboplatin, with combinations of TPZ and cisplatin with either etoposide or navelbine [10], and with combinations of TPZ with carboplatin and taxol [11], or oxaliplatin and Taxol [12].

These preclinical studies have formed the basis for extensive clinical testing of TPZ combined with cisplatin, particularly in advanced non-small-cell lung cancer (NSCLC). To date phase I and II clinical studies have confirmed both the increased anti-tumor activity of TPZ combined with cisplatin compared to historical controls of cisplatin only, as well as the absence of any increased systemic toxicity of cisplatin [13–15]. Recently, results of an international randomized phase III study of TPZ plus cisplatin versus cisplatin alone in stage IIIB and IV NSCLC demonstrated a significant survival advantage of the combination versus cisplatin alone [16]. These clinical studies, therefore, confirm the tumor specific synergy between TPZ and cisplatin and suggest that TPZ may prove to be an effective addition to chemotherapy regimens of solid tumors that contain platinum based anti-cancer drugs.

Mechanism of the tumor specific potentiation of cisplatin by TPZ

Although the original hypothesis that cisplatin and TPZ would be an effective combination in tumors based on their complementary cytotoxicity for aerobic and hypoxic cells respectively, this cannot account for all of the interaction. We have shown, for example, that RIF 1 cells treated in vitro with TPZ under hypoxic conditions are more sensitive to killing by a subsequent dose of cisplatin [9]. We have now performed additional in vitro studies, which have confirmed this increased sensitization of cells by pre-treatment by TPZ under hypoxic conditions [17]. In particular, we have shown that when the two drugs are given at the same time, there is only additive cytotoxicity, whereas when TPZ is given prior to the cisplatin, the interaction is synergistic, as was shown for tumors in vivo. We observed this synergistic interaction with human non-small-cell lung cancer cells, with NIH3T3 mouse cells, and with hamster CHO cells. We have also shown that this interaction depends on the TPZ exposure being under hypoxic conditions; there is absolutely no interaction when the TPZ is given under aerobic conditions.
Figure 3. The number of clonogenic cells per tumor in RIF-1 tumors relative to control tumors. The tumor-bearing mice received injections of TPZ (0.35 mmoles/kg) alone, cisplatin (8 mg/kg) alone or a combination of the two drugs with different times between the two injections. The mice were sacrificed 24 hours after the cisplatin injection. Data redrawn from Dorie & Brown [9].

Figure 4. Clonogenic cell survival of E1A and Hras transformed mouse embryo fibroblasts (MEFs) from p53 wild-type (p53+/+) and p53 knockout (p53−−) mice. The cells were exposed to TPZ under hypoxic conditions for one hour and the survival measured by clonogenic assay.

In an attempt to understand the mechanism for this synergy, we have found that the interaction does not occur in cells that are deficient in DNA cross-link repair, thereby implicating this repair process in the mechanism of the interaction. Indeed, we have shown that cells pretreated with TPZ under hypoxic conditions are more susceptible to cisplatin induced DNA interstrand cross-links [17].

Given the similarity of the schedule dependence of the *in vitro* and *in vivo* results, it seems likely that most, if not all, of the interaction is a result of this cellular phenomenon rather than as a result of complementary cytotoxicity between TPZ and cisplatin in the tumor. The absolute requirement for hypoxia, however, is the basis for the tumor selectivity. The fact that most human tumors have median oxygen levels well below those of normal tissues[18] is the basis for the expectation that this tumor specific interaction will occur in the majority of human tumors.

### TPZ cytotoxicity is independent of p53 status

Since the p53 tumor suppressor gene is mutated in more than half of all solid tumors including NSCLC [19], and mutations in p53 are associated with significantly worse prognosis for these tumors [20], it is pertinent to ask whether cells with mutated p53 are more resistant to TPZ, as has been suggested for many anti-cancer agents [21]. We have tested for this in two different ways.

First, we have measured the surviving fraction using a clonogenic assay for mouse embryo fibroblasts (MEF’s) transfected with the dominant oncogenes Ela and Hras. The mouse embryo fibroblasts were obtained from wild-type (p53+/+) and p53 knockout (p53−−) mice. These same p53−− MEF’s have been shown to be resistant to several anti-cancer drugs, both as cells *in vitro* [22] and as tumors growing in immune suppressed mice [23]. Figure 4 shows the results of this experiment and demonstrates that by clonogenic survival, the p53+/+ and p53−− cells have essentially the same sensitivity to a one-hour treatment of various concentrations of TPZ under hypoxia.

In the second series of experiments, we have performed a similar study with the NSCLC cell lines taken from the NCI panel of 60 cell lines that is used to screen anti-cancer agents. Nine of these eleven cell lines were easily cloned *in vitro*, and their sensitivity by clonogenic assay to various concentrations of TPZ under hypoxia is show in Figure 5. The p53 status of these cell lines has...
recently been published [24], and seven of the nine have
mutant p53. It can be seen from Figure 5, however, that
the two cell lines with wild-type p53 fall within the range
of the sensitivities to TPZ of the cells with mutant p53.
Again, therefore, these data support the view that the
cytotoxicity of TPZ under hypoxia is independent of p53
status.

Although these data do not address the question of
whether the interaction of TPZ with cisplatin is depend-
ent upon p53 status, the fact that cells with mutant p53
have been shown to be more sensitive to cisplatin [25]
suggests that there is little or no basis for expecting that
NSCLC with mutated p53 would be refractory to the
combination of cisplatin with TPZ.

Clinical potential

Tirapazamine (TPZ) is the first drug to enter clinical
practice that exploits a unique feature of solid tumors,
namely their low oxygen tension. The drug is inactive
until it is metabolized to a damaging radical, a process
that only occurs at oxygen tensions lower than those
found in normal tissues but well within the range of
those found in a variety of human solid tumors, includ-
ing brain [26], head and neck [27], lung [28], breast [29],
cervix [30], anal canal [31], and soft tissue sarcomas [32].
All of these data show that the median oxygenation of
these human tumors is considerably less than that of
normal tissues. It is this lower oxygen level that is
responsible for the preferential metabolism of TPZ in
tumors and, as our data indicate, is responsible for the
tumor specific synergy between TPZ and fractionated
irradiation [33] and platinum based chemotherapy [9, 10].
In terms of the interaction of TPZ with cisplatin, clinical
trials have now shown that both response rates and
survival times of patients with advanced non-small lung
cancer can be significantly increased when TPZ is added
to standard cisplatin based chemotherapy. Since pre-
clinical studies have also shown that TPZ is synergistic
with cisplatin, carboplatin and oxaliplatin either alone
or combined with etoposide, nabvbline or with Taxol, this
suggests that the addition of TPZ to these combinations is
likely to prove more effective than any two drug com-
binations presently being employed with this disease.

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References

bioreductive agent with high selective toxicity for hypoxic
1239-42.

exploiting hypoxia in solid tumours. Br J Cancer 1993; 67 (6):
763-70.

3. Fracasso PM, Santorelli AC. Cytotoxicity and DNA lesions pro-
duced by mitomycin C and porfiromycin in hypoxic and aerobic
EMT6 and Chinese hamster ovary cells. Cancer Res 1986; 46 (8):
3939-44.

4. Marshall RS, Rauth AM. Modification of the cytotoxic activity of
mitomycin C by oxygen and ascorbic acid in Chinese hamster
2709-13.

5. Wang J, Biedermann KA, Brown JM. Repair of DNA and
chromosome breaks in cells exposed to SR-4233 under hypoxia

6. Lloyd RV, Duling DR, Rumyantseva GV et al. Microsomal
reduction of 3-amino-1,2,4-benzotriazine-1,4-dioxide to a free

is metabolized to its DNA damaging radical by intracellular

8. Grau C, Overgaard J. Effect of cancer chemotherapy on the
hypoxic fraction of a solid tumor measured using a local tumor

9. Dorie MJ, Brown JM. Tumor-specific, schedule-dependent inter-
action between tirapazamine (SR 4233) and cisplatin. Cancer Res

10. Dorie MJ, Brown JM. Modification of the antitumor activity of
chemotherapeutic drugs by the hypoxic cytotoxic agent tirapazo-

11. Mangold G, Dexter D, Juniewicz P et al. Evaluation of paclitaxel-
ctabinol–tirapazamine combinations in MV-522 human lung
Oncol 1999; Abstr 1704.

tirapazamine–Taxol combinations in the MV-522 human lung
carcinoma xenograft model. Proc Am Assoc Cancer Res 1997;
38: 312 (Abstr 2090).

13. Miller VA, Ng KK, Grant SC et al. Phase II study of the
combination of the novel bioreductive agent, tirapazamine, with
cisplatin in patients with advanced non-small-cell lung cancer.

trial of the combination of tirapazamine and cisplatin in patients

15. Tret J, Haynes B, Johnson E et al. Tirapazamine with cisplatin: A
phase II trial in advanced stage non-small-cell lung cancer

16. von Pawel J, von Roemeling R. Survival benefit from tirazone
carboplatin-tirapazamine combinations in MV-522 human lung

17. Kovacs MS, Hocking D, Evans JW et al. Mechanism of the
tumor-specific enhancement of cell kill by cisplatin produced by
the hypoxic cytotoxic tirapazamine. Proc Am Assoc Cancer Res

18. Vaupel PW, Hockel M. Oxygenation status of human tumors: A
reappraisal using computerized pO2 histography. In Vaupel PW
DKK, Gunderoth M (eds): Tumor Oxygenation. Stuttgart: Gustav


20. Vega FJ, Iribera P, Caldes T et al. p53 exon 5 mutations as a
prognostic indicator of shortened survival in non-small-cell lung

52-53.

22. Lowe SW, Rulye HE, Jacks T, Housman DE. p53-dependent
apoptosis modulates the cytotoxicity of anticancer agents. Cell

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