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SHORT COMMUNICATION

A pharmacokinetic study on *Z*-(±)-2-(1-benzylindole-3-yl-methylene)azabicyclo[2.2.2]octane-3-ol; a novel radio-sensitization agent

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Abstract
Purpose The purpose of this research was to characterize the pharmacokinetic parameters and to evaluate the absolute bioavailability of the targeted compound: *Z*-(±)-2-(1-benzylindole-3-yl-methylene)azabicyclo[2.2.2]octane-3-ol (BMABO), a novel radio-sensitization agent, after oral delivery.
Methods Sprague–Dawley rats received a single oral dose of 20 mg/kg and this was compared with intravenous administration of the compound (1 mg/kg). Blood samples were collected at different time points, and plasma BMABO concentrations were determined using a new sensitive and specific LC/MS analytical method, which utilized electrospray ionization.
Results The bioavailability of orally administered BMABO was determined by comparing plasma concentrations after oral gavage delivery with intravenous delivery. Following delivery of the oral dose, the average C_{max} was 1,710 ± 503 ng/ml, and the AUC-value was found to be 3,561 ± 670 ng min kg/ml mg. Relative to the intravenous dose (100% bioavailability), the bioavailability was 6.2% after oral administration.
Conclusion As the current studies demonstrate the novel radio-sensitization agent BMABO may have potential therapeutic valuable in cancer treatment. Further evaluation of the efficacy and toxicity of BMABO will determine the feasibility of the oral route for future clinical studies.

Keywords Radio-sensitization · Cancer · Pharmacokinetics · Radiotherapy · Therapeutic hyperthermia

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Introduction

In clinical practice, therapeutic hyperthermia (TH) has been used to potentiate radiation-induced cytotoxicity in cancer treatment [1, 3, 8]. TH has proven to be the most potent radiation sensitizer yet identified [3]. Several randomized clinical trials involving different tumor sites have shown benefit from the combined treatment of hyperthermia and radiation [7]. However, in many instances achieving a therapeutic thermal dose can be problematic, resulting in sub-therapeutic levels, and thereby limiting the potential for radio-sensitization [2].

In an attempt to identify and develop small molecules that are non-toxic at physiological temperatures but that function as enhancers of thermal dose at clinically achievable temperatures (i.e., 41°C) we discovered that, *Z*-(±)-2-(1-benzylindole-3-yl-methylene)azabicyclo[2.2.2]octane-3-ol (BMABO) (Fig. 1) is a novel radio-sensitization agent that was able to convert sub-therapeutic hyperthermic exposures into efficacious thermal doses that would yield robust radio-sensitization [5]. The same concept had earlier been

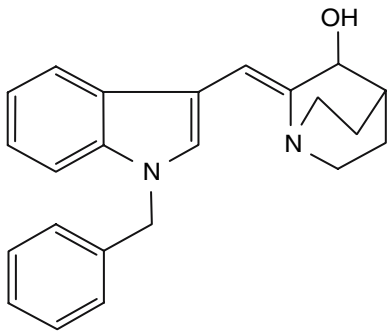

Fig. 1 Chemical structure of *Z*-(±)-2-(1-benzylindole-3-yl-methylene)azabicyclo[2.2.2]octane-3-ol (BMABO)

tested with other structurally related compounds such as indomethacin, which was also shown to lower the threshold thermal exposure of cancer cells to hyperthermic radio-sensitization [4]. BMABO acts as enhancer of heat-induced protein unfolding and denaturation, a mechanism of heat-shock that leads to significant hyperthermic radio-sensitization, which is associated with an increase in the number of cells undergoing a mitotic catastrophe mode of cell-death. We report here the results of a comparative investigation of the blood levels of BMABO delivered intravenously and orally in a rat animal model in an attempt to explore the oral route as a delivery route for future clinical studies.

Materials and methods

Materials

The *Z*-(±)-2-(1-benzylindole-3-yl-methylene)azabicyclo[2.2.2]octane-3-ol was synthesized in our laboratory via a previously reported procedure [6]. Ammonium acetate, sterile water USP, and HPLC grade acetonitrile were obtained from Fisher Scientific, Pittsburgh, PA, USA. Water for HPLC use was passed through a reverse osmosis system (Milli-Q® Reagent Water System) before use. Sodium pentobarbital (50 mg/ml) was obtained from Abbott Labs, North Chicago, IL, USA. Siliconized microcentrifuge tubes, vials, and tips were purchased from Fisher Scientific, Fair Lawn, NJ, USA. Saline (0.9%, injectable) and Heparin sodium (10,000 USP units/ml) were purchased from Baxter Healthcare Corporation, Deerfield, IL, USA. Heparinized caraway capillary tubes were purchased from Baxter Healthcare Corporation, McGraw Park, IL, USA. Polyethylene tubing (ID 0.58 mm) was obtained from the Becton Dickinson Company, Sparks, MD, USA, and silastic tubing (ID 0.50 mm) was obtained from Dow Corning Corporation, Midland, MI, USA. Hamilton syringes 10–100 μl were purchased from Waters Corporation, Milford, MA, USA.

Animals

Male Sprague–Dawley rats weighing between 250 and 300 g (Harlan Laboratories, Indianapolis, IN, USA) were used. The animals were maintained on a standard laboratory diet with water ad libitum and the work was conducted at the University of Kentucky Chandler Medical Center, Division of Laboratory Animal Resources (DLAR). All research and testing activities related to this work were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to the initiation of this research, and during its execution.

In vivo oral and intravenous studies

The bioavailability of the compound BMABO was tested by comparing the kinetic profile after intravenous dosing (100% bioavailable) via the oral gavage technique. For intravenous routing, rats were anesthetized (pentobarbital, 40 mg/kg, IP) and implanted with a jugular cannula under anesthesia in order to administer a 100 μl sterile single dose (1 mg/kg, $n = 3$ rats) of the tested compound formulation (3 mg BMABO/ml in 70% Ethanol) through the cannula followed by a 0.1 ml flush with 10% (v/v) heparin/normal saline solution to keep the cannula patent. Furthermore, for the oral route, utilizing a different set of rats, the tested compound was administered via gavage using gauge 16 stainless steel curved gavage needles (3 and 4″ length) with a ball tip (3 mm ball diameter) to help prevent introduction of the needle into the trachea, and also to prevent trauma to the oral cavity. The feeding needle was inserted through the mouth into the stomach or lower esophagus. Adult animals (post-weaning) were utilized, the needle was passed through the mouth to the back of the throat, and then down the esophagus into the stomach. The tube was attached to a 5 ml syringe, which was used to deliver the tested compound formulation in a volume of 10 ml/kg in doses of 20 mg/kg formulated in 0.5% aqueous carboxymethyl cellulose (CMC) ($n = 3$ rats).

The rats were given a jugular cannulation for blood sampling purposes. Aliquot (150 μl) of blood samples were collected as follows: at baseline; before administration of the test compound (time 0 min), and subsequently at 5, 15, 30, 45, 60, 60, 120, and 180 min following administration of the test compound. The volume of blood removed from the animal after

collection of each sample was replaced with an equal volume of heparin-saline solution. Plasma was separated by centrifugation at 770*g* for 5 min, placed in polypropylene tubes, and frozen at –20°C until the time of analysis.

Sample preparation

Protein in rat plasma samples was precipitated with 300 µl acetonitrile, in 1.5 ml polypropylene test tubes. The samples were vortexed for 30 s and centrifuged at 8,000 rpm for 5 min. About 300 µl aliquot parts of the resulting supernatant were directly transferred to autosampler vials containing low-volume inserts. 10 µl aliquot parts of this final solution were injected onto the HPLC-MS system.

HPLC-MS analysis

Chromatography was performed on a Supelco Supelcosil^{TM} LC-ABZ C_{18} (4.6×50 mm^2, 5 µm) HPLC column with a mobile phase consisting of 90% acetonitrile and 10% 10 mM ammonium acetate. The flow-rate was set at 0.5 ml/min with a total run time of 3 min. The LC-MS system consisted of a Waters 2690 HPLC pump (Waters), a Waters 2695 autosampler, and a Micromass ZQ detector (Waters), which utilized electrospray ionization (ESI). Selected ion monitoring (SIM) was performed in the positive mode, MH+ at *m/z* 345 (dwell time 0.5 s), the capillary voltage was 3 kV and the cone voltage was 35 V. The source block and desolvation temperatures were 100 and 300°C, respectively. Nitrogen was used as the nebulization and drying gas at flow rates of 40 and 400 l/h, respectively. The retention time for BMABO was 1.8 ± 0.15 min. Calibration curves were constructed using a linear regression of the drug peak area versus nominal drug concentrations. The LC/MS analytical method was validated over the concentration range used, and found to have acceptable accuracy, precision, linearity for the determination of BMABO in rat plasma over the concentration range 10–3,000 ng/ml. The limit of quantification (LOQ) was established at 10 ng/ml. MS control and spectral processing were performed using MassLynxTM software, Version 3.5.

Pharmacokinetic analysis

Concentration-time profiles of BMABO after intravenous and oral administration of the formulation solution were evaluated by a non-compartmental model (WinNonlin Professional, Version 4.1, Pharsight Corporation, Mountain view, CA, USA). The pharmacokinetic parameters, such as terminal elimination half-life ($t_{1/2}$), area under the curve from 0 to infinity ($AUC_{0-\infty}$) were estimated using this software.

After a single dose, maximum plasma concentration (C_{max}), and time to reach maximum concentration (T_{max}) were also determined. The absolute bioavailability of the oral formulation was calculated from Eq. 1:

$$F = (\mathrm{AUC}_{\mathrm{PO}}/\mathrm{AUC}_{\mathrm{IV}}) \times (\mathrm{Dose}_{\mathrm{IV}}/\mathrm{Dose}_{\mathrm{PO}}) \times 100, \quad (1)$$

where F is the percent absolute bioavailability, and AUC_{PO}, AUC_{IV}, $Dose_{PO}$, and $Dose_{IV}$ are the area under the curve and corresponding dose for the oral and intravenous administrations, respectively.

Results

Figure 2 illustrates the mean plasma BMABO concentration versus time relationship that resulted after oral dosing through gavage to rats in comparison with IV administration of BMABO. The concentration-time profiles were analyzed by a non-compartmental method, and pharmacokinetic parameters were determined. The mean dose normalized per animal weight AUC-values for BMABO after IV and after oral delivery were 57,833 ± 5,369 and 3,561 ± 670 ng min/ml, respectively (Table 1). The pharmacokinetic parameters for the oral dosing route in comparison with the intravenous route are presented in Table 2.

Following oral administration by gavage, the bioavailability of BMABO was obtained by comparing the

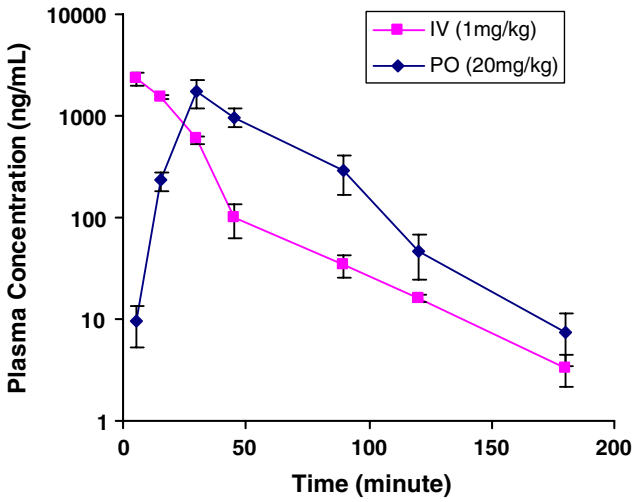

Fig. 2 Mean plasma BMABO levels following intravenous (*IV*) and oral (*PO*) administration at 1 and 20 mg/kg, respectively ($n = 3$). All values show the mean ± STDV

Table 1 Dose normalized area under the curve (AUC) and absolute bioavailability of BMABO formulations in rats ($n = 3$)

Route	Dose (mg/kg)	Dose normalized AUC_{∞}[a] (ng min kg/ml mg) mean ± STDV	Absolute bioavailability (%) mean ± STDV
Intravenous	1	57,833 ± 5,369	100
Oral	20	3,561 ± 670	6.2 ± 0.9

[a] The extrapolation of AUC observed from time zero to last time point to yield AUC_{∞} was 21 and 26% after intravenous and oral dosing, respectively

Table 2 Pharmacokinetic parameters following intravenous administration (IV) and oral administration (PO) of BMABO formulations to rats ($n = 3$)

Parameter	Oral (mean ± STDV)	Intravenous (mean ± STDV)
T_{max} (min)	30 ± 0	5 ± 0
C_{max} (ng/ml)	1,710 ± 503	2,335 ± 333
$t_{½}$ (min)	18.6 ± 1.7	27.5 ± 6.3
CL/F (ml/min kg)	288 ± 57	–
CL (ml/min kg)	17.3 ± 3.4	17.4 ± 1.6
V_d/F (ml/kg)	7,733 ± 1,783	–
V_d (ml/kg)	471 ± 101	680 ± 106

mean AUC after intravenous and oral administration, and was found to be 6.2 ± 0.9% (Table 1).

Discussion

Results of this study showed that the absolute oral bioavailability of BMABO was 6.2%. Due to the fact that many compounds taken orally undergo significant first-pass metabolism by the liver, the relatively low-bioavailability could be attributed to extensive first-pass metabolism by the liver, or could be due to a low-absorption profile. Nevertheless, even though the observed oral bioavailability of BMABO is low, it may still be sufficient to produce an enhancement in radio-sensitization in patients undergoing radiotherapy in conjunction with TH. Furthermore, the possibility for metabolites to be radio-sensitizing as well might not be affected by the compound low-bioavailability. Knowledge about metabolism, metabolites and their activity is therefore a key for the future development of this drug. On the other hand, if the low-oral bioavailability is really due to the fact that the parent compound BMABO is not absorbed from the gastrointestinal tract, and it is mainly excreted unchanged in the rat feces, then this could have consequences for using radiotherapy in the abdominal region. If nearly 95% of the dose of the BMABO radio-sensitizing drug remains concentrated in the gut lumen, the gut would be highly sensitized for any radiation therapy exposure. However, further testing to study efficacy and toxicity will be required to evaluate the dosage regimen of this novel compound and to determine the feasibility of the oral route in future clinical studies.

The time to reach maximal BMABO plasma concentrations (T_{max}) was 30 min after oral administration, which falls within the average time recorded for the oral route. However, the effect of food on the absorption profile was not tested. The elimination half-life ($t_{1/2}$) of BMABO after oral and intravenous administration was 18.6 ± 1.7 and 27.5 ± 6.3 min, respectively. The $t_{1/2}$-values were evaluated using two-tailed t-test statistical analysis, and were found to be statistically insignificant ($P < 0.05$). For the intravenous administration, the last two time points were used by the software to calculate the half-life, while for the oral administration, the last four time points were used. It is possible that the oral half-life may be underestimated due to the absorption phase. The t-test results may be statistically insignificant, but it all comes down to the data points used to calculate the terminal half-life.

The BMABO showed a relatively high volume of distribution (V_d) equal to 471 ± 101 and 680 ± 106 after oral and intravenous administration, respectively. The high-V_d correlates with the log P-value of the compound (log $P = 4.5 \pm 0.4$) calculated using ACD/ChemSkitch software (ACD Labs, Version 6.00, ON, Canada). BMABO is quite lipophilic in nature, and thus will have a tendency to distribute into body tissues and compartments, resulting in high V_d-values. The V_d-values obtained after oral and intravenous administration were evaluated using two-tailed t-test statistical analysis and found to be statistically insignificant ($P > 0.05$).

Taking in consideration the potential ability of BMABO to convert sub-therapeutic hyperthermic exposures into efficacious thermal doses that would yield robust radio-sensitization, BMABO may have therapeutic value in cancer radiotherapy. Based on the above pharmacokinetic data, BMABO may be suitable for oral delivery if proven to be clinically effective and non-toxic to human.

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