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MODE OF ADMINISTRATION DEPENDENT BRAIN UPTAKE OF INDOMETHACIN: SUSTAINED SYSTEMIC INPUT INCREASES BRAIN INFLUX

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Running Title: INDOMETHACIN SUSTAINED SYSTEMIC INPUT INCREASES BRAIN UPTAKE

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The abbreviations used are:

NSAID, Non-Steroidal Anti Inflammatory drug; Aß, Amyloid ß; COX, Cyclooxygenase; GI, Gastrointestinal; VPA, Valproic Acid; OAT, Organic Anion Transporter; OATP, Organic Anion Transporting Polypeptides.

ABSTRACT

NSAIDs, including indomethacin, have been found in both epidemiological and clinical studies to reduce the prevalence and severity of Alzheimer's disease. However, long-term use of indomethacin is limited by significant gastrointestinal and renal toxicities. An indomethacin prodrug that delivers low and continuous blood levels of the drug showed a superior safety profile and similar efficacy in comparison to equivalent dose of free indomethacin due to limited systemic exposure and preferred brain uptake. The purpose of the present investigation was to evaluate whether sustained systemic input causes an increased brain influx in comparison to rapid input of the drug. Oral indomethacin, indomethacin prodrug or intravenous indomethacin infusion were administered to rats. The infusion was designed to mimic the plasma indomethacin levels resulting from the prodrug. The resultant blood levels and brain indomethacin uptake were evaluated. The brain indomethacin concentrations 8hr after indomethacin administration were 0.45, 0.3 and 0.31μg/g following the oral indomethacin, oral prodrug and the intravenous infusion, respectively. The corresponding plasma concentrations were 14.1, 4.1 and 4μg/ml. Therefore, brain vs. plasma indomethacin levels ratios were 2.5-fold higher following slow systemic input of indomethacin in comparison to rapid drug input. In conclusion, indomethacin brain uptake was found to be mode of administration dependent, and a sustained input function increases the drug brain uptake. Thus, these unique results indicate that an appropriate indomethacin controlled release delivery system may induce the desirable brain related pharmacodynamic effects, while avoiding the concentration dependent adverse effects. These findings may contribute to improved therapy in Alzheimer's disease.

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Introduction

NSAIDs, including indomethacin, have been found in both epidemiological and clinical studies to reduce the prevalence and severity of Alzheimer's disease (Veld et al., 2001). Indomethacin inhibits amyloid ß (Aß) plaque formation via γ-secretase inhibition, which is a cyclooxygenase (COX)-independent process (Weggen et al., 2001). In addition, NSAIDs have COX-dependent anti-inflammatory and neuroprotective effects (Halliday et al., 2000; Weggen et al., 2001). However, long-term use of indomethacin for Alzheimer's disease is limited by significant gastrointestinal (GI) and renal toxicities that are concentration dependent (Tabet and Feldman, 2002).

In a previous study, we reported on a novel oral prodrug of indomethacin, comprising the drug attached to the *sn*-2 position of a phospholipid that exhibited superior safety profile and similar efficacy to an equimolar dose of free indomethacin (Dvir et al., 2006). This unique result was derived from the pharmacokinetic properties of the prodrug, which following oral administration resulted in a sustained release profile of the drug in the plasma, with slower absorption rate having a half life value of 23.5 hr in comparison to free indomethacin (10.5 hr). The amount of indomethacin that was absorbed following the administration of an equimolar dose of the prodrug decreased 2-fold, c_{max} decreased 4-fold and t_{max} was delayed 2-fold in comparison to oral administration of the free drug. The unique pharmacokinetics of the prodrug was also related to the disposition of indomethacin to the brain, where despite the lower systemic drug concentrations, elevated brain indomethacin uptake was obtained following the administration of the prodrug to rats in comparison to administration of the free drug. Up to 4-fold higher brain to plasma concentration

ratio of indomethacin was found following oral administration of the prodrug in comparison to oral administration of free indomethacin. Hence, even with the lower systemic indomethacin concentrations, the prodrug did not cause significant reduction in indomethacin brain levels, and resulted in equivalent brain related pharmacodynamic activities.

The purpose of the present investigation was to investigate the factors that caused this unique phenomenon of indomethacin disposition to the brain. Specifically, to evaluate whether the preferred indomethacin brain uptake following the administration of the prodrug in comparison to the free drug was due to the phospholipid complex, or was due to pharmacokinetics reasons, i.e., the input function of indomethacin to the systemic circulation. Therefore, we administered an intravenous infusion in a manner that delivers low and sustained indomethacin plasma concentrations, mimicking the systemic indomethacin profile resulting from oral administration of the prodrug, and evaluated the resultant blood levels and brain indomethacin concentrations.

Materials and Methods

Materials

The indomethacin-phospholipid conjugate was supplied by D-Pharm LTD (Rehovot, Israel). Indomethacin, ibuprofen, formic acid and ammonium acetate were purchased from Sigma Chemical Co. (St. Louis, MO). Saline was obtained from Teva Medical (Ashdod, Israel). Ethanol, methanol, acetonitrile, water and ethyl acetate

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(J.T.Baker, Deventer, Holland) were HPLC grade. All other chemicals were of analytical reagent grade.

Experimental Procedures

All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School Jerusalem. Male Wistar rats (Harlan, Israel), 275-300g in weight, were used for all surgical procedures.

One day before the pharmacokinetic experiment, an indwelling cannula was placed in the right jugular vein of the animals, by a method described before (Hoffman and Levy, 1989). The cannula was tunneled beneath the skin and exteriorized at the dorsal part of the neck. After completion of cannula implantation, the animals were transferred to metabolic cages to recover overnight. During this recovery period and throughout the experiment, food, but not water, was deprived. Animals were randomly assigned to the different experimental groups.

Two groups of animals (n=4 in each group) were administered an equimolar oral dose (0.01 mmole) of free indomethacin or indomethacin-phospholopid prodrug in the same vehicle and volume (1ml/kg) by oral gavage. An additional group of rats (n=4) was administered an intravenous infusion of a commercially available indomethacin i.v. injection (Merck & Co. Inc., Germany). The indomethacin solution was infused through the jugular vein cannula by an automatic infusion pump (PHD 2000 Syringe Pump, Harvard Apparatus Inc., Holliston, MA).

Systemic blood samples (400µl) were taken at 5 min pre-dose, 1, 2, 4 and 8 hours post dose. To prevent dehydration equal volumes of physiological solution were

introduced to the rats following each withdrawal of blood sample. Eight hours after the pharmacokinetic experiment begun, the animals were anesthetized with ether, a systemic blood sample was withdrawn, the animals were sacrificed and the whole brain was obtained and stripped of its external vasculature and meninges. The brain samples were divided into two pieces and accurately weighed (0.75-1 g/brain sample) in order to perform duplicate analysis.

Analytical Methods

A high performance liquid chromatography (HPLC) system (Waters 2695 Separation Module) with a photodiode array UV detector (Waters 2996) was used for determining the amount of indomethacin in plasma and brain, by a method described before with some modifications (Ioffe et al., 2002). To determine brain levels of indomethacin, the brain samples were spiked with 40µl of internal standard solution (ibuprofen, 250µg/ml) followed by extraction (Politron Tissue Homogenizer, 25000 rpm) into 5 ml of ethyl acetate. After homogenizing, samples were centrifuged, supernatant was transferred, evaporated to dryness and redissolved in 80µl of diluent comprising 0.07% ammonium acetate in methanol:acetonitrile:water (88:11:1% v/v, respectively). 20µl of the resulted solution were injected into the HPLC system. The HPLC conditions were as follows: Lichrospher RP-18 column (Merck, Germany), an isocratic mobile phase, 0.1% formic acid in methanol:acetonitrile:water (68:12:20% v/v), at a rate flow of 1ml/min in room temperature.

Duplicate analysis were performed to all brain samples. Separate standard curves were carried out for brain and plasma samples ($R^2 > 0.999$). The minimum quantifiable concentrations for indomethacin plasma and brain samples were

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100ng/ml and 200ng/g, respectively. The inter- and intra-day coefficients of variation were <1.0 and 0.5 %, respectively.

Pharmacokinetic Analysis

Plasma concentrations versus time curves for indomethacin in individual rats were analyzed by means of the noncompartmental analysis model. To achieve the desired indomethacin concentrations in the i.v. infused animals, the rate of the intravenous indomethacin infusion was calculated using the following equation:

$$R = \frac{C_p V_d k}{1 - e^{-kt}}$$

When the plasma concentration (C_p) at any time (t) can be achieved at a constant infusion rate (R) if the volume of distribution (V_d) and elimination constant (k) are known.

Statistical Analysis

All values are expressed as mean ± standard deviation (SD). To determine statistical significantly differences among the experimental groups, the non-parametric Kruskal-Wallis test was used for multiple comparisons, and the two-tailed non-parametric Mann-Whitney *U* test for two-group comparison when appropriate. A *p* value of less than 0.05 was termed significant.

Results

Indomethacin plasma vs. time levels following oral administration of the prodrug or free indomethacin, and following sustained intravenous indomethacin infusion are shown in Fig. 1. It can be seen that the intravenous infusion delivered low and sustained indomethacin plasma concentrations, and managed to mimic the systemic indomethacin profile resulting from oral administration of the prodrug. Indomethacin blood levels were significantly lower, c_{max} decreased 4-fold and t_{max} was delayed 2-fold following the intravenous infusion or the prodrug in comparison to free indomethacin.

Brain and plasma levels of indomethacin 8 hours after oral administration of the prodrug or free indomethacin, and after the initiation of the intravenous infusion are presented in Fig. 2, and summarized in Table 1. It can be seen that the administration of intravenous infusion caused the same indomethacin brain uptake as resulted from oral administration of the prodrug.

Indomethacin brain vs. plasma levels ratio 8 hours after oral administration of the prodrug or free indomethacin, and after the initiation of the intravenous infusion are shown in Fig. 3. In the case of linear kinetic processes, the ratio between plasma and brain concentrations should have been constant in the different experimental groups. Thus, a non-linear brain uptake of indomethacin was detected. Following oral administration of the free drug, brain indomethacin uptake was reduced, and significantly lower brain vs. plasma ratio was obtained (2.5-fold) in comparison to the other experimental groups. Similar brain vs. plasma indomethacin levels ratios were obtained for the animals administered the prodrug and the animals receiving the intravenous infusion.

Discussion

The outcomes of the present investigation show that sustained systemic input of indomethacin increased brain influx. This finding indicates that the disposition of indomethacin into the brain is non-linear, and is mode of administration dependent. Hence, low and constant plasma indomethacin concentrations can cause elevated brain vs. plasma ratio of the drug.

The phenomenon of elevated brain to plasma ratio at lower indomethacin blood concentration can be explained by better uptake of the drug into the brain at lower indomethacin blood concentrations. In a recent publication, Gibbs et al. reported on a biphasic pattern in valproic acid (VPA) brain uptake that resulted from indomethacin. Whereas the presence of 10μM of indomethacin produced a 13% elevation in VPA brain uptake, at higher concentrations of indomethacin (500μM) a 31% reduction in VPA brain uptake was observed. The authors suggested that this biphasic pattern evolved from concurrent inhibition of both uptake and efflux transporters by indomethacin with differing sensitivities, i.e. the efflux transporter being more susceptible to inhibition than the influx transporter (Gibbs et al., 2004). The outcomes obtained in the present investigation may result from the same mechanism. The high indomethacin plasma concentrations resulting from the administration of free indomethacin may inhibit an influx transporter e.g. OATs or OATPs, and therefore reduce brain uptake of the drug, whereas a shallower plasma concentration-time profile avoids this inhibition. On the other hand, the inhibition of efflux transporters, which are more sensitive for inhibition, still occurs at the low indomethacin blood levels produced by a sustained drug input.

A further mechanism that may contribute to this unique indomethacin brain uptake is the cerebral vasoconstriction and consequent reduction in blood flow induced by the drug. A well known and documented adverse effect of indomethacin is the contraction of blood vessels supplying blood into the brain, resulting in hypo-perfusion to brain tissue (McCulloch et al., 1982; Markus et al., 1994). Hence, high blood levels of the drug can induce this brain hypo-perfusion, resulting in decrease in the transport of indomethacin from the blood into the brain. On the other hand, low and continuous levels of the drug minimize this concentration dependent adverse effect and hence, the reduction in the transport of indomethacin into the brain can be avoided.

Oral controlled release products of indomethacin have been introduced before, mainly for reasons of reduced adverse effects. The data presented in this paper suggest that a controlled release product of indomethacin may have an additional advantage over an immediate release product, relating the degree of drug uptake into the brain. A sustained input function can cause reduction in the concentration dependent adverse effects due to limited systemic exposure, and yet deliver preferred brain levels, and maintain brain related pharmacodynamics. Since indomethacin has been shown to be advantageous in the treatment of Alzheimer's disease but its use is limited by adverse effects, this finding may contribute to improved therapy in Alzheimer's disease patients. These conclusions are subject to the usual reservations in extrapolating animal data to man.

Conclusions

In conclusion, we have found that sustained input function of indomethacin to the plasma increase the efficiency of indomethacin brain uptake. The distribution profile of indomethacin into the brain is mode of administration dependent, and a controlled release product of indomethacin may induce the desirable brain related pharmacodynamic effects, while avoiding the concentration dependent adverse effects. These findings may contribute to improved therapy in Alzheimer's disease.

Acknowledgments

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Footnotes

This work is a part of Arik Dahan PhD dissertation. A. Hoffman is affiliated with David R. Bloom Center of Pharmacy.

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Legends for Figures

Fig. 1. Indomethacin plasma concentrations following oral administration of free indomethacin (■), oral administration of the prodrug (■) and intravenous infusion of indomethacin (■). Data presented as average ± SD, n=4 rats in each group. * Significantly different than the two other modes of administrations ($p< 0.05$).

Fig. 2. Brain (■) and plasma (■) levels of indomethacin 8 hours after oral administration of the prodrug or free indomethacin, and after the initiation of the intravenous infusion. Data presented as average ± SD, n=4 rats in each group. * Significantly different than the oral mode of indomethacin administrations ($p< 0.05$).

Fig. 3. Indomethacin brain vs. plasma levels ratio 8 hours after oral administration of the prodrug or free indomethacin, and after the initiation of the intravenous infusion. Data presented as average ± SD, n=4 rats in each group. * Significantly different than the two other modes of administrations ($p< 0.05$).

Tables

	Brain level (µg/g)	Plasma level (µg/ml)	Blood vs. plasma ratio
p.o. free indomethacin	0.45 ± 0.09	14.1 ± 2.6	0.032 ± 0.01
p.o. prodrug	0.3 ± 0.03	4.1 ± 0.8	0.075 ± 0.02
i.v. indomethacin infusion	0.31 ± 0.02	4.0 ± 0.3	0.078 ± 0.01

Table 1. Brain and plasma indomethacin levels 8 hours after oral administration of the prodrug or free indomethacin, and after the initiation of the intravenous infusion. Data presented as average ± SD, n=4 rats in each group.

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Figure 1

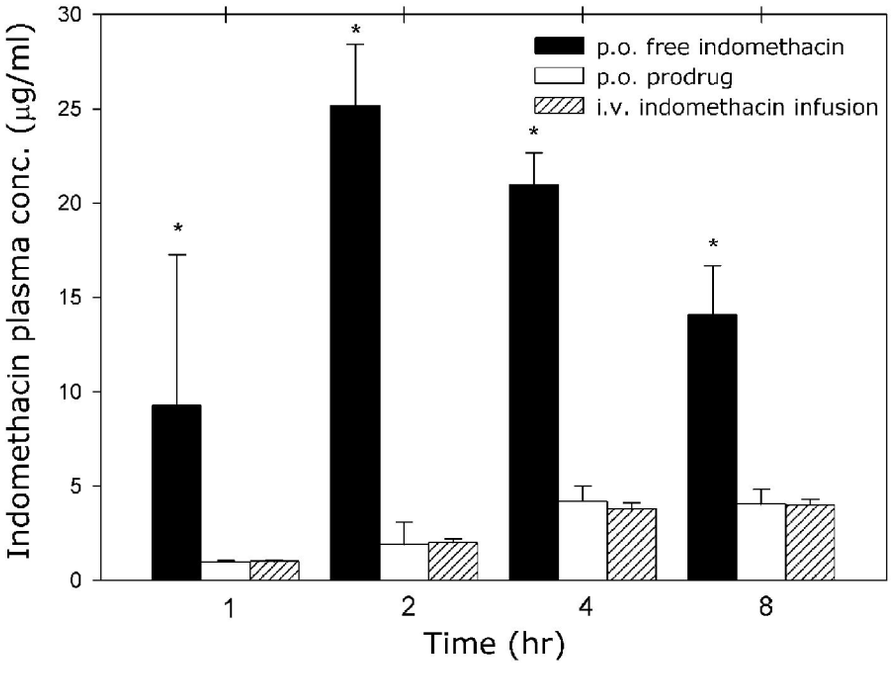

Figure 2

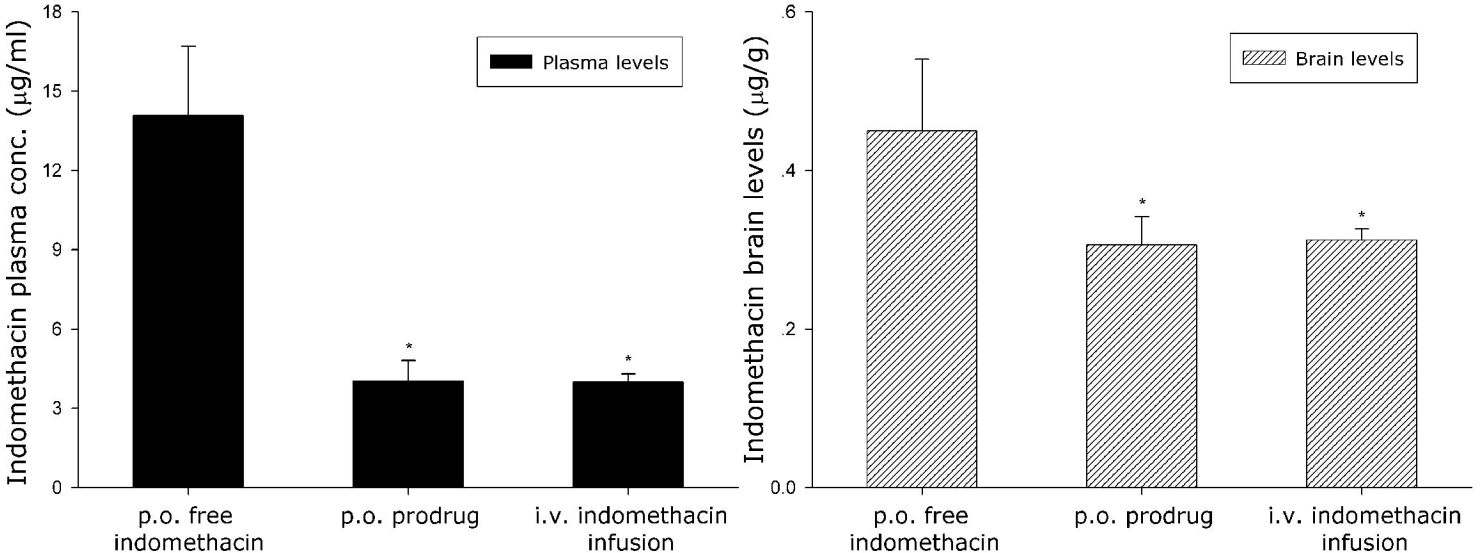
Indomethacin plasma conc. (μg/ml)
18
15
12
9
6
3
0
Plasma levels
*
*
p.o. free indomethacin
p.o. prodrug
i.v. indomethacin infusion
Indomethacin brain levels (μg/g)
.6
.4
.2
0.0
Brain levels
*
*
p.o. free indomethacin
p.o. prodrug
i.v. indomethacin infusion

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Figure 3