P185 COMPUTER-ASSISTED VIDEOMICROSCOPY : EFFECTS OF SOME ANTIHYPERTENSIVES ON GUINEA-PIG ILEUM RESISTANCE VESSELS

G. Baktir[1], E. Bungardt[2], E. Mutschler[2]
1 Department of Pharmacology, Faculty of Pharmacy, University of Istanbul, Istanbul, Turkey
2 Department of Pharmacology, Johann Wolfgang Goethe-University, 60439 Frankfurt, Germany

Submucosal arterioles of guinea-pig ileum, analyzed with computer-assisted videomicroscopy, have recently been shown to possess functional muscarinic M_3, histamine H_1, adrenergic α_{1A}, purinergic P_{2x}, and vasopressin V_1 receptors (1-5). The aims of this study were to investigate ß-adrenoceptor-mediated responses in this tissue and to examine in addition a series of antihypertensive drugs for direct vasodilating properties, e.g. the $ß_1/ß_{2(PAA)}$-adrenoceptor-blocker (±)-celiprolol, the loop-diuretic indapamide, and the potassium channel opener lemakalim. The preparations were obtained from young guinea-pigs of both sexes (250-350 g) and consisted of arteriolar trees embedded in a thin connective tissue sheath. This was pinned out on the base of a small organ bath (0.3 ml) and continuously flowed with gassed Tyrode solution at 32 °C. Arteriolar diameter was monitored with the Diamtrak®-system (6). Outside diameter of arterioles examined in this study ranged from 40-90 µm. Vessels were preconstricted with either (-)-noradrenaline (10 µM) or the thromboxane-A_2-mimetic U-46619 (300-600 nM). The drugs tested were applied in a cumulative fashion; in case of ineffectiveness, the vasodilation induced by the muscarinic agonist arecaidine propargyl ester (1 µM) was used as positive control.

The following results were obtained: lemakalim produced a dose-dependent (glibenclamide-sensitive; data not shown) vasodilation of the preconstricted vessels, whereas indapamide and celiprolol (1 nM-300 µM) were inactive. (±)-Isoprenaline (10-200 µM) produced a further contraction of the U-46619-preconstricted arterioles which, after α-blockade with prazosin (1 µM) converted to a slight dilatation (cf. table).

Compound	EC_{50} ± S.E.M.	Dilatation[1]	
Lemakalim[NA]	12.7 ± 1.48 µM	65.5 ± 3.76 %	n = 9
Indapamide[NA]	inactive	inactive	n = 6
(±)-Celiprolol[U]	inactive	inactive	n = 8
(±)-Isoprenaline[U]	n.d. (> 30 µM)	12.7 ± 3.63 %	n = 14

[1] % of preconstriction; [NA] noradrenaline-preconstricted; [U] U-46619-preconstricted; n.d. = not determined.

A second group of elder animals (700-1100 g; vessel diameter 60-90 µm) was also investigated in view of ß-adrenoceptor-mediated vasodilatory effects. To date, this investigation failed since these preparations showed markedly reduced responses to a large number of vasoconstrictors (i.e. noradrenaline, phenylephrine, U-46619, vasopressin and ATP; n > 30) leading to a non-sufficient preconstriction.

In conclusion, guinea-pig ileum submucosal arterioles displayed only slight vasodilation in response to ß-adrenoceptor-activation. Celiprolol and indapamide showed neither direct nor indirect vasodilatory activity, whereas lemakalim behaved as a vasodilator of moderate potency.

References: (1) Bungardt et al., 1992, Eur. J. Pharmacol. 213, 53; (2) Bungardt et al., 1992, ibid. 221, 91; (3) Bungardt et al., 1992, Naunyn-Schmied. Arch. Pharmacol. 345, R108; (4) Bungardt et al., 1992, Int. J. Purine Pyrimidine Res. 3, 66; (5) Vanner et al., 1990, Circ. Res. 67, 1017; (6) Neild, 1989, Blood Vessels 26, 48.

P186 ABOUT THE PREPARATION OF MYRICETIN-3-GLUCURONIDE AND THE INVESTIGATION OF THE ANTIPHLOGISTIC EFFECT OF ITS METAL SALTS

H. Wadl[1], H.W. Schramm[1], A. Hiermann[2], A. Fleck[1]
1 Institut für Pharmazeutische Chemie, Karl-Franzens-Universität, 8010 Graz, Austria
2 Institut für Pharmakognosie, Karl-Franzens-Universität, 8010 Graz, Austria

Hiermann et al[1] have isolated Myricetin-3-glucuronide **1** as an antiphlogistic priciple from herba *Epilobium angustifolium L.* Because of the little active component contend of the plant, the glycoside was prepared by means of Schramm et al[2]. The biological activity of Myricetin-3-glucuronide was determined by means of carrageenin induced rat paw oedema; the pure substance showd an approximately 500 times higher effect than indomethacine. In addition, slightly modified derivates of **1** were examined for their antiphlogistical effect, too. From the results it could be conclused that the examined more lipophilic derivates like the mono- and dibenzyl-product of **1**, the myricetin-3-glucuronic methylester reacted, losing their high effect as well as the aluminium trichloride complex of **1** in the proportion 1:1 and 1:2 and the calcium- and sodium salts of the glucuronid **1**. In each case, however, the tested compounds showed a considerably higher antiphlogistic effect than indomethacine. The most striking fact was that theese glucuronic-acids derivates which were converted with sodium or calcium carbonate into their salts, despite of application weren't transformed into the free glucuronide **1** with gastric acid and showed less activity than indomethacine. In every case the tested compounds showed a considerable higher antiphlogistical effect than indomethacine.

In conclusion, it may be said that according to our investigations a loss of effect and a composed of the compounds can be excluded; the stability of the compounds against acid agents provides evidence in favour of the compound of metal ions as a complex[3] and against the acid character of the compounds explored respectively.

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2 Schramm H. W., (Plantamed Arzneimittel GmbH) (1992) C.A. **117**: P 111979e (Ger. Offen. DE 4,034,586); Schramm H.W, Wadl H., Reidlinger H. in Vorbereitung.
3 Hiermann A., Schramm H.W., Wadl H. (1992) Sci. Pharm. **60** (3): 203

P187 CELIPROLOL DOUBLE PEAK OCCURRENCE AND GASTRIC MOTILITY: NONLINEAR MIXED EFFECT MODELING OF BIOAVAILABILITY DATA OBTAINED IN DOGS

E. Lipka[1,2], P. Langguth[3], H. Spahn-Langguth[2], E. Mutschler[1], G.L. Amidon[1]
1 College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA
2 Department of Pharmacology, University of Frankfurt, 60439 Frankfurt, Germany
3 Department of Pharmacy, ETH Zürich, 8092 Zürich, Switzerland

Celiprolol shows significant intra- and intersubject variability in its concentration-time profiles - including the occurrence of double peaks - following peroral administration. Simulations as well as experimental studies with other compounds (e.g. [1]) indicated that fasted-state gastrointestinal motility with phases of different activity may be a causative factor for discontinuous input profiles.

The objective of this study was therefore to determine, whether gastric emptying has an influence on the rate and extent of celiprolol absorption and plays a role with respect to double peak formation, and to develop a population pharmacokinetic model that includes gastric emptying.

In mongrel and beagle dogs racemic celiprolol (150 mg) was dosed perorally during four different phases of the fasted state gastric cycle. Intravenous doses (50 mg) were also given to obtain disposition parameters and to determine the absolute bioavailability. Plasma concentrations of both celiprolol enantiomers were determined using a previously published HPLC method [2]. Gastric motor activity was recorded continuously for four hours after dosing using a pneumohydraulic manometric system.

No differences were observed between the two enantiomers during the whole study period, indicating that no relevant stereoselective processes occur in the dog.

The fasted-state motility pattern controls the amount of drug that leaves the stomach and reaches the absorption site and can therefore in part explain the occurrence of double peaks. The data show a significant correlation between the onset of the active motility phase and the celiprolol peak concentrations. In addition to the rate of drug input also the extent appeared to be dependent upon the motility phase, in which the drug was dosed, since the bioavailability tended to be higher with dosage close to the active phase.

Population analysis (employing NONMEM) was performed with a model that includes two input compartments with two lag-times and into which the gastrointestinal motility data were incorporated.

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[2] C. Hartmann, D. Krauss, H. Spahn, E. Mutschler. J. Chromatogr. 496, 387-396 (1989).

P188 COMPARATIVE CELLULAR ASSOCIATION OF PHOSPHODIESTER AND PHOSPHOROTHIOATE OLIGONUCLEOTIDES TO CACO-2 CELLS

G.F. Beck[1], S. Akhtar[1], W.J. Irwin[1], P.L. Nicklin[2]
1 Department of Pharmaceutical Sciences, Aston University, Birmingham B4 7ET, U.K.
2 Drug Preformulation and Delivery, Ciba Pharmaceuticals, Horsham, West Sussex RH12 4AB, U.K.

Characteristics for the cellular association of 20-mer oligonucleotides with phosphodiester (PO) and phosphorothioate (PS) backbone modifications have been compared in the human epithelial cell line; Caco-2. PO and PS oligonucleotides were 5'-labelled with [^{32}P] and purified for experiments by gel electrophoresis.

The cellular association of the PS oligonucleotide was biphasic; a rapid initial association (<15 min) was followed by a slower secondary phase (15-300 min). Meanwhile, the cellular association of the PO oligonucleotide was proportional to time. 5'-[^{32}P]oligonucleotides were stable for at least 15 min on the cell surface, thereafter some degradation was apparent. Subsequent studies were therefore restricted to a 15 min incubation period. At a solute concentration of 0.4 µM the cellular association of PS (3.0%) was an order of magnitude greater than that of the PO oligonucleotide (0.2%). Furthermore, the percentage cellular association of PS was highly saturable (0.9 % at 5 µM) whereas that of PO was only slightly saturable (0.2% at 5 µM, 0.1% at 200 µM). Cross-inhibition studies showed that the cellular association of the PO oligonucleotide was reduced in the presence of 20 µM PS (50% inhibition by 50-fold molar excess). Conversely, the cell-associated PS oligonucleotide was not influenced by PO. The cellular association of PS and PO oligonucleotides demonstrated differential temperature-dependence; PS was temperature-independent whereas PO decreased between 37°C (0.2%) and 18°C (0.05%) and plateaued through to 4°C (0.05%). The ability of NaCl washes to remove surface bound PS oligonucleotide indicated their binding was ionic in nature. This was not observed for PO oligonucleotides.

Using the Caco-2 cell line, PS oligonucleotides have a highly saturable, temperature-independent cellular association which is susceptible to the ionic environment. In contrast, PO oligonucleotides have a poorly saturable, temperature-dependent cellular association which is insensitive to the ionic environment. These observations suggest that PS oligonucleotides have a high affinity, capacity limited binding to cell surface components which is not apparent for PO oligonucleotides.