Beyond relief : biomarkers of the anti-inflammatory effect and dose selection of COX inhibitors in early drug development

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Chapter 2

PKPD correlations and biomarkers in the development of COX-2 inhibitors

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ABSTRACT

The mechanism by which COX inhibitors exert their analgesic effect is well established. However, data show no direct correlation between drug concentrations in plasma and the analgesic or adverse effects in chronic inflammatory conditions. This represents a major problem in the development of COX inhibitors, since it is difficult to predict the appropriate dosing regimen for the treatment of chronic inflammatory pain, based upon information from pre-clinical studies and eventually early clinical studies. The factors that determine response in inflammatory pain must be understood in order to make predictions about the time course of the analgesic effect. In this review the determinants of drug response and their variability will be discussed: physicochemical properties, pharmacokinetics (PK), pathophysiology and disease progression.

From a mechanistic point of view, endogenous mediators of inflammation might be used as a biomarker for the analgesic effect and safety assessment. Such a biomarker can be an intermediate step between drug exposure and response. In addition, its concentration-effect relationship could be characterised by pharmacokinetic-pharmacodynamic (PK/PD) modelling. Indeed, recent investigations have shown that COX-2 inhibition, as determined by modelling of PGE_2 levels in the whole blood assay *in vitro* can be used as a marker to predict drug effects (analgesia) in humans. A model-derived parameter, IC_{80}, (total and unbound) was found to correlate directly with the analgesic plasma concentration of different COX inhibitors varying in enzyme selectivity. These findings indicate that PGE_2 and TXB_2 inhibition can be used to predict and select efficacious doses in humans.

BIOMARKERS

When the outcome of pre-clinical or clinical experiments is very difficult to quantify and show large variability, as observed in pain measurements, a biomarker of drug effect might be useful (1). Biomarkers have been defined by the Biomarkers Definitions Working Group as physical signs or laboratory measurements that may be detected in association with a pathological process and that have putative diagnostic and/or prognostic utility (2). A biomarker that is intended to substitute for a clinical endpoint is a surrogate endpoint. Biomarkers can be evaluated in different models, i.e. *in vitro* assays, *in vivo* animal models, clinical trials in healthy subjects and patients, epidemiological studies and simulated biological systems.

During the early stages of drug development, biomarkers can be used as a guide for dose selection, dose escalation and safety monitoring. In diseases in which the pathogenic mechanism is poorly understood, biomarkers might also be useful. In fact, biomarkers can play a crucial role in understanding on a mechanistic level the differences in clinical response between subjects (3). Biomarker measurements can also help explaining empirical results of clinical trials by relating the effect of interventions on molecular and cellular pathways to clinical response.

The validation of biomarkers is, therefore, very important; particularly if one bears in mind their relevance for decision-making and regulatory purposes. Their use as surrogate endpoint is evaluated by considering those factors that are related to the ability to accurately substitute for a clinical endpoint, including face, criterion and construct validity (4). In addition, practicality, specificity and innovation are three important aspects in the development of biomarkers or surrogate endpoints. Typically, for innovative products limited information is available on the relationship between biomarker versus clinical response. This may limit the utility of biomarkers in the early development phases. In contrast, the availability of a validated biomarker may greatly facilitate the development of compounds that act via well-established mechanisms of action (4).

There is growing evidence that the evaluation and validation of biomarkers according to mechanism-based PK/PD modelling and simulation can increase the predictive power of biomarkers and their use in drug development.

A seven-point mechanistic classification based on the location of the biomarker in the chain of events, from the underlying subject's genotype or phenotype throughout to clinical scales has been proposed by M. Danhof at the Ninth European Federation for Pharmaceutical Sciences (EUFEPS) Conference on Optimizing Drug Development in Basel, Switzerland, December 10-12, 2001 (2). This mechanism-based classification consists from distal to proximal to clinical endpoints. The different types of biomarkers are type 0 biomarkers: genotype or phenotype, type 1 biomarkers: concentration, type 2 biomarkers: target occupancy, type 3 biomarkers: target activation, type 4 biomarkers: physiologic measures or laboratory tests, type 5 biomarkers: disease processes and type 6 biomarkers: clinical scales.

In this paper, first we discuss the determinants of treatment response to COX inhibitors and the role of PK/PD modelling in the development of COX inhibitors. Secondly, the role of biomarkers to correlate the non-linear relationship between analgesia and exposure, as measured in different clinical and pre-clinical models will also be evaluated. Focus will be placed onto the pre-clinical *in*

vitro and *in vivo* models and subsequent scaling of concentration-effect relationships from pre-clinical to clinical settings.

COX INHIBITORS

COX inhibitors are effective anti-inflammatory, antipyretic and analgesic agents, which are commonly used in the treatment of acute and chronic pain, rheumatoid arthritis and osteoarthritis. They act by inhibiting cyclo-oxygenase (COX) activity and consequently the formation of pro-inflammatory mediators like prostaglandins (PG) and thromboxanes (TXB) (figure 1) (5). Since the early 90s, it is generally accepted that cyclo-oxygenase exists in two isoforms. Cyclo-oxygenase-1 (COX-1) is a housekeeping enzyme responsible for modulating physiological events and is present in most tissues including stomach, kidney and platelets, whereas cyclo-oxygenase-2 (COX-2) is highly induced in various cells by pro-inflammatory stimuli, mitogens and cytokines (6). Recently, a third isoform (COX-1v) has been identified, but its function is not clear yet. Continuous COX-1 inhibition is thought to be principally responsible for gastrointestinal adverse effects following prolonged administration of non-selective COX inhibitors, whereas selective COX-2 inhibition accounts for the anti-inflammatory, anti-pyretic and analgesic efficacy (7). Recent investigations demonstrate that the roles of COX-1 and COX-2 are oversimplified. Data from those studies suggest that COX-2 is present under non-pathological conditions in tissues such as kidneys, brain and the spinal cord, playing an important role in the maintenance of physiological homeostasis (8). Previous findings have also shown that selective inhibition of COX-2 only partially reduces the level of PG at the site of inflammation in comparison with non-selective COX inhibitors, which reduce PG to undetectable levels (9). This suggests that COX-1 may contribute to the pool of PG at the site of inflammation. Yet, COX-2 does seem to have emerged as the dominant (albeit not exclusive) source of PG formation during inflammation (10).

By reducing PG and TXB synthesis, COX inhibitors block the nociceptive response to endogenous mediators of inflammation, with the effect being greatest in tissues that have been subjected to injury or trauma (figure 1). COX inhibitors also exert their analgesic effects through a central mechanism. Central neuronal tissues have been shown to synthesize PGs, and spinally administered COX inhibitors have been shown to reduce peripherally induced hyperalgesia (11).

Rational drug therapy is based on the assumption that there is a causal relationship between dose, dosing regimen, and/or exposure to a medication on one hand and its therapeutic response as well as adverse effects on the other. Hence, it has been one of the major goals of clinical pharmacology to find systematic ways to identify the dosing regimen that produces clinically relevant analgesia (12). An important question that remains to be answered is how much and how long COX-2 and COX-1 should be inhibited to ensure an optimal risk-benefit ratio, allowing for sustained analgesic response and appropriate safety margin. To date, the dose selection of COX inhibitors has been based primarily on clinical endpoints for analgesia, an approach which disregards the impact of maximum, long-lasting blockade of either enzyme systems.

Despite a clear analgesic response upon administration of COX inhibitors, no direct correlation is observed between the drug concentrations in systemic fluids (blood, plasma) and the magnitude of

the pharmacological effect. Lack of such a direct correlation does not mean one cannot identify the optimal dose required to achieve a predefined treatment response. Adequate insight into the pathophysiology of pain in conjunction with tools such as nonlinear mixed effect modelling can enable the characterisation of nonlinear relationships between pharmacokinetics and pharmacodynamics.

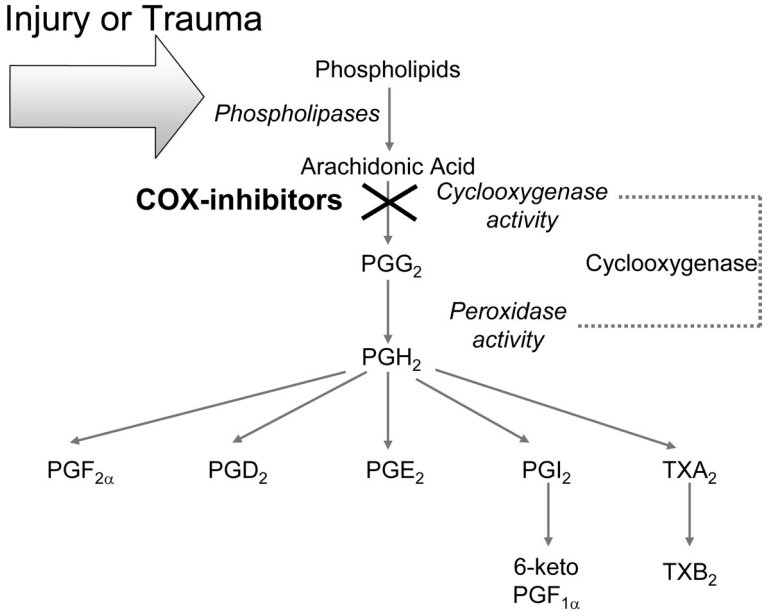

Figure 1. Prostaglandin synthesis pathway after injury or trauma. After an injury or trauma, arachidonic acid is formed by phospholipase activity. Arachidonic acid is converted to PGH_2 via PGG_2, which is the common precursor for the synthesis of PGs. COX-inhibitors block the activity of the enzyme cyclo-oxygenase, thus inhibiting the formation of PGG_2.

COMPLEX SIGNAL TRANSDUCTION PROCESSES OF PAIN

Understanding of the processes and mechanisms underlying pain arousal and transduction is essential to establish a link between analgesic effect and exposure to anti-inflammatory drugs. However, most attempts to correlate the analgesic effect with drug exposure have been based on absolute and relative scales of pain perception or relief, instead of assessing the rate and extent of change in mediators responsible for pain signalling and transduction. Such measures are independent of numerous external factors that can affect pain perception and pain relief. In addition, high variability in pain measurement in animals and humans, especially when compared with other sensory systems, has been reported in an abundant number of articles. Progress has been made in the understanding of pain physiology, but pain perception in animals and humans nonetheless displays considerable and unexplained inter-individual variability (13). Moreover, inter-individual variability greatly exceeds intra-individual variability in pain measurements (13).

Physiological pain occurs when electrical signals are transmitted via A-mechanothermal and C-polymodal fibres to the thalamus and higher centres of the brain (figure 2). The afferent nociceptors are peripheral nerve endings, with a high threshold for activation (14). In inflammatory pain, hypersensitivity is the consequence of alterations in transduction sensitivity of the nociceptors,

activity-dependent changes in excitability of spinal neurons and phenotypic changes in sensory neurons innervating the inflamed tissue (15). Tissue injury results in the release of inflammatory mediators from damaged or infected cells, increasing the transduction of painful stimuli. Among these mediators, a second series of signals is that generated or initiated by inflammatory cytokines such as, tumour necrosis factor (TNF-α), interleukins (IL-1β, IL-6). Cytokines act on and between inflammatory cells, inducing some of the features of the inflammatory response. They also mediate some of the systemic effects of inflammation, such as fever or cachexia (15). The induction of cytokines can lead to the expression of the inducible form of nitric oxide synthase (iNOS), which in turn provokes the release of excessive amounts of nitric oxide (NO) that may participate in the pathogenesis of tissue injury. However, the interplay between different cytokines and inflammatory mediators like prostaglandins is not fully understood yet (16). In addition to local cellular events, potassium, prostaglandins, bradykinins, ATP and other mediators from damaged cells trigger the nociceptors to send afferent impulses via the dorsal root ganglion to the spinal cord. Via second-order neurons in the dorsal horn, afferent information is transmitted through the spinothalamic tract to the thalamus and to the sensory cortex (17). These higher centres are responsible for the perception of pain. At different levels of the pain pathway, a complex system of compensatory neuroinhibitory mechanisms is involved in the perception of pain.

Table 1. Different chemical mediators in FCA model of chronic inflammation in rats. Sample type and brief description of time-concentration profile is presented.

Chemical mediator	Sample type	Time of highest concentration after FCA injection	References
Calcitonin gene-related peptide	Dorsal root ganglion	Day 21	(115)
COX-2	Paw tissue	Day 14-25	(9)
Galanin	Dorsal root ganglion	Day 21	(115)
Interleukin-1β	Paw tissue	6 hours	(15)
Interleukin-1β	Serum	Day 1	(122)
Interleukin-6	Serum	Day 11-25	(9)
Mu-opioid receptor	Spinal cord	Day 7	(49)
Nerve growth factor	Paw tissue	3 hours – day 5	(15)
Neurokinin-1receptor	Spinal cord	Day 4	(49)
PGE_2	Paw tissue	Day 14-25	(9)
Substance P	Ankle joints	Day 21	(114)
Substance P	Dorsal root ganglion	Day 21	(115)
Tumor necrosis factor-α	Paw tissue	Day 1 and day 5	(15)
Tumor necrosis factor-α	Serum	Day 1	(122)

The inflammatory process is a dynamic process with varying phases depending on the status of the disease. Typically, the release of chemical mediators is variable in onset and duration. In pre-clinical experiments in rats, concentration-time profiles of a spectrum of chemical mediators released in peripheral tissue, plasma and central nervous system after intraplantar injection of Freund's Complete Adjuvant (FCA) show wide variability (table 1). Variability in the time to maximum concentration of different mediators after FCA injection in table 1 ranges from within a few hours until several weeks after inflammation. Furthermore, increased production of mediators may last from one to several days. Interestingly, the time course of the elevation of certain mediators may also show biphasic behaviour. For example TNF-α shows a peak level 24 hours after FCA injection

followed by a decrease till baseline levels until the TNF-a levels rises 5 days after FCA injection. This might indicate a dual role for TNF-α in the onset and maintenance of the inflammatory response.
In addition to fluctuations in chemical mediators, measurements of pain (algesia) reveal large variability over time. The analgesic effects of COX inhibitors have been studied in a wide range of animal models. The main findings of these investigations have been summarized in table 2. In this table, the effects of Ca^{2+}-channel blockers and opioids are shown, for comparative purposes. Comparison of drug effects reveals large differences in the ED_{50} values, the dose that produces 50% of the maximum effect (table 2). For example, aspirin ED_{50} values vary between 51.8 and >200 mg/kg for the different animal models of inflammatory pain. Since it is unclear whether animal models truly reflect inflammatory disease states in humans, it's not adequate to make direct extrapolations from the efficacious dose in the FCA model to predict therapeutic dose in human rheumatoid arthritis. Moreover, variability in ED_{50} could be due to differences in PK between different animal models, which are not taken into account when the parameter ED_{50} is compared. A more scientific approach should consider the assessment of drug exposure, i.e. EC_{50}, the concentration that produces 50% of the maximum effect rather than ED_{50}. However, large variability in analgesic effects and algesia exists in both animal and human data, which makes it difficult even to compare the different concentration-effect relationships of COX inhibitors. Scaling and parameterisation of the data based on a biomarker, which is likely to show less variability itself,

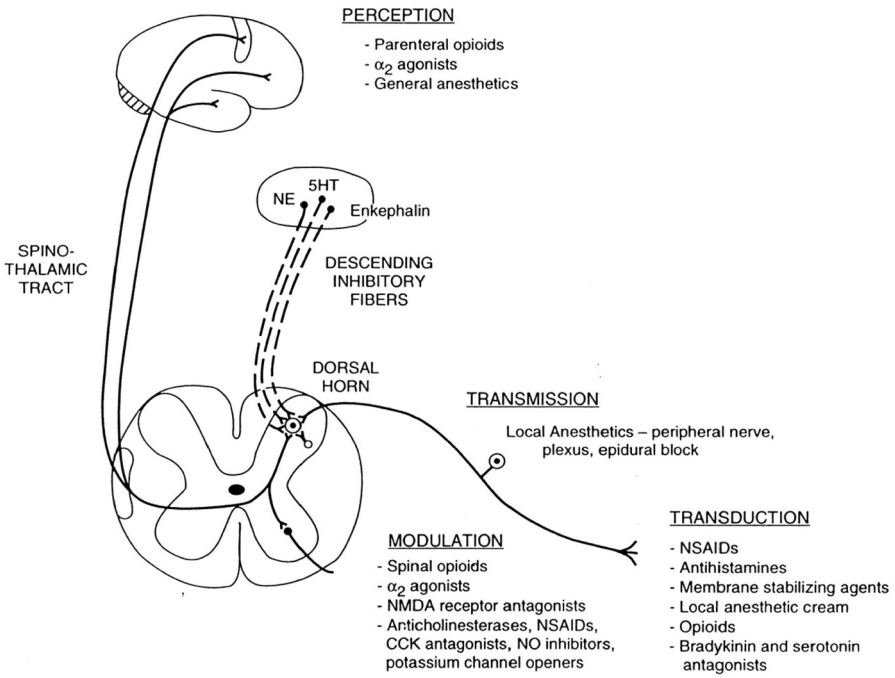

Figure 2. Diagrammatic representation of the four processes involved in the sensory pathway: transduction, transmission, perception, and modulation. Primary afferent neurons transmit information from the periphery to the dorsal horn of the spinal cord. Afferent information is then transmitted via the spinothalamic tracts by second-order neurons to the thalamus and to the sensory cortex. The descending inhibitory fibres (interrupted lines) modulate the afferent input at the dorsal horn. Also represented are the agents that can modify the sensory input of each of the four processes[1].

[1]Reprinted from Canadian Journal of Anesthesia, Vol. 48, 2001, pp1000-1010, Kelly *et al*: "Pre-emptive analgesia I: physiological pathways and pharmacological modalities" with permission from Canadian Journal of Anesthesia.

Table 2. Hyperalgesia ED_{50} (mg/kg) values of different compounds in different rat models of nociceptive, inflammatory and neuropathic pain. Carrageenan and FCA were administered by intraplantar injection. Carrageenan model consists of data from intraplantar injection in the hind paw and data from the airpouch model. Not enough data was found on injection of capsaicin, kaolin injection, Sciatic Cryoneurolysis (SCN), Sciatic Nerve section (SNS) and paw incision. In general, COX-inhibitors are not effective on the normal nociceptive response threshold or latency as reflected in the tail flick and hotplate test results. Nor are they effective in models of neuropathic pain, however, in combination with Ca^{2+}-channel blockers and opioids supra-additive effects have been reported (68;69). Opioids show efficacy on normal threshold, whereas Ca^{2+}-channel blockers have high efficacy in neuropathy. In acute and chronic inflammatory pain, a dose-dependent effect of COX-inhibitors is observed [a]pooled severity index (PSI) from arithmetic average of the volume, [b]determined in mice, [c]ED_{30} (mg/kg) in paw oedema, [d]ED_{50} (mg/kg) in paw oedema, [e]prophylactic treatment, fcold water test, [g]antipyretic. *References: (20;22;31;70-113-122).*

Abbreviations: 6-MNA, 6-methoxy-2-napthylacetic acid; CCI, Chronic Constriction Injury; DDE, dose dependent effect; Eff., effective; FCA, Freund's complete adjuvant; inj., injection; Nd, not determined; Not eff., not effective; MPE, maximal possible effect; PSN, Partial sciatic nerve; SNL, Segmental spinal nerve ligation.

Compounds	Drugs	Hot plate test	Tail flick test	Brewer's yeast inj.	Acetic acid writhing test	Formalin test	Carrageenan model	FCA model	CCI model	PSL model	SNL model
COX-2 inhibitors	Celecoxib	Nd	Nd	Nd	Nd	Not Eff.	7.9	0.37	Nd	Nd	Nd
	DFP	Eff.	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Etodolac	Nd	Nd	9.24	3.67	Nd	6.99[c]	1.61	Nd	Nd	Nd
	Etoricoxib	Nd	Nd	Nd	Nd	Not Eff.	0.34	0.6	Nd	Nd	Nd
	Mefenamic Acid	Nd	Nd	Nd	20.7[b]	Nd	56[d]	9.2[e]	Nd	Nd	Nd
	Meloxicam	Not Eff.	30.5%[b]	Nd	6.5[b]	2.8 µmol/kg	5.8	0.4	Nd	Nd	5.53 µg
	Nimesulide	Nd	5.62[b]	Nd	11.2[b]	Nd	7.0[b]	Eff.	Nd	Nd	Nd
	Rofecoxib	23.4% MPE	Nd	Nd	Nd	Eff.	1.0	0.7	Nd	Nd	Nd
	Valdecoxib	Nd	Nd	Nd	Nd	Nd	Nd	0.036	Nd	Nd	Nd
Non-specific COX-inhibitors	6-MNA	Nd	Nd	Nd	Nd	Nd	12[d]	175[e]	Nd	Nd	Nd
	Aspirin	Not Eff.	2% MPE	51.8	67.5	>150	167[c]	>200	Nd	Nd	Nd
	Diclofenac	Not Eff.	21.4%[b]	3.80	1.54	34.5 µmol/kg	1.6	0.99	Nd	Nd	Nd
	Fenoprofen	Nd	Nd	Nd	3.7[b]	Nd	58[d]	15[e]	Nd	Nd	Nd
	Ibuprofen	Not Eff.	Not eff.	Nd	80	19	0.6	44[e]	Nd	Nd	Nd
	Indomethacin	Not Eff.	3% MPE	3.47	0.71	Eff.	1.5	0.42	Nd	Nd	0.24 µg
	Ketoprofen	nd	2% MPE	0.63	1.34	Not Eff.	1.12[c]	1.24	Nd	Nd	96.3% MPE
	Naproxen	Nd	Eff.[b]	2.78	9.83	Nd	1.83	9.38	Nd	Nd	Nd
	Oxaprosin	Nd	Nd	Nd	200	Nd	136[d]	150[e]	Nd	Nd	Nd
	Piroxicam	Nd	19.4%[b]	1.94	0.92	Not Eff.	1.31, 1.0	0.26	Nd	Nd	Nd
	Sulindac sulfide	Nd	Nd	Nd	1.7	Nd	33[d]	1.6[e]	Nd	Nd	Nd
	Tolmetin	Nd	Nd	38[g]	1.3[b], 1.42 µg/kg	Nd	55[d]	283[e]	Nd	Nd	Nd
COX-1 inhibitors	Flurbiprofen	Nd	Nd	Nd	3.3	2.1 nmol	2.6[d]	0.8[e]	Nd	Nd	Nd
	Ketorolac	Eff.[a], Not Eff.	18% MPE	Nd	3	5.2 nmol	0.29	9.4[e]	Nd	Nd	Not Eff.
Ca^{2+} channel blockers	Gabapentin	Not Eff.	Small effect	Nd	DDE	88	19.2, 50, 0.74 mg/ml,	>250	103[f], 87	>250, >300 I.t.	45.9 µg, 34
	Lamotrigine	Not Eff.[b]	>100 µg	Nd	Nd	28 nM[b]	207	Nd	11	Nd	Nd
Opioids	Morphine	9.8	1.8[b], 9.5	0.12, 1.93	0.3 , 0.21[b]	0.2, 2.3	1.5	58[a]	Nd	Nd	DDE, 4.6
	Buprenorphine	DDE, 0.4569 µmol/kg[b]	4 nmol/l , 0.16 , 1.6	0.12	0.07[b]	nd	Nd	Not Eff.[a]	Nd	Nd	nd

Table 3. Characteristics of different COX-inhibitors; protein binding, total and unbound therapeutic plasma concentrations and potencies on COX-1 and COX-2, as determined by the whole blood assay *in vitro*. NA means not applicable.
References: (43;50-60).

Drug	Therapeutic Dose (mg/day)	Therapeutic PlasmaConcentration (μM)	Whole Blood COX-1 IC_{50} (μM)	Whole Blood COX-1 IC_{80} (μM)	Gastric Mucosa COX IC_{50} (μM)	Whole Blood COX-2 IC_{50} (μM)	Whole Blood COX-2 IC_{80} (μM)	COX-2 Selectivity in Whole Blood (ratio of IC_{50})	COX-2 Selectivity in Whole Blood (ratio of IC_{80})	Degree of Plasma Protein Binding (%)	Unbound Plasma Concentration (μM)	Unbound IC_{50} (μM)	Unbound IC_{80} (μM)	Ratio of Total Conc. to COX-2 IC_{50} (μM)	Ratio of Free Conc. to COX-2 IC_{50} (μM)
6-MNA	1000 - 2000	155.4	31.01	146	0.48	19.84	580	1.56	0.25	NA	NA	NA	NA	7.83	NA
Aspirin (high dose)	1200 - 5200	111.0	4.45	8	0.03	13.88	>100	0.32	NA	60	44.4	5.55	NA	8.00	4.32
Aspirin (low dose)	81 - 325	15.0	4.45	NA	0.03	13.88	NA	0.32	NA	60	6	5.55	NA	1.34	0.72
Celecoxib	100 - 200	0.8	10.0 - 20.0	28	NA	0.3	6	60	4.67	97	0.024	0.009	0.18	2.67	0.10
DFP	NA	NA	> 100	> 100	NA	0.3	4	333	NA	NA	NA	NA	NA	NA	NA
Diclofenac	150 - 200	0.8	0.26	1	0.23	0.01	0.27	26	3.70	99.8	0.0016	0.00002	0.00054	80.00	1.00
Etodolac	600 - 1200	37.6	19.58	69	3.20	2.47	8	7.93	8.63	99	0.376	0.025	0.08	15.22	0.02
Fenoprofen	800 - 3200	89.5	2.73	23	0.17	14.03	100	0.19	0.23	99.7	0.2685	0.042	0.3	6.38	0.02
Flurbiprofen	200 - 300	53.2	0.41	1	0.23	4.23	24	0.10	0.04	99.96	0.02128	0.0017	0.0096	12.58	0.005
Ibuprofen (high dose)	1200 - 3200	111.0	5.9	58	0.70	9.9	67	0.60	0.87	99	1.11	0.099	0.67	11.21	0.11
Ibuprofen (low dose)	800 - 1200	38.8	5.9	NA	0.70	9.9	NA	0.60	NA	99	0.388	0.099	NA	3.92	0.04
Indomethacin	75 - 200	3.0	0.16	0.46	0.85	0.5	5	0.32	0.09	90	0.3	0.05	0.5	6.00	0.60
Ketoprofen	100 - 300	9.4	0.11	1	0.08	0.88	22	0.13	0.05	99	0.094	0.0088	0.22	10.68	0.11
Ketorolac	10 - 40	2.0	0.27	0.0034	0.33	0.18	4	1.50	0.00	98	0.04	0.0036	0.08	11.11	0.22
Mefenamic Acid	500	6.0	1.94	>100	0.70	0.16	>100	12.13		75	1.5	0.04	NA	37.50	9.38
Naproxen	500 - 1000	253.0	32.01	110	0.52	28.19	260	1.14	0.42	99.7	0.759	0.085	0.78	8.97	0.03
Nimesulide	100 - 200	3.4	10.48	41	1.49	0.18	7	58.22	5.86	99	0.034	0.0018	0.07	18.89	0.83
Oxaprosin	1200	681.9	14.58	NA	2.62	36.67	NA	0.40		99.5	3.4095	0.18	NA	18.60	0.09
Piroxicam	20	16.6	2.68	15	0.87	2.11	31	1.27	0.48	97	0.498	0.063	0.93	7.87	0.38
Rofecoxib	25 - 50	1.9	13	>100	NA	0.59	6	30	NA	87	0.247	0.077	0.78	3.22	NA
Sulindac sulfide	300 - 400	14.6	41.26	38	NA	24.94	100	1.65	0.38	98.5	0.219	0.37	1.5	0.59	0.01
Tolmetin	1200 - 1800	126.9	1.08	0.82	3.50	2.25	43	0.48	0.02	99.4	0.7614	0.014	0.258	56.40	0.36
Valdecoxib	NA	NA	86	NA	NA	0.285	NA	302	NA	NA	NA	NA	NA	NA	NA

might enable mechanism-based extrapolation between the different models and species. In addition, biomarker analysis in combination with pain measurements might help to understand variability in pain perception.

PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF COX INHIBITORS

The primary objective of pharmacokinetic-pharmacodynamic (PK/PD) modelling is the characterisation and prediction of the time course of drug effects under physiological and pathological conditions *in vivo* (18). Such PK/PD models consist of (1) a pharmacokinetic model, describing the time course of drug in blood or plasma, (2) a pharmacodynamic model, describing the relationship between drug concentration and effect and (3) a link model, which serves to account for the often observed "delay" of the effect relative to the plasma concentration (18).

A limitation of the currently available PK/PD models for COX inhibitors is that they are descriptive and do not take into account the pathophysiology of inflammation and the role of disease progression. Specifically, changes in disease status as a function of time (e.g. as reflected by varying concentrations of endogenous mediators) and the mechanism of COX-inhibition need to be taken into account in order to fully understand the non-linearity in the relationship between PK and analgesic effects during acute and chronic inflammation (figure 3).

MEASUREMENT OF PAIN

ANIMAL MODELS OF PAIN

Thus far, it has been assumed that the pre-clinical models that mimic clinical inflammation in humans can be used to predict the analgesic effect of COX inhibitors. According to current understanding of the underlying mechanisms, pain models can be broadly classified into three types: a) physiological (acute nociception), b) inflammatory and c) neuropathic pain models (19). Application of noxious stimuli results in an increased pain response in models of neuropathic, inflammatory and postoperative pain syndromes compared to the healthy situation, which is called hyperalgesia. Pain evoked by an application of innocuous stimuli is called allodynia.

In models of physiological pain, a noxious stimulus, mostly noxious heat, is used to measure the behavioural response of naïve animals (20). In the hot plate and tail-flick tests, the paw or tail is heated by a radiant heat source and the latency time to withdrawal is measured (21). These models can be used to characterise the pharmacological effects of analgesic and anti-hyperalgesic drugs. However, they have the disadvantage that drug administration occurs prior to heat stimulation. In general, COX inhibitors do not affect the normal nociceptive response threshold or latency time in the hot plate or tail flick tests (table 2).

Models of inflammatory pain produce a state of hyperalgesia that is evident from a few hours to weeks after peripheral injection of chemical irritants (21). Alternatively, inflammatory pain can also be induced by exposure of the rat hind paw to UV irradiation, which produces highly reliable and persistent allodynia (20). Similarly to the physiological models mentioned previously, drugs are often administered pre-emptively, i.e., before application of pain stimuli. Mechanical hyperalgesia due

inflammatory pain can be reduced in a dose-dependent manner by COX inhibitors (table 2) (22). In most of the mono-neuropathy models sciatic nerve injury is produced which leads to abnormal pain in the hind paw. Today, three different models of neuropathic pain are most frequently used (1) the chronic constriction injury (CCI) model as proposed by Bennet and Xie (23); (2) the single tight ligation of the partial sciatic nerve (PSL) model by Seltzer (24) and (3) the segmental spinal nerve ligation (SNL) model by Kim and Chung (25). In these models, hyperalgesia and allodynia typically

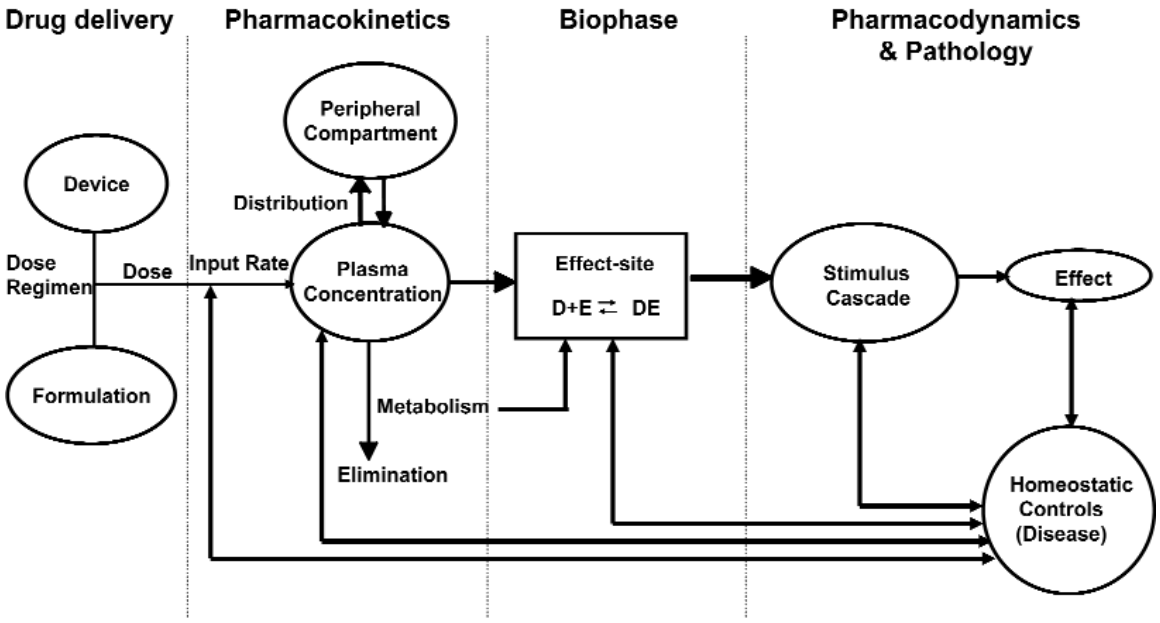

Figure 3. Schematic representation of the pharmacokinetic-pharmacodynamic processes and homeostasis that determine the relationship between the administered dose and the resulting effect intensity. Pharmacokinetic parameters like absorption, distribution, metabolism and elimination determine the relationship between dose and drug concentration in the biophase. In the biophase, the drug and its active metabolites interact with its receptor(s) and the pharmacological effect is accomplished by cascade of events leading to (an) effect(s). Homeostatic control mechanisms can influence the pharmacokinetics and pharmacodynamics of the drug and its metabolites.

develop over several weeks. However, the onset and extent of each component is different between the models. Only few articles have been published showing that COX inhibitors attenuate hyperalgesia in models of neuropathic pain (26).

CORRELATION BETWEEN THE POTENCY OF COX-INHIBITORS IN ANIMALS AND HUMANS

Although animal models of inflammatory and neuropathic pain have similarities to human disease conditions, extrapolation from pre-clinical animal models is rather difficult. Dubinsky *et al.* observed a correlation between the average single oral human therapeutic dose for pain relief and the oral ED_{50} dose in the joint flexion assay in arthritic rats (27). Mukherjee observed a correlation between the ED_{50} for paw oedema in the carrageenan model and the effective oral human daily dose for an array of COX inhibitors. These results indicate that information from of pre-clinical pharmacodynamic models can be of value for the prediction of the analgesic dose of COX inhibitors in humans (22).

$$Ln(ED_{50,human}) = A \cdot Ln(ED_{50,animal}) + B \quad (1)$$

In both analyses of Dubinsky and Mukherjee, a linear relationship was observed according to in which ED_{50}, human is the therapeutically effective dose in humans, ED_{50}, animal is the efficacious dose in animals, B is the intercept of y-axis, and A determines the steepness of the curve. In this equation A and B are empirical and mechanistically unknown scaling factors. Therefore, the ED_{50}, animal does not reflect the underlying concentration-effect relationships and pre-clinical analgesic doses cannot be considered a predictor of clinical analgesia. A more scientific approach for interspecies extrapolation is scaling based on drug exposure or biomarker concentration.

MODELS TO INVESTIGATE SELECTIVITY ON COX-1 AND COX-2

As an alternative to *in vivo* measurements, *in vitro* methods may enable the assessment of concentration-effect relationships that reflect in a strict quantitative manner the anti-inflammatory response *in vivo*.

Different models have been developed to study the selectivity of COX inhibitors on cyclo-oxygenase activity *in vitro* (28). These models include purified or microsomal recombinant enzymes (29;30), homogenised cells (31;32) or intact cells (33;34). The advantages and drawbacks of each individual test have been analysed elsewhere (35) and will not be discussed in such detail in this review. As standardisation is not available, experimental conditions can vary widely among the various assays. In these assays arachidonic acid can be supplied from endogenous or exogenous sources, which can affect the apparent potency of competitive (Class 1) COX inhibitors. It has been demonstrated that these models can be useful to guide the synthesis of selective COX-2 inhibitors (28), but such models do not consider protein binding of COX inhibitors and other aspects of the kinetics of drug-enzyme interaction (6). In fact, some sources of variability have been identified, like time-dependent binding for certain compounds, which could be adapted when investigating different classes of COX inhibitors. Therefore, assay systems that reflect physiological conditions and account for differences in pharmacological properties provide the most appropriate models (36).

A more pathophysiological model to study the selectivity on COX-1 and COX-2 is the human Whole Blood Assay (hWBA), which was first described by Patrignani and colleagues (28). In the hWBA, the production of prostaglandin E2 (PGE_2) by bacterial lipopolysaccharide (LPS) stimulation in leukocytes in the whole blood is used to characterise drug effects on COX-2, whereas the production of thromboxane B2 (TXB_2) by platelets in the whole blood sample during one-hour whole blood clotting is used as an endpoint to determine drug effects on COX-1. The hWBA has several advantages over other *in vitro* systems, as it uses whole cells, takes into account intra-cellular transport of drugs and provides a physiological plasma protein level. Moreover, the method a can be used *ex vivo* following *in vivo* administration of COX inhibitors at therapeutic doses (37). A reflection of therapeutic efficacy is achieved when the hWBA is used to determine the effects of COX inhibitors in healthy volunteers who have taken the drug for several weeks (36). Moreover, this model enables calculation of IC_{50}'s. A drawback of this system is still the high biological variability in

measurements, but that does not surpass its advantages as long as sample size is properly considered (37). In his publication, Blain has found that the level of COX-1 and COX-2 inhibition achieved *in vivo* cannot be predicted universally from the corresponding *in vitro* dose-response curves, which could be a major drawback of the model (37). As Blain states, the predictability of the hWBA was suitable for diclofenac, acceptable for ibuprofen, but inadequate for meloxicam. However, the investigators only plotted the average concentration-inhibition curves of 24 healthy volunteers without evaluating information derived from between-subject variability. Nonlinear mixed-effects modelling of individual concentration-inhibition curves as proposed in this review was not applied to that data set.

The estimated IC_{50} values for COX-1 and COX-2 of different COX inhibitors have been shown to vary between models. Yet, the ranking order of selectivity remains approximately the same from one study to another. COX inhibitors with the highest selectivity for COX-1 and the lowest for COX-2, and those with the lowest selectivity for COX-1 and the highest for COX-2, yield the same results, irrespective of the preparation used for the assay (38). In addition, the hWBA has been used for the determination of COX-1 and COX-2 selectivity in humans, rat and guinea pig blood (28;39;40). Despite methodological variations, when comparing all the different assay systems, the COX-2/COX-1 ratios of different COX inhibitors are able to classify the COX inhibitors according to their selectivity into selective COX-2, non-selective and selective COX-1 inhibitors (41). However, the assessment of COX-1/COX-2 selectivity ratio *ex vivo* after drug administration to humans has provided evidence that selectivity behaves as a continuous variable. Thus, it will reflect the selectivity *in vitro*, but more importantly the dose and frequency of administration. This is further amplified by an important intra-subject variability in both COX-1 and COX-2 inhibition after dosing.

SELECTION OF COX-INHIBITORS

In the early phases of drug development, the selection criteria for new COX inhibitors are primarily based on the inhibition profile for COX-1 and COX-2. Typically, drug selectivity is assessed by *in vitro* assays during screening, as these assays are relatively rapid and simple. In these models, the selectivity of action of COX inhibitors is determined by the ratio of IC_{50} estimates (i.e., the concentration that produces 50% of the maximal inhibitory response) for the inhibition of COX-1 and COX-2, respectively. Comparison of the IC_{50} ratio is frequently used to determine the relative biochemical selectivity of the various compounds (42). Despite the widespread use of these assays for screening purposes, translation of the results from selectivity profiles to the analgesic effect *in vivo* has not been reported.

To date, the dose selection for COX inhibitors is targeted to maximal inhibition of COX-2. Typically, no attention is given to the dose range at which the therapeutic effect occurs without loss of selectivity of action, or the minimal level of inhibition required to produce a therapeutic effect. This approach may lead to an undetectable increase in safety risks. As depicted in figure 4C, adapted from (42), a typical COX inhibitor has an *in vitro* COX-1/COX-2 ratio of about 18, which indicates that the compound can be classified as a selective COX-2 inhibitor. At a single therapeutic dose of 50 mg

or 100 mg, however, (fig 4A and B) the compound causes a significant inhibition of COX-1 at drug levels that suppress COX-2 by > 90%. This shows that this drug cannot be considered a preferential COX-2 inhibitor after administration of "therapeutic" doses (42). Figure 4C shows that at a concentration resulting in 90% inhibition of COX-2, COX-1 is inhibited for at least 40%, which accurately reflects the drug profile *in vivo*. In this respect it is important to realize that the use of an *in vitro* ratio based on IC_{50} values does not capture differences in the shape of the concentration-inhibition curves for COX-1 and COX-2, which can be very different, as indicated by different slopes. As a consequence, the actual selectivity ratio varies with the concentration, making it incorrect to determine the selectivity of action on COX-1 and COX-2 solely based on the ratio of IC_{50} values. In fact, this ratio should only be used as an approximation of selectivity in the absence of information on the slope of the concentration-inhibition curve. It's not yet understood which mechanisms determine the slope of the drug concentration-inhibition relationship.

Table 3 shows the dosing range of different COX inhibitors, IC_{50} values for the inhibition of COX-1 and COX-2 *in vitro* (both in μM), and the therapeutic plasma concentration in humans. The

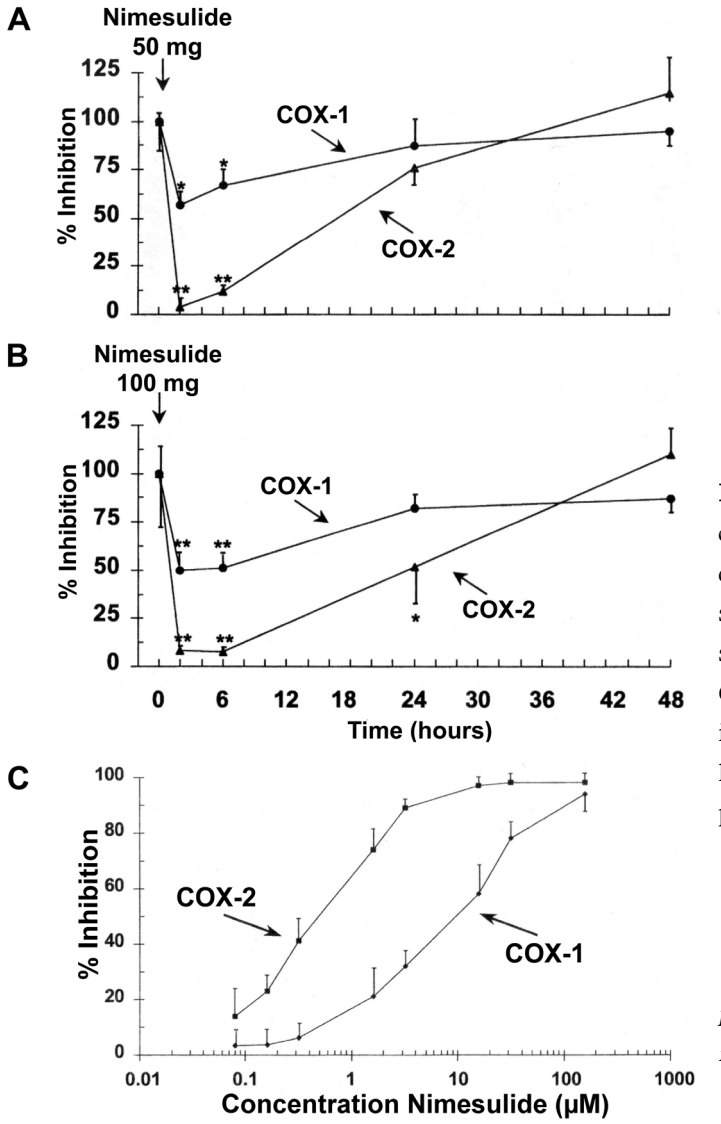

Figure 4. Inhibition of COX-1 and COX-2 activity over time by a COX-inhibitor. A single therapeutic dose of 50 mg or 100 mg (A and B) causes a significant inhibition of COX-1 at drug levels that suppress COX-2 by >90%. Despite an *in vitro* IC_{50} COX-1/COX-2 ratio of about 18 (C), this agent inhibits platelet COX-1 activity at therapeutic plasma levels, and therefore could not be considered a preferential COX-2 inhibitor[1].

[1]*Reprinted from Clinical Pharmacology and therapeutics, Vol. 63 Issue 6, 1998, pp672-681, Panara M R et al.: "Effects of nimesulide on constitutive and inducible prostanoid biosynthesis in human beings" with permission from Elsevier.*

therapeutic plasma concentration in μM is the average concentration in plasma at which analgesia is

achieved in patients. Typically at the therapeutic analgesic dose, COX-1 is inhibited by more than 80%, except for COX inhibitors that display more than 1.56 fold selectivity for COX-2. This demonstrates that an *in vitro* IC_{50} ratio can predict the inhibition *in vivo*. If a compound is more than 1.56 fold selective for COX-2 *in vitro* (as assessed by the IC_{50} ratio), only COX-2 will be inhibited *in vivo* at therapeutic plasma concentrations. Although this relationship has been observed between *in vitro* and *in vivo* COX-1 and COX-2 inhibition, no direct correlation has been established for analgesic effects of COX inhibitors. Understanding of other relevant factors is required to enable early, accurate prediction of therapeutic doses.

Determinants of the PK/PD relationship of COX inhibitors in acute and chronic inflammatory pain
One may need to consider more than just the pharmacokinetics and pharmacodynamics to determine time course of the treatment response of COX inhibitors in inflammatory pain. Specifically, time-dependent factors in the pharmacodynamics of enzymatic inhibition and the role of disease progression must be taken into account.

Pharmacokinetics. The physicochemical properties of a drug contribute to its pharmacokinetics. In general, COX inhibitors are lipid-soluble, weakly acid, drugs which bind extensively to plasma proteins, with albumin as the major binding protein (43). Typically, COX inhibitors have a low extraction ratio and are subject to restrictive elimination (i.e., only clearance of the unbound fraction occurs). Thus, changes in plasma protein binding can have significant effect on drug disposition, volume of distribution (Vd), clearance (Cl) and pharmacological activity (43). Therefore, the unbound fraction of a drug, f_u, is a critical parameter in describing and understanding the pharmacokinetics of COX inhibitors.
The pK_a, the acid dissociation constant, of COX inhibitors is another important determinant of treatment response, as it drives the absorption rate after oral administration. In reality, it has been hypothesized that the pK_a value can be an important determinant of the distribution of COX inhibitors. It appears that unionized COX inhibitor concentrations in the extracellular fluid increase with increasing pK_a values, which allows enhanced diffusion of drug to the site of action (22). Generally, COX inhibitors have a relative small volume of distribution, which indicates preferential distribution to certain body compartments. Selective tissue accumulation of COX inhibitors at the site of inflammation may explain their superior anti-inflammatory analgesic activity, but is also known to cause gastrointestinal and kidney toxicity (44).

The lipid solubility of drugs is another determinant of the ease with which drugs cross cellular membranes. The octanol-water partition coefficient, determined at pH 7.4, could be the best indicator for the trans-membranal diffusion of COX inhibitors. On the other hand, Mukherjee found that the octanol-water partition coefficient at pH 7.4 and therefore the diffusion to intracellular sites is not a limiting factor for this class of drugs (22).

Equilibration kinetics. Physicochemical properties contribute to the affinity and efficacy of COX

inhibitors. To date, it is generally accepted that COX inhibitors can be classified on their mode of inhibition of COX in three classes. Class 1 inhibitors are simple competitive inhibitors, Class 2 COX inhibitors are time-dependent inhibitors and Class 3 COX inhibitors are irreversible inhibitors (38). In contrast to classical non-selective COX inhibitors, which are Class 1, 2 or 3 for both COX enzymes, all diarylheterocyclic COX-2 selective inhibitors like celecoxib and rofecoxib are Class 2 for COX-2 and Class 1 for COX-1. Thus, selective COX-2 inhibitors are Class 1 inhibitors for COX-1, binding to COX-1 for a short period, but are Class 2 inhibitors for COX-2, and therefore display time-dependent binding to COX-2. On the other hand, it has been demonstrated that drugs like nimesulide and meloxicam, which have time-dependent binding to COX-2, also display time-dependent binding to COX-1 (29). However, these classical COX inhibitors bind differently to the enzyme than the newly developed coxibs.

Class 3 inhibitors irreversibly inhibit COX. Aspirin is the only example of a marketed class 3 inhibitor. The pharmacodynamics of aspirin depends on the synthesis and degradation of the COX-enzyme. Yamamoto found that after administration of a single dose (325 mg) of aspirin to healthy volunteers, aspirin concentrations were measurable for 8 hours in plasma, whereas COX-1 was inhibited for approximately 200 hours (45). This clearly shows that the turnover of COX can be a major determinant of the pharmacodynamics of COX inhibitors. To what extent such time-dependencies influence the pharmacodynamics of COX inhibitors is not yet clear, but obviously it depends on the relationship between drug clearance, distribution and offset (k_{off}) of the binding to COX. For class 1 COX inhibitors, no influence of COX turnover is expected since the binding is more rapid than the elimination half-life.

Gierse hypothesized that determination of the selectivity based on the ratio of IC_{50} values is not supportable if the underlying binding kinetics of COX inhibitors is different to each isoform of the COX-protein. This underscores the need to characterise the *in vivo* selectivity of action under physiologically relevant conditions (46).

Disease progression. Disease progression can be defined as changes in disease state as function of time (47). Drug effects may be symptomatic (i.e. no change in the disease progression), protective (i.e. the drug causes a slower rate of the disease progression) or curative (i.e. the drug abolishes the disease progression).

Disease progression analysis describes in a strict quantitative manner the effect of drug treatment on the progression of disease. This is important in the following situations, (1) when the drug has no direct observable pharmacological effect, (2) when the drug is intended to modify disease progression and (3) when disease severity influences the drug response.

The dynamics of disease progression determines to which extent a drug can yield a pharmacological response (figure 5). The absolute acute effect of a drug depends on the status of the disease. In figure 5, at time T, an absolute acute effect of A can be reached, whereas at time T+1, a maximal effect of B can be reached. In order to predict the effect of a drug in a chronic disease, the time course of disease must be characterised. In addition, if a drug is given chronically, a separation must be made between symptomatic and disease modifying effects of the treatment. In contrast to opioids, the

analgesic effects of COX inhibitors have been primarily studied under chronic disease conditions because COX inhibitors have no hypoalgesic effects. However, this does not preclude the assessment of underlying mediators of inflammation, which could be performed in animals and humans. Melli investigated the levels of PGE_2, a marker for COX-2 activity, in chronic inflammatory processes in rats (48). The plasma levels of PGE_2 showed oscillations over time, indicating that the amount of COX-2 protein is not constant or that the metabolic rate varies under disease conditions. These findings suggest that the effects of COX inhibitors in the treatment of chronic inflammatory conditions may be variable, if inhibition levels are not optimal. Consequently, the effects of COX inhibitors upon the chronic inflammatory processes could be different from those observed in acute conditions.

BIOMARKERS

The nature and complexity of the interaction between various factors that determine the analgesic response of COX inhibitors require biomarkers to explain and understand variability in the treatment effect. The use of a biomarker in pain measurements is an important step in the development of new COX inhibitors, as it can link PK to the analgesic effect and eventually provide a proxy for safety evaluation. Given the nature of the inflammatory response and the mechanism of action of COX inhibitors, a number of mediators can be used as an intermediate step between PK and analgesia. In conjunction with non-linear mixed effect modelling, the relationship between biological marker, pain measurement and safety can be characterised. Primary candidates for such a role are prostaglandins and thromboxanes (figure 1). The mechanism of inflammation in animals and

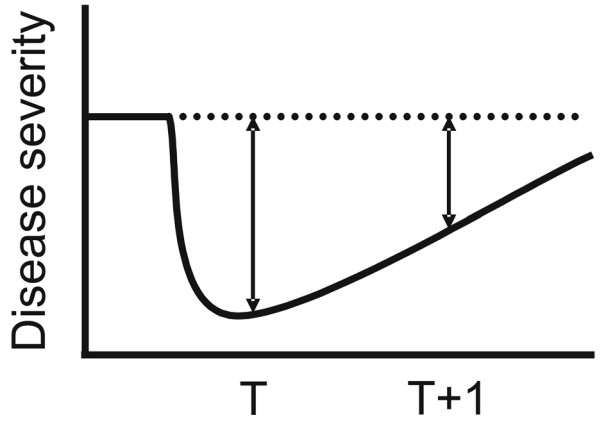

Figure 5. The absolute maximal acute effect of a drug upon the disease progression depends on the status of the disease. The first arrow indicates an absolute effect of 80 at time is T, as the disease progresses the effect of a drug can be maximally 40 or less at time is T+1 (second arrow). In order to predict an effect of a drug in a chronic disease, the progress of the disease has to be known. The solid line indicates the progression of disease, the dashed line indicates the healthy condition.

humans is similar, which might facilitate the extrapolation of this biomarker from pre-clinical to clinical data. In the next paragraph, literature findings of biomarkers and correlation with pain response in presented. Pre-clinical data is limited and therefore, the next step is to use clinical data to search for a suitable biomarker to predict analgesic response.

BIOMARKER AND PAIN RELATIONSHIP

Limited information is available on the time course of the pain intensity and biomarkers in animal and human studies. Figure 6 shows the correlation between pain response and spinal reorganization over time following FCA injection and in the CCI model of neuropathic pain. The maximal increase

in neurokinin-1 receptor density is correlated with the maximal pain response four days after FCA injection. The same correlation between pain response and receptor density is observed in the CCI model (49). These results indicate that pain response can be linked to a specific substrate in chronic diseases in animals. However, the use of receptor occupancy or tissue samples in the context of drug development is laborious and a systemic biomarker might be preferable. A systemic biomarker, however, should reflect the underlying processes in the target tissue or a mechanistic extrapolation between target tissue and systemic exposure has to be demonstrated. To date, no literature findings of systemic biomarkers for pain measurements is available from animal studies, and therefore we

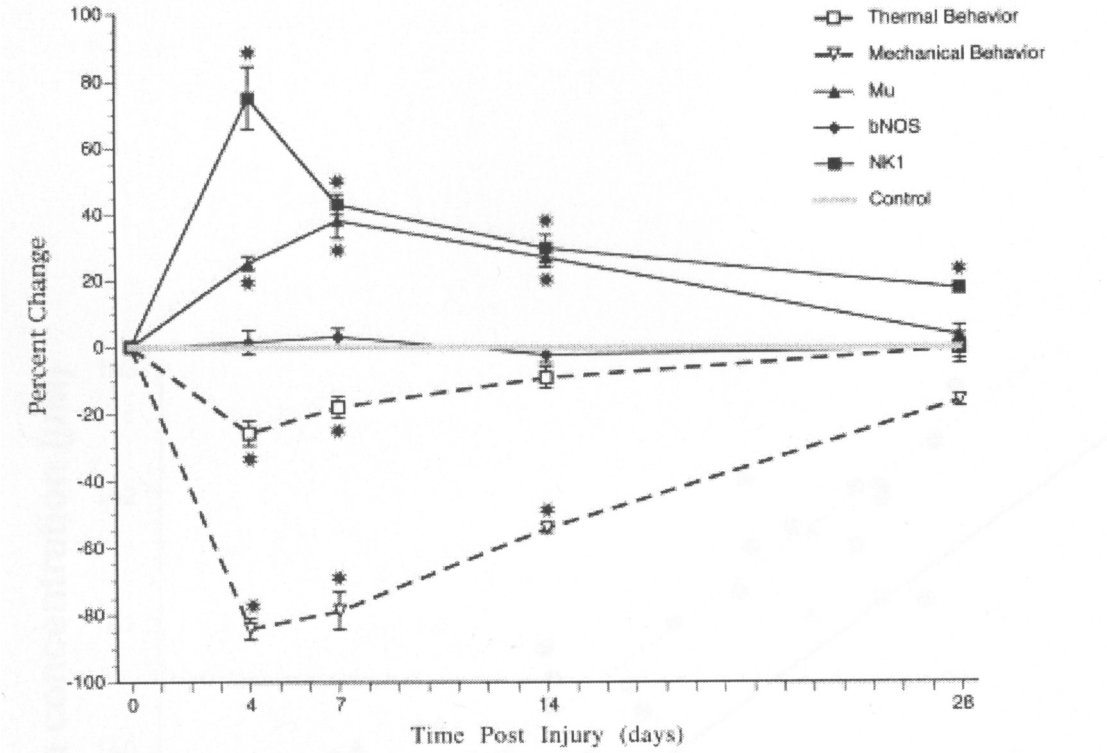

Figure 6. Time course of behavioural and anatomical changes in animals treated with complete Freund's adjuvant. Dashed lines represent the per cent change (ipsi/contra) in behaviour. The percent change in the immunolabelling of the neurokinin-1 (NK1) receptor, mu-opioid receptor, and the cell count data for brain nitric oxide synthase (bNOS) is represented by continuous lines[1] (*P<0.05).

[1]*Reprinted from Neuroscience, Vol. 82, Issue 2, 1998, pp559-574, Goff et al, "Reorganization of the spinal dorsal horn in models of chronic pain: correlation with behavior" with permission from Elsevier.*

proceeded with clinical data. Cryer and Feldman found a direct correlation between the gastric IC_{50} and COX-1 IC_{50} in blood, as measured by the hWBA of both selective and non-selective COX inhibitors in humans (50). In biopsies, total gastric COX activity was measured by PGE_2 production. Inhibition of COX-1 can be used to predict the gastric dose, however it is not clear whether this gastric dose truly reflects side effects like ulcer formation. Still, this is a first step in the development of a systemic biomarker for pain measurements.

CURRENT WORK ON BIOMARKER AND PAIN RELATIONSHIP

In conjunction with modelling, we have explored the predictive value of *in vitro*-derived parameters for clinical dose estimation of selective and non-selective COX inhibitors. Systemic PGE_2 and TXB_2

production were measured using the hWBA. From a mechanistic point of view, investigating COX-2 inhibition as marker for the analgesic effect might be limited in view of the fact that recent data showed that COX-1 is, to some extent, involved in inflammation. Pharmacokinetics, pharmacodynamics and *in vitro* properties of selective and non-selective COX inhibitors were analyzed. The data were obtained from published articles (table 3) (43;50-60). The plasma concentrations of COX inhibitors at which analgesic therapeutic effect was achieved, were correlated to *in vitro* estimates for the inhibition of COX-2 in humans. The IC_{50} values for COX-2 inhibition, as obtained from the *ex vivo* whole blood assay, were selected as a link to analgesic concentrations (figure 7). In addition, the role of protein binding was investigated; IC_{50} values and therapeutic plasma concentrations were corrected for free fraction (f_u).

A correlation was found between IC_{50} parameter and corresponding therapeutic plasma concentration of 22 different COX inhibitors (figure 7). Total and unbound IC_{50} values showed to correlate with therapeutic plasma concentration of COX inhibitors. However, data shows that more than 50% inhibition is required to achieve analgesia, as indicated by the data points that are approximately 10 fold above the line of unity. Hence, we re-parameterised the model to obtain IC_{80} estimates (the concentration that produces 80% inhibition). As shown in figure 8, analgesic therapeutic plasma concentration is directly correlated with IC_{80}. From this correlation it is evident that at least 80% inhibition of COX-2 is required to produce analgesia. Therefore, this *in vitro* derived parameter can be used to predict the exposure levels of COX inhibitors that yield analgesic effect.

A first attempt to understand the role of COX-1 and COX-2 inhibition and its relation with clinical efficacy has been initiated by H. Fenner. His data show that meloxicam and naproxen inhibited COX-2 by 80% or more at their therapeutic dose (36). Our findings are also comparable with published data from Warner *et al.* Based on steady state plasma concentration of more than 40 COX inhibitors, Warner found that COX inhibitors are used therapeutically at doses that produce more than a 50% reduction in prostanoid formation (60). With regard to selectivity, we can conclude that selective COX-2 and non-selective COX inhibitors induce analgesia by PGE_2 inhibition.

Our approach shows that model-derived parameters provide a valuable tool for the prediction of therapeutic dose in an early stage of drug development.

Safety. Recently, the withdrawal of rofecoxib (Vioxx™) from the market by Merck & Co., Inc. because of the increased risk of myocardial infarction and stroke associated with chronic treatment has caused scepticism about the future of COX-2 inhibitors. This event will certainly lead to changes in the regulatory policy concerning dose rationale and safety assessment of new and old COX inhibitors. It also highlights the need for innovative approaches in drug development, compatible with the latest proposals published by the FDA, in which the importance of biomarkers is emphasised and pharmacokinetic-pharmacodynamic modelling is recommended (61).

Unclear is whether the cardiac toxicity of rofecoxib is a purely compound-related issue or a class-specific one that was due to a long-lasting, excessively high COX-2 inhibition achieved at the

marketed doses. Thus far, several lines of evidences have supported that cardiac toxicity may be

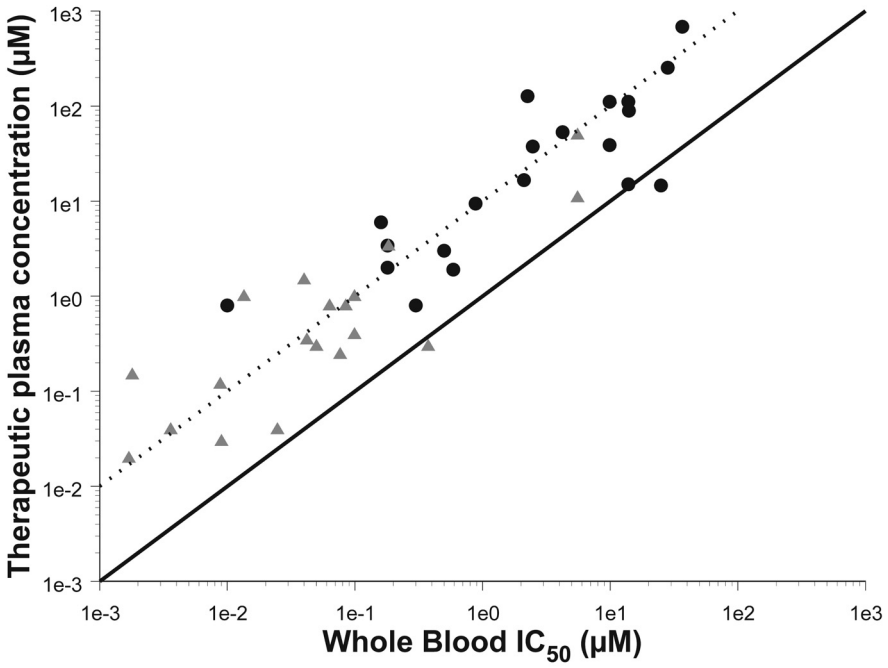

Figure 7. *In vitro* IC_{50} values of COX-2 inhibition of 22 different COX-inhibitors versus their analgesic therapeutic plasma concentration. IC_{50} values are determined in whole blood of healthy volunteers using the hWBA. Total (black dots) and unbound (grey triangles) IC_{50} values and total and unbound therapeutic plasma concentrations are depicted. Black line denotes Y=X, dashed black line denotes Y=10*X.

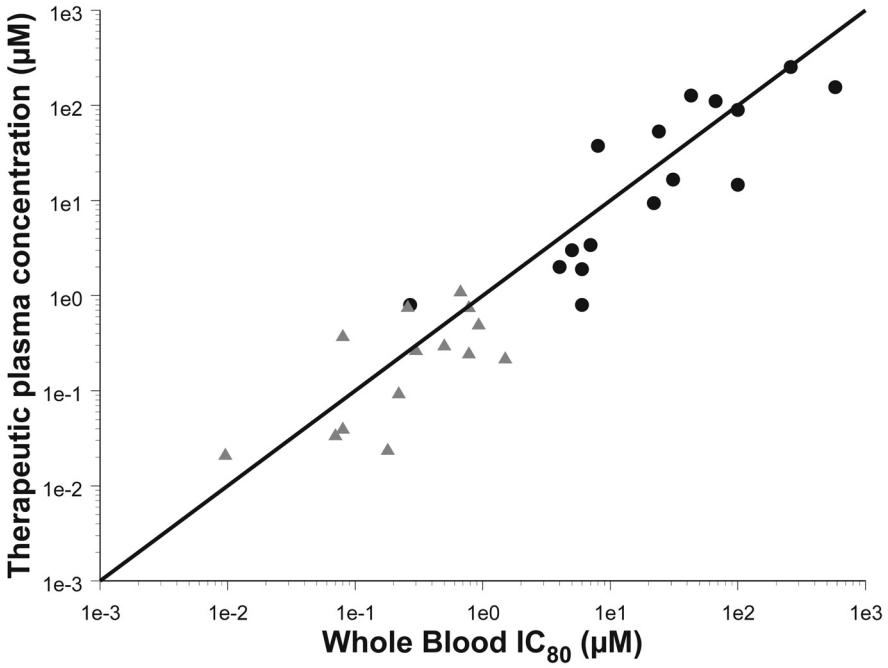

Figure 8. *In vitro* IC_{80} values of COX-2 inhibition of 22 different COX-inhibitors versus their analgesic therapeutic plasma concentration. IC_{80} values are determined in whole blood of healthy volunteers using the hWBA. Total (black dots) and unbound (grey triangles) IC_{80} values and total and unbound therapeutic plasma concentrations are depicted. Black line denotes Y=X.

associated with the use of other COX-2 inhibitors (i.e., valdecoxib and celecoxib) corroborating a mechanism-based effect (62). Thromboxane A2 (TXA_2) is a vasoconstrictor and platelet agonist. Inhibition of TXA_2 by COX-1 has displayed cardiovascular protection properties. So far, only aspirin at low dose has displayed these properties (63). In contrast, prostacyclin (PGI_2) is a vasodilator that inhibits platelet function. COX-2 is the major source of PGI_2 under physiological conditions in humans. In human syndromes of platelet activation, the biosynthesis of PGI_2 is increased, and it has been suggested to function as a homeostatic response ligand to accelerated platelet-vessel wall interaction. Furthermore, Cheng et al. established that endogenous PGI_2 modulates the cardiovascular actions of TXA_2 *in vivo* in mice (64). Thus, selective COX 2 inhibitors depress prostacyclin (PGI_2) but not COX-1-derived TXA_2. The effects of TXA_2 would be exaggerated during treatment with COX-2 inhibitors, potentially predisposing patients to heart attack and stroke. However, many factors will contribute to the overall hazard associated with the treatment with COX-2 inhibitors: 1) clinical substrate (i.e., intrinsic risk for cardiovascular disease), 2) selectivity attained *in vivo*, 3) dose, total exposure and duration of dosing, and 4) between-subject variability.

On the other hand, potential differences in cardiovascular outcome after prolonged treatment with selective COX-2 inhibitors might be explained by intrinsic molecular, pharmacokinetic and pharmacodynamic properties, i.e., not a class effect, but a compound-related effect (65). Evaluation of the latter hypothesis is not as straightforward as it seems. In fact, it is quite difficult to detect differences in safety from comparative clinical trials as those studies are designed for testing non-inferiority and statistically powered for the primary endpoints. From a statistical perspective, differences will remain undetected unless the effect size and variability of the secondary endpoint is comparable to the one for which the study was originally powered. Finally, it is important to realise that in addition to drug properties, treatment duration is probably a major determinant in the differences of cardiovascular side-effects. Most coxibs were initially approved as single doses based on the results of randomised clinical trials.

The rationale for dose selection, dosing regimen and treatment duration with coxibs in chronic inflammatory arthritis will have to account for long-term cardiovascular adverse events. In such circumstances, the use of a biomarker for analgesia and side-effects becomes highly relevant. A therapeutic window needs to be constructed based on COX-1 and COX-2 inhibition curves for compounds in development. Treatment efficacy can be determined by COX-2 inhibition (IC_{80}) whilst inhibition of COX-1 can be used to predict GI safety. And if the hypothesis of class-specificity for cardiovascular toxicity is true, i.e., that the interplay between COX-1 and COX-2 inhibition modulates the long-term cardiovascular effects, constant and complete COX-2 inhibition without COX-1 inhibition in a minority of patients may lead to cardiovascular events. COX-2 and COX-1 inhibition curves may therefore be a suitable proxy for long-term safety. However, further investigation is needed to clarify the role of chronic selective COX-2 inhibition in cardiovascular toxicity. Thus, it is important to identify whether adverse events derive from efficacious but unsuitable doses, which cause excessive, long-lasting COX-2 inhibition during chronic treatment or

whether there is a minority of patients susceptible to maximum inhibition of vascular COX-2 in the presence of unaltered platelet TXB_2.

Whatever the case may be, knowledge about the PK/PD relationships for PGE_2 and TXB_2 inhibition can be used to quantitatively differentiate new compounds as well as other currently available coxibs at the marketed doses. For instance, rofecoxib and etoricoxib are highly selective COX-2 inhibitors with relatively long elimination half-lives. Plasma concentrations after administration of the approved therapeutic doses are proper to fulfil a continuous and profound inhibition of COX-2 (>90%) that translates into a clinical analgesic effect when administered once daily. Taking into account treatment duration and pharmacokinetic variability, it is conceivable that therapeutic doses could produce permanent, total suppression of COX-2 activity in a number of patients, the consequences of which may relate to the recent safety findings. In contrast, plasma levels achieved after administration of once daily doses of celecoxib and lumiracoxib should be insufficient to sustain appropriate analgesia throughout the dosing interval, since circulating concentrations decrease rapidly due to their relatively short half-life ($t_{1/2}$ is 11 h for celecoxib and 3-6 h or lumiracoxib). Thus, a twice-daily dosing regimen for both drugs should be used to overcome the limitations of such a short half-life. Instead, administration of single high doses has been selected for these drugs. In fact, celecoxib doses cause peak concentrations that are 6 to 10-fold higher than those necessary to inhibit inducible COX-2 activity by >80% throughout the dosing interval. Thus, theoretically the long-term effects of celecoxib administered at a dose of 200 mg daily on renal and cardiovascular function should be minor because its impact on renal and vascular COX-2 activity can be short-lasting. For lumiracoxib, the mean peak plasma concentration after a single dose of 200 mg to healthy volunteers is 14 μM (66), a value that is >10-fold higher than the concentrations necessary to achieve analgesia. This dosing regimen for lumiracoxib can cause a profound suppression of PGE_2 and vascular PGI_2, but it can be hazardous and has been associated with higher hepatotoxicity than non-selective COX inhibitors (67).

FUTURE PROSPECTS

The WBA has been frequently used in rats, guinea pigs and humans only to identify selectivity of compounds. From the current data, we have shown that biomarker inhibition yields information not only on selectivity, but also on analgesic concentration ranges. In addition, it seems that there is no need for an allometric scaling factor between those species. The only confounding factor that we have been able to observe is protein binding. Another potential factor is the turnover rate and total pool of COX enzymes under pathophysiological conditions. However, based on the current standardisation of the WBA, this does not seem to play a role. Likewise, non-selective activity of COX-2 inhibitors on COX-1 production can be characterised by TXB_2 inhibition. As TXB_2 do have a physiological role in coagulation homeostasis, which is different among species, pre-clinical data may not be directly correlated with human data, i.e. an allometric scaling factor may be required. Therefore, we recommend that dose selection and escalation of new compounds in clinical development be based on biomarker inhibition in human blood rather than *in vivo* pre-clinical models of pain. Refinement

of the therapeutic dose of new compounds, particularly if large inter-individual variability is observed in PK and PD *in vitro*, can be achieved by further evaluation of the biomarkers *ex vivo* in the target patient population.

CONCLUSIONS

In this review, we discussed several aspects important to the understanding of pharmacology in pain research. As there is no direct correlation between PK and analgesic effects of COX inhibitors, a biomarker becomes a valuable tool in the development of these compounds. PK/PD analysis reveals the relevance of the COX-1/COX-2 assays *in vitro*, as they can be linked to plasma kinetics and clinical effects *in vivo*. Our findings indicate that model-derived parameters for the inhibition of COX-2 (IC_{80}) as determined by hWBA can accurately predict therapeutic analgesic effects in humans. An efficient approach to the development of new compounds ought to be based on the characterisation of PGE_2 and TXB_2 inhibition.

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