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Standards of laboratory practice: analgesic drug monitoring

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Analgesics are the most commonly consumed over-the-counter preparations in the United States. They are used in the treatment of various pain syndromes and other medical conditions. Although analgesics are generally perceived to be safe agents, serious toxicity may occur in the setting of acute overdose, chronic abuse, or overuse. The indications for therapeutic drug monitoring in patients using these medications appropriately is as yet not well defined. The emphasis of this discussion, therefore, is on recommendations for monitoring in situations where toxicity is suspected. Preanalytical, analytical, and practice issues including drug interactions, frequency of monitoring, pertinent ancillary tests, reporting, and special patient groups at risk for toxicity are reviewed. Recent information from a major manufacturer of evacuated tubes arguing against the use of gel tubes for blood collection for drug monitoring is included. Colorimetric/enzymatic/immunoassays for the routine/stat monitoring of acetaminophen and salicylate and diflunisal cross-reactivity with most of the currently used salicylate assays are presented. Achiral and chiral chromatographic assays and newly introduced columns such as restricted access media and/or automated chromatographic systems are reviewed for the analysis of ibuprofen, naproxen, and the recently introduced tramadol. Finally, concepts regarding future directions including drug chirality and chiral analysis are presented.

Analgesic drugs comprise the largest category of pharmaceutical agents consumed by individuals in the United States. The number of over-the-counter formulations available to the consumer is staggering. Acetaminophen alone is estimated to be present in >200 brand name, over-the-counter preparations today *(1)*. In addition, hundreds of products containing aspirin, amounting to 20 billion tablets, are consumed annually in the United States *(2)*. Combinations with other analgesics, antihistamines, opioids, stimulants, and sedatives are available. With the recently expanded sales and marketing of over-the-counter ibuprofen and naproxen, additional increases in the already widespread use of nonsteroidal antiinflammatory drugs (NSAIDs)[3] is likely. One major concern is that the analgesic class of drugs accounted for more exposures reported to the American Association of Poison Control Centers in 1995 than did any other category of pharmaceutical agents *(3)*. The serious nature of these exposures is underscored by the fact that they also led to the highest number of pharmaceutical drug-related deaths during that same year *(3)*.

Analgesics are used in the treatment of a wide variety of medical conditions. Their use in treating both acute and chronic pain syndromes such as cephalgia or dysmenorrhea is well recognized. With the exception of acetaminophen and tramadol, the above analgesics, also classified as NSAIDs, are indicated for the treatment of acute or chronic inflammatory diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute pericarditis, and Kawasaki's disease. Antipyresis is another common indication for the administration of these agents, particularly in the pediatric age group. Acetylsalicylic acid may be useful for the prevention of thrombosis in patients with coronary artery or cerebrovascular disease, given its antiplatelet effect, and salicylate may have a role in minimizing tissue hypoxia-reperfusion injury after organ transplantation *(4)*. Finally, increasingly important clinical applications are being realized by both NSAIDs and opioid analgesics for the treatment of postoperative and cancer pain *(5)*.

Therapeutic drug monitoring (TDM) of NSAIDs was

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[3] Nonstandard abbreviations: NSAID, nonsteroidal antiinflammatory drug; TDM, therapeutic drug monitoring; CYP, cytochrome; CSF, cerebrospinal fluid; GC, gas chromatography; GC-MS, gas chromatography–mass spectrometry; NAPQI, *N*-acetyl-*p*-benzoquinonimine; and CNS, central nervous system.

previously reviewed *(6)*. This discussion focuses on the clinical and analytical issues surrounding the monitoring of commonly used analgesic drugs. Emphasis is on clinical, pharmacologic, and analytical issues applicable to the practice of TDM and toxicology *(7, 8)*. Analgesics discussed are those readily available in over-the-counter preparations, such as acetaminophen, salicylates, ibuprofen, and naproxen. Other areas briefly presented include emerging concepts with regard to cytochrome (CYP) P450 metabolism and other phase I and II metabolic pathways, the contribution of both active and inactive metabolites to drug toxicity and drug-drug interactions, chirality, and confounding issues surrounding workplace drug testing for certain opioids *(9–12)*. This review does not extensively cover traditional opioid analgesics, such as morphine and codeine, that have been adequately addressed in the arenas of clinical toxicology and forensic urine drug testing/workplace drug testing *(13)*.

Indications for Monitoring

Although definitive indications for therapeutic monitoring of the analgesics are as yet not well defined, the need for drug concentration determination becomes critical in situations where overdose, abuse, or toxicity is suspected. Additionally, screening may be used in situations when compliance or abuse of these drugs is questioned. On the basis of the proposed rationale for therapeutic and toxicologic drug monitoring and a review of the recently published clinical pharmacology literature, analgesics monitoring may be appropriate for the following proposed criteria:

1. To confirm or identify suspected drug toxicity in chronic use, therapeutic misadventure, or accidental or intentional acute overdose. Recommendations within this category may vary from drug to drug and are proposed as follows by agent:
   - A. Acetaminophen concentrations are recommended in the following situations:
     - suspected dose-related drug toxicity
     - acute overdose
     - chronic abuse
     - suspected patient noncompliance
     - change in liver or renal function
     - screening for acetaminophen as a co-ingestant (advised in all patients with intentional drug over dose)
   - B. Salicylate concentrations are recommended in the following situations:
     - suspected dose-related drug toxicity
     - acute overdose
     - chronic abuse
     - suspected noncompliance
     - change in renal function, mental status, acid-base status, or pulmonary status in patients using salicylates chronically
     - after the addition of a second drug that alters salicylate pharmacokinetics
     - screening as a co-ingestant after intentional drug overdose
   - C. Ibuprofen and naproxen concentrations are rarely indicated but could be considered in the following situations:
     - suspected noncompliance
     - change in renal or hepatic function in a patient using these medications chronically
   - D. Opioid monitoring, specifically for meperidine or propoxyphene/norpropoxyphene is recommended in the following situations:
     - suspected noncompliance
     - presence of symptoms suggestive of dose-related propoxyphene/norpropoxyphene toxicity (i.e., a change in mental status, seizures, cardiac arrhythmias, or electrocardiographic changes)
     - presence of symptoms suggestive of dose-related meperidine/normeperidine toxicity (i.e., myoclonic twitching, seizures, or changes in mental status or renal function)
     - opioid screening may be a useful adjunct to monitoring those patients enrolled in programs or undergoing workplace drugs-of-abuse testing

2. To aid in the identification of an unknown drug ingested in unknown quantities, drug identification and/or quantitation may be performed as an adjunct for patient management, in consultation with the clinical staff.

3. To monitor selected patient groups at greater risk for analgesic drug toxicity or drug-drug interaction, i.e., geriatric or alcoholic patients.

4. To confirm complete drug absorption and adequate drug elimination as an adjunct in drug overdose management.

Preanalytical Issues

To ensure sample stability and valid interpretation, attention should be given to criteria for acceptable specimens (i.e., serum, plasma, or others), free drug concentration, the anticoagulant used, collection device or tubes, and timing, including circadian rhythms.

Because the majority of analgesics-monitoring is for toxicity, serum or plasma would be acceptable specimens. However, for salicylates, use only red-topped, heparin, or EDTA tubes *(14)*. Table 1 shows sampling considerations and selected pharmacokinetic parameters, modified from the published data of Wilson *(15)* and Cannon et al. *(16)*. Therapeutic monitoring of analgesics requires steady-state trough samples. Samples and timing requirements in the overdose setting are dependent on the analgesic and time of ingestion. Generally, serum and/or urine are the samples of choice. Because of the clinical status of the overdosed patient, serum samples might precede urine samples. For urine collection, a noninvasive procedure is more "popular" for workplace or drug rehabilitation screening. Because the majority of the analgesics are excreted as urinary metabolites (see Table 1), urinary

Table 1. Sampling considerations and selected pharmacokinetic parameters for analgesics monitoring.[a]

Analgesics	Chirality	Sample	Half-life	Excreted in urine	Time to peak	Therapeutic dose	Therapeutic concentrations	Time of sampling
Salicylate (Acetylsalicylic acid)	NA[b]	Serum, plasma (heparin or EDTA), urine, saliva, synovial fluids	3–20 h (~ dose) (saturation kinetics) (10–30 min, ASA)	5% (SA)	1–2 h[c]	0.5–1 g[d] 5–6 g[f]	20–100 mg/L[d,e] 100–250 mg/L[f]	After acute OD: on presentation, then every 2 h until peak, and every 4–6 h thereafter
Diflunisal	NA	Serum or plasma	5–12 h	<10%	2–3 h	250–500 mg	50–200 mg/L	NA
Acetaminophen	NA	Serum/plasma (EDTA),[g] urine, CSF	1–3 h 3.2 h	<5%	0.5–1.0 h >4 h for OD[h] 4 h	0.5–1 g	10–20 mg/L >Plasma	4 h after a single acute ingestion; 4–8 h following ER OD; 2 levels needed, if co-ingested, to assure complete absorption
(*S*)-(+)-Ibuprofen (*R*)-(−)-Ibuprofen	Eutomer Distomer	Serum, plasma, urine	1–3 h	<10%	1–2 h	200–800 mg	15–30 mg/L	NA
(*S*)-(+)-Naproxen	Eutomer	Serum, plasma, urine	9–22 h	10%	1–4 h	275–1500 mg	50–100 mg/L	NA
(+/−)-Tramadol	?	Serum, plasma, urine	6.3 h	30%	2–3 h	50–400 mg	NE (300 μg/L, C_{max} for 100-mg dose)	NA
(+/−)-M1-tramadol metabolite[i]	?		7.4 h	<60%			NE (55μg/L, C_{max} for 100-mg dose)	

[a] Modified from Wilson *(15)* and Cannon et al. *(16)*.
[b] NA, not applicable; SA, salicylic acid; ASA, acetylsalicylic acid; OD, overdose; and NE, not established.
[c] Later in overdose, especially if drug concentrations occur.
[d] Analgesic.
[e] Antipyretic.
[f] Antiinflammatory.
[g] Connected with cyclosporine.
[h] Longer for Tylenol ER™ (extended release).
[i] Mono-*O*-desmethyltramadol.

screening may be more practical in some settings. Although there are no active metabolites for acetaminophen, salicylic acid, or ibuprofen *(17)*, meperidine and propoxyphene are hepatically metabolized to the toxic substances normeperidine and norpropoxyphene/dinorpropoxyphene, respectively.

Timing of the samples should be, if the time of ingestion is known, postabsorptive after the peak concentration for acetaminophen. Refer to the Rumack nomogram *(18)*. Because toxic ingestion of acetaminophen could produce delays in peak concentrations of up to 4 h, sampling should be performed 4 h postingestion if the ingestion time is known. If the ingestion time is unknown, collect the sample and perform the assay as soon as possible. Serial monitoring may be necessary to ensure that either the initial or the subsequent samples would have been collected at the postabsorptive phase *(19)*. Although timing of salicylate concentrations after overdose was previously recommended in the postabsorptive phase or ~6 h postingestion, concentrations drawn on presentation and then every 2–4 h allow for a more proactive approach toward treatment.

The use of gel tubes for analgesics monitoring has not been systematically substantiated, and recently a major manufacturer advised against its use for drug monitoring in general. Furthermore, the use of gel tubes is contraindicated for basic drugs such as tricyclic antidepressants. Because tramadol is a basic drug with a tertiary amine group similar to those of tricyclic antidepressants, it would be advisable for the laboratory to establish the clinical efficacy of gel tubes for tramadol monitoring. A recent in vitro study showed that ibuprofen piconol hydrolysis to ibuprofen is highly dependent on tube anticoagulants, with hydrolysis half-lives ranging from 2.5 h without anticoagulant, 8 h with citrate, and 15.5 h with heparin, to 162 h with EDTA *(20)*. This might imply that for a "hypothetical" patient medicated with (*RS*)-(+/−)-ibuprofen piconol, with blood collection using EDTA shortly thereafter, the pro-drug (*RS*)-(+/−)-ibuprofen piconol might still be detectable. This would require its differentiation from ibuprofen. It would be advisable for the laboratory to establish the clinical efficacy of various tube anticoagulants. Most of the analgesics are administered orally. If an analgesic is administered intravenously, venipuncture should be performed using the median cubital vein from the opposite arm.

Collected specimens such as serum/plasma and urine, when refrigerated, have been shown to be suitable for analysis for a length of time ranging from several hours to the usual storage time of up to 2 weeks. There are no systematic studies, such as those published for immunosuppressants, on the effects of long-term storage at room temperature on analgesic concentrations.

Apart from serum/plasma and urine, other samples, including saliva, synovial fluid, and cerebrospinal fluid (CSF) have been used for monitoring acetaminophen, and saliva and synovial fluid for monitoring some NSAIDs. Salivary acetaminophen concentrations show linear pharmacokinetics for doses of 18 mg/kg of body weight *(21)*. Acetaminophen serum and saliva concentrations were significantly correlated, but the agreement of limits and mean values were poor *(22)*. For children with juvenile chronic arthritis or chronic liver disease, saliva provides meaningful pharmacokinetic data for acetaminophen *(23)*. For routine TDM and toxicology, CSF is seldom appropriate for analgesic monitoring. However, a recent study shows that analgesia may be correlated with CSF acetaminophen concentrations *(24)*. Propacetamol, a prodrug hydrolyzed to acetaminophen with $t_{½}$ of 7 min, was administered intravenously to 43 patients with nerve-root compression pain. CSF drug concentrations peaked at 4 h compared ~2 h for plasma. Thereafter, CSF drug concentrations were higher than the plasma concentrations. The estimated $t_{½}$ for plasma and CSF were 2.4 and 3.2 h, respectively.

Generally, circadian rhythm is not an issue for monitoring analgesics. However, a recent study showed that after a single AM 1.5-g dose of acetaminophen, the urine contained twice the amount of acetaminophen glucuronide than that collected after a single PM dose *(25, 26)*. This temporal variation may be caused by a decreased absorption rate of acetaminophen. Circadian rhythms may have clinical implications in the use of aspirin and other NSAIDs. Diurnal variations of (*S*)-(+)-naproxen after oral administration of a PM dose of 500 mg of (*S*)-(+)-naproxen to 12 healthy males showed delayed peak serum concentrations compared with the same AM dose *(27)*. This may necessitate dosage adjustment for arthritic patients. Ingestion of aspirin at different times of the day led to significantly different peak salicylate concentrations and half-lives in healthy volunteers *(28)*. Circadian variations in peak plasma concentrations of indomethacin *(29)* and ketoprofen *(30)* have been noted and may impact the tolerance to and analgesic effectiveness of indomethacin *(31)*.

When used as an adjunct in management of the acutely poisoned patient, the reliability of a single analgesic measurement is poor. Utilization of a single drug concentration is especially problematic in overdoses involving sustained-release or enteric-coated products. Serial monitoring is suggested to ascertain adequate drug elimination and to rule out ongoing drug absorption. Formerly, half-life calculations in acute acetaminophen overdose settings were felt to be predictive of toxicity and were used when the exact time of ingestion was unknown *(1)*. More recent data suggests, however, that a prolonged acetaminophen half-life does not reliably correlate with hepatic or renal toxicity *(32)*. The utility of a single drug concentration in patients therapeutically taking acetaminophen or salicylates is such that the physician could consider changing dosing on the basis of a single (increased) concentration.

Table 2. Proposed assay criteria for analgesics monitoring.

Analgesics	Method	Samples[a]	Qualitative	Quantitative	TAT[b]	Sensitivity	Precision, CV
Salicylate	Trinders	S/P/U	X		<1h		
(Acetylsalicylic acid)	Colorimetric	S/P/U		X	<1h	28 mg/L	
	Immunoassays	S/P/U		X	<1h	50 mg/L	5–10%
	HPLC, GC	All		X	1–4h		
Diflunisal	Trinders	S/P/U	X		<1h		
	TLC[c]	S/P/U	X		<1h		
	HPLC, GC	All		X	1–2h	50 mg/L	5–10%
Acetaminophen	TLC	U	X		<1h		
	HPLC	All		X	1h	10 mg/L	5–10%
	FPIA	All		X	<1h	10 mg/L	5–10%
(*RS*)-(+/−)-Ibuprofen	HPLC, GC	All		X	1–2h	15 mg/L	5–10%
	Chiral HPLC	All		X			
(*S*)-(+)-Naproxen	TLC	U	X		<1h		
	HPLC, GC	All		X	1–2h	50 mg/L	5–10%
	Chiral HPLC	All		X			
(+/−)-Tramadol	TLC	U	X		<1h		
	HPLC, GC	All		X	1–2h	25 μg/L[d]	20%[d]
	HPLC (REMEDi)	U	X		<1h		
	GC–MS	All	X	X	1–2h		

[a] CSF, saliva, serum (S), plasma (P), and urine (U).
[b] Turnaround time.
[c] TLC, thin-layer chromatography; and FPIA, fluorescence polarization immunoassay.
[d] Suggested–based on tricyclic antidepressant HPLC assays.

Analytical Issues

Analgesic and metabolite monitoring/screening may be achieved by simple color spot tests, colorimetric tests, enzymatic methods, immunoassays, thin layer chromatography, gas chromatography (GC), HPLC, and automated HPLC such as REMEDi. Table 2 shows the characteristics of these assays. The clinical efficacies should be verified by subscribing to survey programs.

The currently used colorimetric assays and immunoassays for acetaminophen and salicylate have provided satisfactory performance according to the survey results of the College of American Pathologists. Diflunisal, because of its structural similarity to salicylate, cross-reacts in colorimetric assays and immunoassays *(33)*. Percentages for the automated clinical analyzer colorimetric and the fluorescence polarization immunoassay are 61% and 230%, respectively. Other colorimetric tests would probably show similar interferences. Thus, in a recent study of two patients co-medicated with diflunisal and aspirin for rheumatoid arthritis, erroneous toxic concentrations were caused by diflunisal interference in the nonspecific salicylate assays *(34)*. The authors suggested that these assays should not be used and that HPLC should be used to differentiate salicylate toxicity. Diflunisal interference with salicylate may be detected and can be avoided ideally by drug information included in the test order form, and/or by checking the patient's medication records.

Generally, HPLC and GC offer quantitation of parent drugs and metabolites. The procedures usually require sample preparation either by protein precipitation or by extraction. Gas chromatography–mass spectrometry (GC-MS) may be readily used for screening and quantification of newer analgesics such as tramadol and metabolites. Chromatographic assays for acetaminophen and salicylate are well established, but they are seldom used except for reference purposes. However, for the other analgesics, chromatographic assays offer clinically efficacious screening and/or quantitation. With the advent of novel column technology, such as the restricted access media and chiral stationary phases; automated HPLC such as REMEDi, ASPEC/ASTED, Prep-Station™, and others; automation such as Prep-Station for GC and GC-MS; and the availability of chemometrics for controlling instrumentation and data processing, both achiral and chiral analgesic analyses may be readily performed *(35, 36)*. These would enhance the monitoring of newly introduced analgesics. For example, an HPLC assay for ibuprofen and its major metabolites in urine involved acidification and hexane-propanol extraction, followed by back-extraction by sodium bicarbonate and neutralization *(37)*. Analysis was performed by an initial isocratic mode, followed by an abrupt gradient. The detection limit for ibuprofen was ~2.5 mg/L in 100 mL of urine. Using GC-MS, the hydroxy- and carboxy-metabolites of ibuprofen, as well as the parent drug, may be detected for up to several days after a single 400-mg oral dose *(38)*. (*S*)-(+)-naproxen, (*S*)-(+)-6-*O*-desmethylnaproxen, and five conjugates were determined using HPLC with a silica column and cetyltrimethylammonium ions in the mobile phase *(39)*. Re-

cently, direct sample analysis using novel column technologies such as restricted access media have obviated the need for sample preparation *(36)*. Ibuprofen and (*S*)-(+)-naproxen in plasma were analyzed by direct injection into a restricted access medium–a column bonded with α_1-acid glycoprotein–which is a biocompatible external surface with the internal surface of the pores bonded with C_8 or C_{18} for hydrophobic interactions *(40)*. System pressure did not increase even after several hundred plasma samples were analyzed. Linear calibration was established.

Icteric samples, with increased bilirubin concentrations of 50–200 mg/L (5–20 mg/dL) showed a decrease of 10–50 mg/L of salicylate in some colorimetric assays. In this setting, other useful specimens such as saliva and synovial fluids for arthritic patients may be tested. These concentrations may correlate with the plasma free drug concentrations, thus serving as a useful guide to correlate with responses.

Free drugs are monitored using ultrafiltration or equilibrium dialysis of serum. CSF and synovial fluid have been used in some research studies. For the analysis of free drugs in synovial fluid or saliva, various methodologies have been published in the literature. When equilibrium dialysis was used, protein binding of free (*S*)-(+)-naproxen in plasma was independent of pH *(41, 42)*. When equilibrium dialysis was used in combination with HPLC, the coefficient of variation of this method was 7.4%. (*S*)-(+)-naproxen, ibuprofen, and diclofenac in plasma and synovial fluids were determined by HPLC for osteo-rheumatoid arthritis patients *(43)*. Salicylic acid in saliva was determined by solid-phase extraction, followed by HPLC–fluorescence detection *(44)*. For children with juvenile chronic arthritis, a noninvasive method of monitoring saliva salicylic acid was achieved by HPLC *(45)*. Chiral analysis for (*S*)-(+)-naproxen and (*S*)-(+)-6-*O*-desmethylnaproxen in biological fluids was achieved using an α_1-acid glycoprotein column *(46)*. More recently, a molecularly imprinted polymeric HPLC column was used for the chiral analysis of (*RS*)-(+/−)-naproxen *(47)*.

Table 3. Analgesic medications metabolized by cytochrome P450.

Medication class	Family	Medication class	Family
Acetaminophen	1A2, 2E1		
NSAIDs		Opioids	
Diclofenac	2C9 (probe)	Codeine	2D6
Ibuprofen	2C9	Methadone	3A4, 2D6
Mefenamic acid	2C9	Fentanyl	3A4
Naproxen	2C9, 1A2	Tramadol	2D6
Piroxicam	2C9	Hydrocodone	2D6
Lornoxicam	2C9	Oxycodone	2D6
Aceclofenac	2C9	Sufentanyl	3A4
Flurbiprofen	2C9	Dihydrocodeine	2D6
Enoxicam	2C9	Alfentanil	3A4
Pirprofen	2C9		

Practice Issues

DRUG INTERACTIONS

Both the laboratory scientist and the clinician should be aware of numerous drug interactions with the analgesics. Refer to Table 3 for CYP P450 metabolism of analgesics.

Acetaminophen. Although chronic ethanol abuse increases hepatotoxicity in those who ingest acetaminophen, *(48–52)* acute ethanol co-ingestion is hepatoprotective *(53–56)*. Diflunisal increases acetaminophen concentrations *(57)*. Acetaminophen increases the hypoprothrombinemic effect of anticoagulants *(58)*, and concomitant administration of isoniazid has produced hepatotoxicity *(59–61)*. Chronic use of anticonvulsants (phenytoin, carbamazepine, or phenobarbital) may predispose patients to hepatotoxicity, given the ability of these drugs to induce P450 2E1. In a study using saliva, halothane decreased the $t_{½}$ of acetaminophen from 2.1 to 0.96 h and increased the clearance rate from 8.7 to 17 mL · min · kg *(62)*. Thus, halothane may enhance the hepatic metabolism of acetaminophen. Metyrapone increases the $t_{½}$ of acetaminophen, prevents glucuronidation of acetaminophen, and may increase oxidation to the toxic metabolite *N*-acetyl-*p*-benzoquinonimine (NAPQI) *(63)*. Inhibitors of CYP P450-mediated oxidation, such as cimetidine, have not been effective antidotes after acetaminophen overdoses in humans.

Salicylates. The following section is modified from Dromgoole and Furst *(64)*. Antacids decrease the absorption of and enhance renal clearance of salicylates. Acetaminophen, metoprolol, and caffeine increase salicylate concentrations. Salicylates decrease acetazolamide secretion; potentiate the hypoprothrombinemic effects of anticoagulants; increase plasma concentrations of cyclic antidepressants; enhance the hypoglycemic effect of chlorpropamide/tolbutamide; decrease the antihypertensive effect of angiotensin-converting enzyme inhibitors and *B*-adrenergic blockers; increase or prolong methotrexate concentrations and half-life, leading to methotrexate toxicity; and may increase the area under the curve of numerous other NSAIDs. Carbonic anhydrase inhibitors increase the penetration of salicylates into the central nervous system (CNS); corticosteroids increase the renal clearance of salicylates; and indomethacin causes blockade of the irreversible acetylation of platelets by aspirin. (*S*)-(+)-naproxen, instead of high doses of aspirin, is suggested for patients treated with valproic acid. Patients taking high-dose aspirin therapy should be carefully monitored when corticosteroids are coadministered.

Ibuprofen and naproxen. Concomitant use of these NSAIDs with tacrolimus or triamterene may increase the risk of acute renal tubular necrosis *(65, 66)*, produce a loss of blood pressure control from antihypertensive agents *(67)* through decreased diuresis and natriuresis, increase digoxin and lithium concentrations *(68, 69)*, increase peri-

operative bleeding *(70)*, and enhance methotrexate-mediated myelosuppression and gastrointestinal toxicity *(71)*. Co-medication of NSAIDs with anticoagulants should be avoided. Probenecid may induce naproxen toxicity by interfering with its elimination *(72)*. Monitoring of the patient's clinical response and for drug toxicity would also be recommended when NSAIDs are used in combination with phenytoin, oral hypoglycemic agents, or aminoglycosides. For treatment of rheumatoid arthritis, cyclosporine A and a NSAID such as (*S*)-(+)-naproxen together would produce greater renal impairment than the individual agents, possibly due in part to renal vasoconstriction *(73)*. Monitoring of plasma NSAIDs and whole blood cyclosporine A, along with other renal function testing, such as the glomerular filtration rate, effective renal plasma flow, and serum creatinine, may be useful. Cimetidine, ranitidine, and famotidine decrease both $t_{½}$ β and α of (*S*)-(+)-naproxen *(74–77)*. Cimetidine decreased (*S*)-(+)-naproxen $t_{½}$ by 39–60% *(74)*. Thus, NSAIDs and antacids or cholestyramine should be given at different times.

Propoxyphene. Concomitant use of propoxyphene with other CNS depressants or cardiovascular agents may produce enhanced CNS or respiratory depression or cardiac arrhythmias. Ethanol coadministration, for example, is a major cause of drug-related death *(78, 79)*. Significant increases in carbamazepine concentrations have been noted and have produced moderate-to-severe neurotoxicity *(80–83)*. The use of warfarin with propoxyphene/acetaminophen combinations may enhance its hypoprothrombinemic effect *(84, 85)*. Toxic metabolite accumulation (norpropoxyphene) may occur when nephrotoxic drugs are coadministered.

Meperidine. The coadministration of other centrally acting drugs, such as tricyclic antidepressants or phenothiazines, may cause exaggerated sedation or respiratory depression. Drugs that produce increased serotonin neurotransmission, such as serotonin re-uptake inhibitors, monamine oxidase inhibitors, or tramadol, if administered concurrently, may produce a life-threatening "serotonin syndrome," composed of altered cognition, neuromuscular activity, and autonomic function *(86)*. Cimetidine may decrease the clearance of meperidine by up to 22% *(87)*.

TURNAROUND TIME

Another critical area facing the laboratory scientist is turnaround time. Stat turnaround (≤1 h) is required for acetaminophen concentration reporting. Non-stat concentrations are not appropriate, given the urgent need for antidote (*N*-acetylcysteine) administration in settings of both acute and chronic toxicity. With regard to salicylate concentrations, 1-h (stat) concentration reporting is recommended, given the potential urgency to begin treatment (alkalinization of the blood and urine or hemodialysis) in scenarios of both acute and chronic toxicity. Stat ibuprofen concentrations would potentially allow more rapid triage of the patient who has overdosed into a "low-risk for toxicity" category and hence allow for possible earlier discharge after acute overdose, but such concentrations are generally not available and have not been widely used or tested *(88)*. Meperidine/normeperidine concentrations would ideally be available on a stat basis, because hemodialysis could be considered in patients with impaired renal function and signs of severe neurotoxicity from this agent.

FREQUENCY

Very little data exist on which to base recommendations for frequency of monitoring of acetaminophen or salicylate concentrations during therapeutic use. Routine monitoring has been used for patients requiring high-dose salicylate or diflunisal therapy for rhematologic diseases. In fact, nomograms, albeit rarely used, have been developed for therapeutic salicylate monitoring *(89)*. Recent trends in lower dosing of salicylates, based on the observation that an average aspirin dose of only 2.665 g/day has an excellent safety profile, and is cost-effective *(90)* may obviate the need for routine monitoring of this agent. High-dose therapy may be replaced by new therapeutic dosing schedules based on symptomatic and disease-modifying antirheumatic drug therapy, rather than the previously established "antiinflammatory" doses. If changes are made to the regimen of patients on chronic salicylate therapy, it should be noted that a new steady-state is reached more than 1 week after the dosing change. Other useful monitoring parameters in the treatment of rheumatoid arthritis include the number of tender or swollen joints, visual analog scales, acute-phase reactants (erythrocyte sedimentation rate or C-reactive protein), duration of early morning stiffness, activity of daily living, and pain during movement and at rest *(91)*. Concentrations of other NSAIDs, such as ibuprofen or naproxen, are not monitored during routine therapy. Instead, clinical parameters in the patient with arthritis may be monitored, as noted above for salicylates *(92)*.

The frequency with which analgesic drug concentrations should be monitored in hospitalized or emergency department patients is delineated in the following discussion. After acute acetaminophen overdose, one stat concentration is to be obtained at 4 h after ingestion, if the exact time of ingestion in known. This allows for hepatotoxicity risk categorization via a Rumack nomogram plot *(18)*. This initial concentration is followed by one additional concentration every 2 h until a peak occurs if co-ingestants that would impair absorption or decrease gut motility are present (i.e., opioids or anticholinergic medications). Very little data is available with regard to the laboratory diagnosis and management of sustained-release acetaminophen (Tylenol ER) overdose. Currently, the manufacturer recommends that concentrations be obtained at both 4 and 8 h after an acute ingestion *(93)*. Both concentrations are plotted on the Rumack nomo-

gram *(18)*. If either concentration falls above the lower line, antidote therapy is warranted. Obtaining one additional concentration at the completion of antidote therapy to assure concentrations are nondetectable before stopping *N*-acetylcysteine administration may be considered, given the unknown but theoretical ability of this formulation to form bezoars or concretions.

The frequency of monitoring salicylate concentrations in hospitalized patients is as follows: a concentration should be obtained initially and then every 2 h after an acute overdose until a peak occurs, then every 4–6 h thereafter until concentrations are <200 mg/L (assuming unaffected acid-base and mental status).

Monitoring ibuprofen or naproxen concentrations after an overdose is generally not indicated. A nomogram purported to predict toxicity from ibuprofen has been developed *(88)*, but because concentrations are not readily available, this nomogram has not gained widespread popularity. No data with regard to naproxen concentrations in overdose exist.

ancillary clinical and laboratory monitoring

Ancillary clinical and laboratory monitoring is essential after acute overdose or suspected chronic toxicity from analgesic drugs. After acute or chronic acetaminophen overexposure, transaminases, creatinine, coagulation studies, bilirubin, and acid-base status are monitored closely. The mental status is monitored for onset of encephalopathy, which heralds a poor prognosis. Other signs of irreversible hepatotoxicity include a bilirubin concentration >40 mg/L (4 mg/dL), a creatinine concentration >33 mg/L (3.3 mg/dL), arterial pH <7.3, increased factor VIII to V concentrations, or a prothrombin time >1.8 × baseline *(94–96)*. With chronic therapeutic use, renal function and liver function testing may be necessary on a periodic but as yet undefined basis.

After an overdose or chronic overuse of salicylates, liver function, renal function, acid-base status, coagulation function, calcium, glucose, and electrolytes are monitored closely. Mental status and pulmonary status are also monitored, because aberrations would probably warrant hemodialysis. With chronic use, the onset of tinnitus or hearing loss is an unreliable marker of toxicity *(97)*. Symptoms of salicylism, which occur during chronic use (headache, confusion, tinnitus or hearing loss, nausea, vomiting, hyperpnea, or fever) warrant immediate plasma concentration, electrolyte, arterial blood gas, and renal function determinations. Otherwise, guidelines for the frequency of plasma concentration monitoring during chronic therapy have not been clearly established. Compliance with antiplatelet therapy may also be monitored with periodic platelet aggregation studies *(98)*.

After acute ingestion of >3 g of ibuprofen, renal function should be checked at baseline and repeated within 1–2 weeks *(88)*. For any symptomatic patient who has acutely overdosed on ibuprofen, arterial blood gas analysis should be considered, along with a baseline hemogram and renal function tests (regardless of the dose ingested). Although some some studies suggest that NSAIDs have a good safety profile *(99)*, ancillary monitoring at the initiation of ongoing therapy with ibuprofen or naproxen in healthy patients could be considered and includes an initial hemogram and fecal occult blood test within 3 months of starting the NSAID, then every 6–12 month thereafter. Patients at high risk for gastrointestinal bleeding should have the above performed within 1 month and every 3–6 months thereafter. Healthy patients should have an initial sodium, potassium, blood urea nitrogen, creatinine, and urinalysis within 3 months of initiating NSAID treatment, then every 6–12 months. For patients at high risk for nephrotoxicity, the same tests are recommended, but they should be repeated within 1–3 weeks of the initiation of therapy and then every 3–6 months thereafter. In healthy patients, an initial alanine aminotransferase analysis within 3 months of starting therapy is recommended and then every 6–12 months thereafter. Patients at high risk for hepatotoxicity should have alanine aminotransferase analysis within 1 month of therapy initiation and then every 3–6 months thereafter *(100)*.

For patients acutely or chronically abusing propoxyphene, both acetaminophen and salicylate concentrations should also be performed, because many formulations include these drugs. (See *Salicylates* and *Acetaminophen* above.) The patient on repeated doses of meperidine, particularly if orally administered, must be monitored closely for the onset of tremors, myoclonic jerking, or seizures indicative of normeperidine accumulation. Such accumulation occurs in the presence of renal dysfunction or in the settings of other medical conditions, such as malignancy or sickle cell anemia *(101)*.

Reporting Issues

The clinician may find additional information necessary for proper interpretation of drug concentrations. Refer to Tables 1 and 4 for selected pharmacokinetic and toxicokinetic parameters, modified from published data by Wilson *(15)*, and Cannon et al. *(16)*. Before the interpretation

Table 4. Selected toxicokinetic parameters for analgesics monitoring.[a]

Analgesics	Toxic dose	Toxic concentration
Salicylate (acetylsalicylic acid)	140 mg/kg (acute ingestion)	>300 mg/L[b]
Diflunisal	7.5 gm (adults)	800–1000 mg/L
Acetaminophen	140 mg/kg (acute ingestion)	150 mg/L[c]
(*RS*)-(+/−)-Ibuprofen	>3 g or 100 mg/kg	200–500 mg/L
(*S*)-(+)-Naproxen	Variable	200–400 mg/L

[a] Modified from Wilson *(15)* and Cannon et al. *(16)*

[b] Toxic salicylate concentrations are dependent on chronicity of use, volume status, arterial pH, time since ingestion, and formulation ingested.

[c] Toxic acetaminophen concentrations are dependent on time since ingestion [acute overdose (OD); see Rumack Nomogram] and chronicity of use.

of an isolated acetaminophen concentration, the following questions should be answered: (*a*) What time period has elapsed since the acute ingestion? (*b*) Are there co-ingestants that may alter gut motility, absorption, and the time to peak concentration? (*c*) Is the ingestion acute, chronic, or acute-on-chronic? (*d*) Is there a history of alcohol abuse, liver disease, malnutrition, or the use of medications that induce CYP1A2 or 2E1? Similarly, addressing the following questions will allow more meaningful interpretation of salicylate concentrations: (*a*) How much time has elapsed since the acute ingestion? (*b*) What formulation of salicylate was ingested, i.e., methyl salicylate, enteric coated, or effervescent? (*c*) Is the scenario one of acute overdose or chronic use or abuse? (*d*) What is the volume status and acid-base status of the patient? (*e*) Is the presence of co-ingestants, which may alter gut motility, suspected? (*f*) Is there substantial CNS, pulmonary, or renal dysfunction? When faced with the interpretation of opioid concentrations, the time since ingestion, chronicity of use, renal status of the patient, and tolerance to the medication should be considered. Additionally, synthetic opioids such as fentanyl, hydrocodone, or hydromorphone may not be reliably detected with common assays.

When critical (high) values for acetaminophen, salicylates, meperidine, propoxyphene, or norpropoxyphene are obtained by the laboratory in hospitalized patients or outpatients, the results should be called immediately to the treating physician. Results that indicate drug concentrations below the therapeutic range do not require special notification but may suggest noncompliance. Critical concentrations for acetaminophen are dependent on the time because ingestion, (see the Rumack nomogram) *(18)* and are dependent on the factors discussed above. Critical concentrations for salicylates are dependent on the chronicity of use. After acute salicylate overdose, volume status, arterial blood pH, time after ingestion, formulation ingested, estimated completeness of absorption, and potential effects of co-ingestants on gut motility are taken into consideration. These factors markedly limit the utility of the Done Nomogram *(102)*, which has been purported to predict toxicity after a single acute ingestion of salicylates in patients with unaffected renal function, volume, acid-base status, and without co-ingestants. This tool has more recently been reported to have poor predictive value *(19)*. Furthermore, the Done Nomogram should not be used to estimate toxicity associated with chronic overuse or for patients who have ingested liquid or enteric-coated forms of salicylate. Critical concentrations for ibuprofen are dependent on time after ingestion, and the Hall Nomogram *(88)* has been developed to predict toxicity in the overdose setting. This nomogram is of limited utility, however, because ibuprofen concentrations are generally not available within the relevant initial 4-h period after ingestion. Data regarding critical concentrations with outpatient therapeutic use of ibuprofen or naproxen are not available. Critical concentrations for opioids such as propoxyphene and normeperidine are not well defined, given the limited data availability in this area and the wide variation in tolerance to such medications. In general, patients ingesting high doses of propoxyphene demonstrated toxic effects at serum concentrations >1 mg/L (or 3 μmol/L) *(103)*. Meperidine toxicity is reported with serum concentrations in the range of 10–30 mg/L. Normeperidine CNS toxicity is reported with serum concentrations in the range of 450–800 μg/L *(104)*.

If the sample was collected before peak concentration, as shown by Table 1 or the nomograms, request another sample collection at the postabsorptive phase. Perform serial monitoring to ascertain adequate drug elimination.

Subtherapeutic values should be considered as a potential indication of patient noncompliance. Subtherapeutic values may also be the result of inappropriate early sample collection, drug interaction from induction by another drug metabolized by similar enzymes, and for salicylate, icteric samples.

Interpretive comments by the laboratory may be of use to clinicians. For example, it should be indicated that "normal" ranges do not apply in acetaminophen overdose or chronic overuse settings. Similarly, toxicity from salicylates may be present at reference values or below reference values with chronic use situations or in patients with altered volume or acid-base status. A reminder to the clinician interpreting opioid concentrations could point out that tolerance develops with chronic use. Finally, in the overdose or chronic overuse setting, a laboratory comment might point out that poor reliability is given to a single acetaminophen or salicylate concentration.

The length of time that medical information from a collected specimen remains relevant varies among analgesics. Toxicity from acetaminophen would be expected to peak within 72 h of acute ingestion, and information beyond that time frame would rarely be useful. It should be noted that stat, i.e., <1 h, turnaround times for acetaminophen are essential because the antidote, *N*-acetylcysteine, is universally effective if administered within 8 h of acute ingestion. With regard to ibuprofen overdose, all patients who become acutely ill do so within 4 h of ingestion, and estimation as to whether substantial toxicity is likely to occur will be made within that time frame. Because ibuprofen concentrations are rarely available on a stat basis, however, this markedly limits their clinical utility in the overdose setting.

Other Issues Relating to Special Populations, Free Drug Concentrations, and Dosing

CONSIDERATIONS IN PEDIATRICS AND PREGNANCY

Acetaminophen crosses the placenta, placing the fetus at risk for hepatotoxicity after maternal overdose. Therefore, cord-blood concentrations may be indicated, if the child is delivered, to determine the need for antidote therapy. Acetaminophen metabolism and elimination are unchanged in pregnancy *(105)*. No age-related differences are reported in adults vs children with regard to interpre-

tation of concentrations. The free fraction of salicylate increases to 12% during pregnancy *(106)*. Finally, salicylates may displace bilirubin from plasma proteins in neonates, producing kernicterus *(107)*.

CONSIDERATIONS IN ALCOHOLICS

Alcoholics ingesting "upper" therapeutic doses (2.5–4 g) of acetaminophen may sustain hepatic necrosis. This may occur, in part, because of the induction of hepatic P450 CYP2E1 by ethanol, thus increasing the formation of the toxic metabolite NAPQI. Glutathione stores in the liver and other susceptible tissues may be diminished in alcoholic patients, rendering them more susceptible to the oxidant effect of NAPQI *(108)*.

DOSING ISSUES

Although dosing for acetaminophen and salicylates *(16)* should be based on ideal body weight, dosing for ibuprofen should be based on actual or total body weight *(109)*. In geriatric patients, the free fraction of diflunisal, (*S*)-(+)-naproxen, and salicylate may increase by >50% *(110)*. Age, gender, and obesity impact salicylate pharmacokinetics, such that clearance of the free fraction of salicylate is lowered in elderly females; accumulation occurs, and lower dosages are necessary *(111)*. In a limited study of geriatric patients treated with 500 mg of naproxen twice daily, higher mean predose concentrations, areas under the curve, and reduced clearance were noted *(112)*. Although protein binding (99.8%), is about the same as for the younger group, the free concentration was significantly higher. In the absence of excessive side effects, a lower starting dose was recommended.

FREE DRUG CONCENTRATIONS

Because salicylate dose is increased or as concentrations rise above 400 mg/L (400 μg/mL), disproportionate increases in the concentration of unbound drug are seen, and the bound fraction decreases to 76% *(113–115)*. Although it has been suggested that free salicylate concentrations may correlate better with therapeutic effect, *(116)* the therapeutic range of unbound salicylate has not been established. Calculations may be used to correct total concentrations of the highly protein-bound salicylates in patients who are known to be hypoalbuminemic *(117)*. The following formula has been used:

$$Cp_{true} = Cp^1/[(1- \alpha)(p^1/p_n) + \alpha]$$

where:

α is the usual free fraction (0.16);
p^1 is the patient's albumin concentration;
p_n is the reference interval albumin (4.4); and
Cp^1 is the patient's plasma drug concentration.

Future Directions

POTENTIAL CLINICAL APPLICATIONS OF CHIRAL PHARMACOLOGY AND RACEMATE ANALYSIS

Chiral pharmacology of analgesics, as in other areas of clinical pharmacology, has demonstrated greater pharmacological activity of one enantiomer of a drug, such as (*S*)-(+)-ibuprofen, as shown in Table 1 *(15, 16)*. This may indicate the need to reexamine the correlation of plasma concentrations and response and suggests that there may be merit in monitoring the pharmacologically active enantiomers. For analgesics administered as racemates or as single enantiomers, the chiral (specific)/pharmacologically active enantiomers–eutomers–may exhibit "unique" pharmacokinetic and pharmacodynamic properties *(118–125)*. Furthermore, in vivo epimerization would change the concentration of the pharmacologically active enantiomer. Of the five commercially available R-aryl propionic-class NSAIDs, *rac*-ibuprofen and *rac*-fenoprofen undergo substantial epimerization in humans *(126)*. Furthermore, a recent animal study showed (*R*)-(−)-naproxen in vivo epimerization to (*S*)-(+)-naproxen *(46)*. For 46 patients with nerve-root compression pain, 800 mg of *rac*-ibuprofen was administered, and chiral analysis showed that plasma enantiomers peaked at ~5 h, earlier than those of CSF at 3 h *(127)*. After 90 min, the enantiomeric CSF concentrations were higher than those of plasma. The elimination $t_{½}$ values for plasma (*R*)-(−)- and (*S*)-(+)-ibuprofen were 1.7 and 2.5 h and were 3.9 and 7.9 h for CSF, respectively. A recent study showed that (*R*)-(−)-naproxen is not a stereoselective substrate of human orthologous UDP-glycuronosyltransferase *(128)*.

For 237 patients with osteoarthritis treated with (*S*)-(+)-naproxen, the free concentrations were not correlated to clinical efficacy and adverse effects *(41, 42)*. Female free concentrations were higher than those of male. Also for females, the unbound fraction was higher in older females. Furthermore, the (*S*)-enantiomers of NSAIDs may account for the most of the antiinflammatory effect, and the binding of the (*S*)-enantiomer with proteins such as albumin may also differ from that of the other enantiomer. Thus, the antiinflammatory effect of NSAIDs may correlate better with the free fraction of the (*S*)-enantiomer in synovial fluid–the site of action *(129)*.

NEW ANALGESIC

Tramadol is an atypical, binary analgesic possessing both opioid and nonopioid characteristics *(130)*. It binds to the mu receptors for opioids and blocks norepinephrine and serotonin re-uptake. Tramadol is metabolized in the liver by CYP2D6 to a major metabolite, mono-*O*-desmethyl metabolite (M1), and other N-demethylated metabolites and undergoes additional glucuronidation and sulfation. Tramadol concentrations in renal patients may be increased because of impaired renal excretion. Inducers of CYP2D6, such as carbamazepine and rifampin, lower the elimination $t_{½}$ of tramadol. After absorption, enantiomers of both tramadol and M1 have parallel time courses. M1 binds more strongly than tramadol to the opioid receptors and accounts for more of the analgesic activity. Animal studies showed that M1, as an analgesic, is ~6 × more active than its parent drug, tramadol. (+/−)-Tramadol and its major metabolite, M1, are racemates. However,

there is a <10% difference in the enantiomeric concentrations. Currently, there is a lack of data showing the correlation of plasma concentration of tramadol with analgesic response. Thus, tramadol monitoring awaits further study.

Conclusion

In summary, although the role of TDM for analgesics is controversial and not clearly defined, guidelines for the identification of suspected drug toxicity with chronic use, therapeutic misadventure, and accidental or intentional acute overdose have been proposed. The need for monitoring in selected patient groups at greater risk for analgesic drug toxicity or drug-drug interaction, for confirmation of complete drug absorption and adequate drug elimination as an adjunct in drug overdose management, and the use of screening when compliance or abuse of these drugs is questioned have been reviewed.

Future areas of importance include the potential clinical application of chiral pharmacology and definition of the need for monitoring of newer analgesics such as tramadol.

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