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PARACETAMOL: MECHANISM OF ACTION, APPLICATIONS AND SAFETY CONCERN

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Abstract: Paracetamol / acetaminophen is one of the most popular and most commonly used analgesic and antipyretic drugs around the world, available without a prescription, both in mono- and multi-component preparations. It is the drug of choice in patients that cannot be treated with non-steroidal anti-inflammatory drugs (NSAID), such as people with bronchial asthma, peptic ulcer disease, hemophilia, salicylate-sensitized people, children under 12 years of age, pregnant or breastfeeding women. It is recommended as a first-line treatment of pain associated with osteoarthritis. The mechanism of action is complex and includes the effects of both the peripheral (COX inhibition), and central (COX, serotonergic descending neuronal pathway, L-arginine/NO pathway, cannabinoid system) antinociception processes and "redox" mechanism. Paracetamol is well tolerated drug and produces few side effects from the gastrointestinal tract, however, despite that, every year, has seen a steadily increasing number of registered cases of paracetamol-induced liver intoxication all over the world. Given the growing problem of the safety of acetaminophen is questioned the validity of the sale of the drug without a prescription. This work, in conjunction with the latest reports on the mechanism of action of paracetamol, trying to point out that it is not a panacea devoid of side effects, and indeed, especially when is taken regularly and in large doses (> 4 g/day), there is a risk of serious side effects.

Keywords: paracetamol, acetaminophen, toxic effects, mechanism of action, cyclooxygenase, cannabinoid, serotonergic, prostaglandin-endoperoxide synthases

Paracetamol (an international name used in Europe) and **acetaminophen** (an international name used in the USA) are two official names of the same chemical compound derived from its chemical name: N-a**cet**yl-**para**-**am**inophen**ol** (the segment ''cet'' inserted between ''para'' and ''amino'') and N-**acet**yl-para-**aminophen**ol. This drug has a long history and, as it often happens with important discoveries, it was found by chance. In the 80s of the 19th century, two young doctors at the University of Strasburg, in order to eradicate worms by mistake dispensed acetanilide to a patient instead of naphthalene (Fig. 1). They noticed that the drug had a small impact on intestinal parasites, however, it significantly decreased high temperature. Young doctors - Arnold Chan and Paul Heppa - quickly published their discovery and acetanilide was introduced into medical practice in 1886 under the name of antifebrin (1). Soon it appeared that although the production of this drug was very cheap, acetanilide

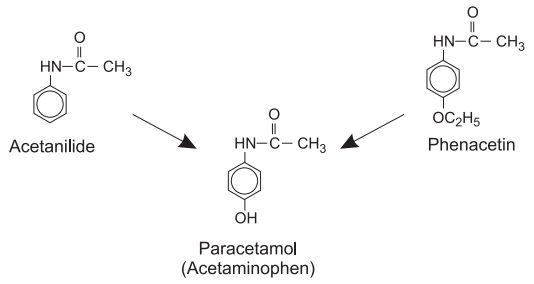

Figure 1. Chemical structure of analgesics - aniline derivatives. Phenacetin until the 80s of the 20th century was included in the composition of numerous mixtures. Saridon (Roche firm) and the so-called in Polish "tablets with cross" produced by Polpharma SA in Starogard Gdański (previously Starogardzkie Zakłady Farmaceutyczne Polfa) and Marcmed from Lublin are the most well-known preparations. Due to its carcinogenic action damaging the kidneys and the liver as well as the patients' tendency towards an overuse, the drug was withdrawn from the American market in 1983 (in Saridon, phenacetin was replaced by paracetamol). In Poland, it happened as late as in 2004

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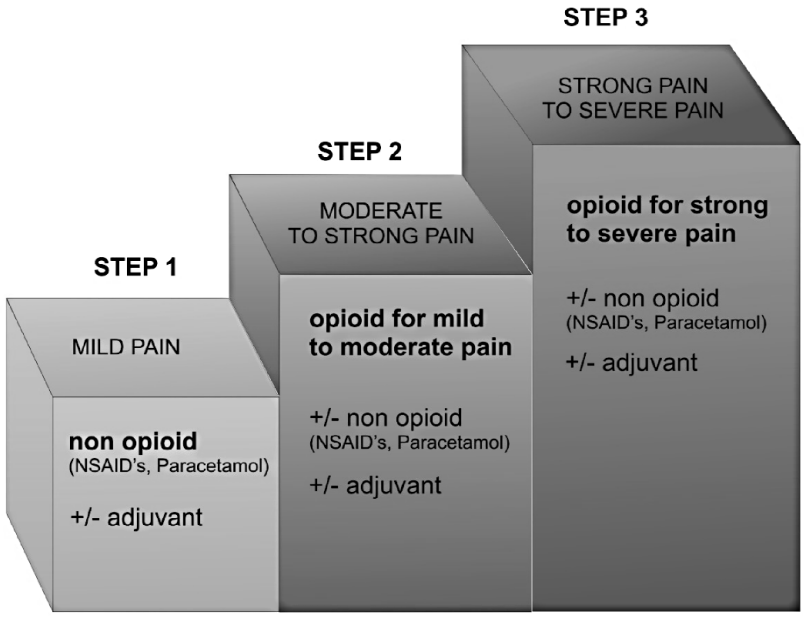

Figure 2. Paracetamol on the WHO analgesic ladder (the rules for using analgesics, which consider individual intensity of pain).

could not be used as an antipyretic medicament due to its high toxicity, the most alarming of which was methemoglobinemia. This resulted in a great deal of research on less toxic derivatives of acetanilide. Phenacetin and N-acetyl-p-aminophenol appeared to be the most satisfying compounds, which had been earlier synthesized by Harmon Northrop Morse in 1878 (Fig. 1) (2). The first clinical trials with those two acetanilide derivatives were performed by a German pharmacologist Joseph von Mering. On the basis of the obtained results, a faulty conclusion was drawn that paracetamol was characterized by high toxicity similar to acetanilide, therefore phenacetin was the first derivative to be introduced into medical practice in 1887. Phenacetin was widely used in analgesic mixtures until the time when it was associated with the development of analgesic nephropathy after a prolonged usage (3). In Poland, phenacetin was used as a component of very popular and available everywhere analgesic ''tablets with the cross''. In fact, acetaminophen/paracetamol became popular half a year later in 1948 when Bernard Brodie and Julius Axelrod demonstrated that paracetamol was the main active metabolite of acetanilide and phenacetin responsible for their analgesic and antipyretic action and that methemoglobinemia was induced by another metabolite, phenylhydroxylamine (4). That discovery revolutionized the pharmaceutical market of analgesic drugs and since then paracetamol has started its staggering career.

The use of paracetamol

Paracetamol was introduced into the pharmacological market in 1955 by McNeil Laboratories as a prescribed analgesic and antipyretic drug for children under its trade name *Tylenol Children's Elixir* (the name *tylenol* derives from its chemical name – N-ace**tyl**-p-aminoph**enol**). One year later, 500-mg tablets of paracetamol were available over the counter in Great Britain under the trade name of *Panadol,* which were produced by Frederick Stearns & Co, the branch of Sterling Drug Inc. In Poland, paracetamol became available in 1961 and since then it has belonged to the one of the most frequently sold analgesic medications. There are about a 100 preparations in the trade offer, which contain paracetamol alone or in combination with other active substances.

The paracetamol place on the WHO analgesic ladder, which precisely defines the rules for application of analgesic drugs, is impressive. This drug has been placed on all three steps of pain treatment intensity. In different pains of moderate intensity, paracetamol as a weak analgesic together with nonsteroidal analgesic drugs or coanalgesics (e.g., caffeine) is a basic non-opioid analgesic (the first step of the analgesic ladder). When pain maintains or increases, paracetamol is used as an additional analgesic with weak (e.g., caffeine, tramadol) or strong (e.g., morphine, phentanyl) opioids from the second and third step of the analgesic ladder, respectively,

Fig. 2). Paracetamol, if efficient, is a recommended oral analgesic of a first choice to be used for a long time, e.g., in symptomatic treatment of slight and moderate pain occurring in osteoarthritis as well as in muscle or tendon pains. Moreover, it is a drug of choice in patients in whom application of non-steroidal anti-inflammatory drugs (NSAIDs) are contraindicated, e.g., in the case of gastric ulcers, hypersensitivity to aspirin, impairments in blood coagulation, in pregnant women, nursing mothers and children with fever accompanying a disease (5). The use of paracetamol in children requires special care and maintain in an adequate dosage (based on age), which significantly differs from standard adult. The recommended dosage for children consider the metabolism of paracetamol, which determines the toxicity of the drug, especially hepatotoxicity (see below). In children, paracetamol metabolism changes with age: in younger children the sulfation pathway is dominated route of paracetamol elimination (which is mature at birth); the glucuronidation pathway takes about two years to mature. The oxidation of paracetamol, which takes place mainly with the participation of the enzyme CYP2E1 in neonates is negligible, because the activity of CYP2E1 increases with age, reaching the adult value at age 1-10 years. For comparison, in adults, paracetamol is metabolized mainly in the liver *via* glucuronidation (50-60%), sulfation (25-30%) and oxidation (< 10%) (see below in the section on adverse effects). Therefore, according to Ji et al. (6), the proposed dosage of paracetamol in children up to 12 years is as follows:

- under 2 years – no recommended dose; treatment under the supervision of a physician;
- 2-3 years – 160 mg (daily dose divided into two dose units, i.e., 2 × 80 mg); total dose corresponds to 1/2 of a single dose for an adult, i.e., 325 mg;
- 4-6 years – 240 mg (daily dose divided into three dose units, i.e., 3 × 80 mg); total dose corresponds to 3/4 of a single dose for an adult;
- 6-9 years – 320 mg (daily dose divided into four dose units, i.e., 4 × 80 mg); total dose is the same as a single dose for an adult;
- 9-11 years – 320-400 mg (daily dose divided into four-five dose units, i.e., 4-5 × 80 mg; total dose corresponds to 1-1 1/4 of a single dose for an adult;
- 11-12 years – 320-480 mg (daily dose divided in the four-six dose units, i.e 4-6 × 80 mg; total dose corresponds to 1 – 1 1/2 of a single dose for an adult.

According to the 20th edition of Drugs of Contemporary Therapy (Polish), the acetaminophen dosage schedules in pediatric patients should be as follows: 10-15 mg/kg oral dose and 15-20 mg/kg rectal dose every 4-6 h, maximum of 5 doses/day; in newborns orally or rectally 10 mg/kg of body weight every 4 h or 15 mg/kg every 6 h (maximum daily dose in newborns is 60 mg/kg).

Mechanism of action

Although paracetamol was discovered over 100 years ago and has been widely used in medical practice for more than half the century, its mechanism of action has not been elucidated until now (7). It has analgesic and antipyretic properties similarly to NSAIDs, but contrary to them, it does not possess any anti-inflammatory activity. When applied in recommended doses, it does not induce typical for NSAIDs gastrointestinal side effects. However, it suppresses prostaglandin production likewise NSAIDs.

Due to lack of an anti-inflammatory component, paracetamol has not been regarded as a member of the NSAIDs family in pharmacological textbooks, although what is interesting, it has been always discussed together with these drugs. Therefore, the discussion on the mechanism of action of paracetamol should begin from the analysis of NSAIDs action.

All conventional NSAIDs inhibit the convertion of arachidonic acid (AA) into prostaglandin H - PGH_2. The stage is catalyzed by prostaglandin H synthase (PGHS), at present referred to as cyclooxygenase (COX) within which isoenzymes COX-1 (PGHS-1) and COX-2 (PGHS-2) occur (8). The prevalence and the role of the third isoenzyme COX-3 is the subject of ongoing to date discussions (read further). PGHS is a bifunctional enzyme and possesses two different enzymatic activities: cyclooxygenase and peroxidase (POX). The conversion of AA→PGH_2 involves two reactions: cyclization of AA to unstable 15-hydroxyperoxide (PGG_2) with the involvement of a cyclooxygenase component and double oxidation in position 9 and 11; whereas the reduction of PGG_2 molecule to its 15-hydroxy analogue, unstable structure of PGH_2, takes place due to peroxidase activity of PGHS (POX).

Prostaglandin H_2 (PGH2) is a substrate for specific synthases, tissue-dependent isomerases catalysing its further conversions into different endogenous regulators, namely: prostaglandins of the D (PGD_2), E (PGE_2), F (PGF_2) series and prostacyclin (PGI_2; prostacyclin is not a prostaglandin and a commonly used abbreviation is historically conditioned) and thromboxanes (TXA_2 and TXB_2). They all are characterized by different biological activity and

many of them have anti-inflammatory properties. Thus, the action of NSAIDs, which inhibits the stage of conversion AA→PGH_2, and also the formation of the aforementioned regulators, have some favorable (anti-inflammatory, analgesic and antipyretic) and side effects (associated with the inhibition of synthesis of particular regulators in different tissues). A precise mechanism of NSAID action together with therapeutic and side effects has been presented in the recently published large study by Nowak and Dzielska-Olczak (9) and Nowak (10, 11).

While traditional NSAIDs and selective COX2 inhibitors inhibit cyclooxygenase (PGHS) through competing with arachidonic acid for the active site of the enzyme (12), paracetamol is likely to act as a factor reducing a ferryl protoporphyrin IX radical cation (Fe^{4+}=$OPP^{\bullet+}$) within the peroxidase site of the PGHS enzyme. In turn, the Fe^{4+}=$OPP^{\bullet+}$ generates tyrosine radicals in the place of PGHS cyclooxygenase, which are essential for catalyzation of AA oxidation reaction (12-16) (Fig. 3). Due to a fact that hydroperoxides of fatty acids, like PGG_2 (reduced by POX), oxidize porphyrin within the peroxidase site of the enzyme, cyclooxygenase inhibition by paracetamol is difficult in the presence of high peroxide levels. Graham and Scott suggested that paracetamol should be classified to the group of the so-called atypical NSAIDs, determined as peroxide sensitive analgesic and antipyretic drugs (PSAAD) (17).

For the last decades, it was thought that paracetamol reveals analgesic and antipyretic properties by acting centrally and its inhibitory effect on COX-1 and COX-2 activity, i.e., prostaglandin synthesis was low. This concept was based on the original research carried out by Vane and colleagues, which was published at the beginning of the 70s of the previous century. Those authors observed that paracetamol decreased prostaglandin synthesis ten times stronger in the brain than in the spleen (18).[1]

At that time COX isoforms were not known because isoenzyme, COX-2, was identified only at the beginning of the 90s of the previous century (25, 26). Ten years later, the experiments performed on the dog's brain tissue revealed the presence of the third COX isoform, COX-3, which demonstrated special sensitivity to paracetamol (27). However, it soon appeared that so sensitive to paracetamol COX-3 does not function in the human organism. The human analogue of dog's COX-3, which occurs in some tissues especially of the central nervous system, is an alternative splice variant of COX-1 without a preferential sensitivity to paracetamol, encoding proteins of amino acid sequence different from COX and not exhibiting COX activity (28-30). Thus, COX-3 involvement in the mechanism of action of paracetamol in humans has not been justified, which has been confirmed by Kis et al. as well as by Hinz and Brune (15, 29). However, the discussions regarding a potential role of identified three COX isoenzymes in the mechanism of paracetamol action are still being continued (31-34).

The concept regarding COX-dependent central mechanism of paracetamol action has not stood the test of time (29). Firstly, the studies by Graham and Scott have shown that paracetamol really inhibited prostaglandin synthesis in well-functioning cells, however, it did not exert the same effect in the tissue/cell homogenate, where the concentration of arachidonic acid is low (35). Secondly, paracetamol has been found to have an inhibitory impact on COX-1 and COX-2 activity in peripheral tissues, although not to the same extent, since a stronger effect was always observed in relation to COX-2, especially in the cells of the vascular endothelium.

[1]In numerous academic textbooks including those published during the last decade, the central mechanism of paracetamol action has been discussed emphasizing its weaker inhibitory effect on the cyclooxygenase activity and prostaglandin production as compared to NSAIDs. The early study by Flower and Vane from 1972 in the prestige magazine *Nature* announced the mechanism of paracetamol activity even in its title: "*Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol)*" (18). Scientific prestige of the future Nobel prize winner, John R. Vane, was so high that despite later published articles, which did not completely confirm the original results of the British researchers (19-21) that study was still citied and its results were considered the substantial basis of the mechanism of paracetamol action for many pharmacologists and doctors.

Flower and Vane indicated that prostaglandin production in the brain was 10-fold more sensitive to paracetamol action than in the spleen (18). At that time, John R. Vane, the future Noble Prize winner in physiology and medicine (John R. Vane, Sune K. Bergstrom and Bengt I. Samuelsson – "Nobel Prize" in 1982 for discoveries on prostaglandin and related biologically active substances) was the author of many other essential for medicine innovative observations that were published in prestige magazines, e.g. *"Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs*" (22). John Vane, using a guinea pig lung homogenate in his study, concluded that analgesic, antipyretic and anti-inflammatory action of aspirin, indomethacin and salicylate is associated with a lower prostaglandin production resulting from cyclooxygenase inhibition (COX). Other articles published in the same magazine *Nature* by Vane et al.: "*Indomethacin and aspirin abolish prostaglandin release from spleen*" and by Smith and Willis: "*Aspirin selectively inhibits prostaglandin production in human platelets*" contained the results confirming those observations (23, 24). It is worth remembering that COX isoenzymes were not discovered at that time.

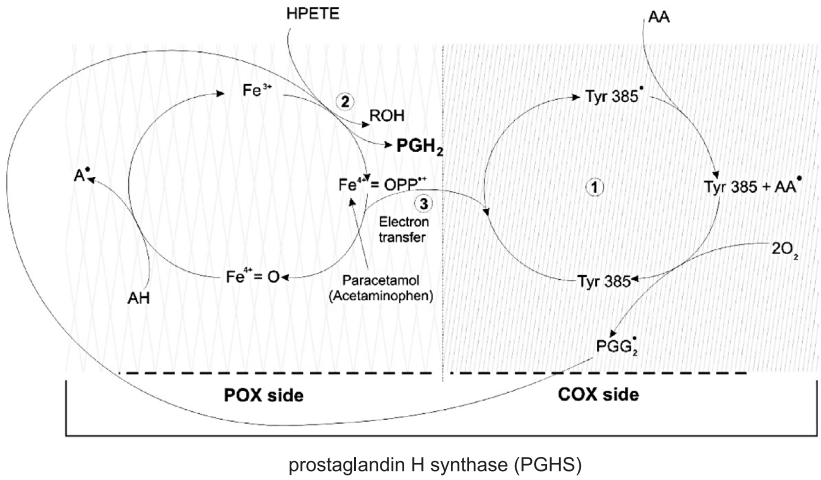

Figure 3. The complex of prostaglandin H synthase (PGHS) including two components: cyclooxygenase (COX) and hydroperoxidase (POX) is a bifunctional enzyme, responsible for the metabolism of arachidonic acid (AA) to prostaglandin PGH_2. The reaction occurs *via* two stages: **1.** AA oxidation to PGG_2 depends on tyrosine radical ($Tyr385^{\bullet}$) in the COX site. **2.** PGG_2 undergoes reduction to PGH_2 in the POX site, which results in the oxidation of the peroxidase heme radical. **3.** A formed ferryl protoporphyrin IX radical cation (Fe^{4+}=$OPP^{\bullet+}$) generates $Tyr385^{\bullet}$ radicals. Thus, the POX part is "self-sufficient", whereas COX depends on POX. Paracetamol reduces an iron cation in protoporphyrin IX radical (Fe^{4+}=$OPP^{\bullet+}$) in the POX part, which contributes to a lower amount of $Tyr385^{\bullet}$ radical formation. Abbreviations: AA – arachidonic acid; $AA^{\bullet}$ – arachidonic acid radical; $A^{\bullet}$ - oxidized cosubstrate; AH – reduced cosubstrate; Fe^{3+} - enzyme at rest; Fe^{4+}=O – protoporphyrin IX (heme); Fe^{4+}=$OPP^{\bullet+}$ - protoporphyrin radical IX; HPETE – hydroperoxides of fatty acids; $PGG_2^{\bullet}$ - prostaglandin G_2 containing peroxide radical; PGH_2 – prostaglandin H; ROH – alcohol; $Tyr385^{\bullet}$ – tyrosine radical (12, 16, 38).

Hinz et al. indicated that orally administered paracetamol at a dose of 1 g inhibited 80% of the COX-2 activity in human blood monocytes (36). The results of extensive studies by Hinz and Brune published in the years 2006-2012 reveal that paracetamol is a preferential inhibitor of COX-2 isoenzyme, however, its effect depends to a great extent on the state of environmental oxidation/reduction (redox) (15, 37).

Among other possibilities of the central action of paracetamol, its stimulating effect on descending serotoninergic pathways, which are involved in inhibition of pain sensations has been discussed. This theory has been confirmed by *in vivo* studies on animals as well as on humans. Alloui et al. carried out the study on analgesic and anti-inflammatory action of paracetamol in rats which were given caragenin. No anti-inflammatory effect of paracetamol was observed, however, central antinociceptive effect of this drug with the involvement of the $5\text{-}HT_3$ subtype of serotonin receptors was detected (38). The study on healthy volunteers in whom the pain was induced through electrical stimulation of the median nerve showed that analgesic action of paracetamol was completely blocked in the group of subjects treated with paracetamol combined with tropisetron or granisetron ($5\text{-}HT_3$ receptor antagonists) (39, 40). Data concerning central action of paracetamol through its effect on descending serotoninergic pathways do not exclude a hypothesis assuming the presence (or coexistence) of the inhibition of prostaglandin synthesis (35). Prostaglandin PGE_2 modulates numerous physiological processes and can also modulate nociceptive and autonomic processes *via* its influence on descending serotoninergic antinociceptive system (41).

Novel studies on the mechanism of action of paracetamol regard it as a pro-drug, which due to its active metabolites demonstrates an association with the endocannabinoid system. It has been observed that in mouse brain and spinal cord, paracetamol is subject to deacetylation to p-aminophenol that in turn reacts with arachidonic acid affected by fatty acid amide hydrolase (FAAH), resulting in the formation of an active metabolite of the drug, the fatty acid amide N-arachidonoylphenolamine (AM404) (42, 43). AM404 does not act directly on cannabinoid receptors, however, it increases activity of endocannabinoid system in an indirect way (44). On one hand, this compound is a strong activator of the vanilloid receptor subtype 1 (TRPV1), being a ligand of receptors for cannabinoids CB_1, and on the other hand, it leads to an increase in the endogenous pool of these compounds as an inhibitor of the

endogenous cannabinoid (anandamide) reuptake (45). Endogenous cannabinoids, e.g., anandamide, act antinociceptively both at the level of the spinal cord as well as the brain. The study on rats performed by Bertolini et al. presented that an earlier administration of the CB_1 receptor inhibited AM404 activity and completely blocked analgesic action of paracetamol in the animals (46). Moreover, cannabinoids considerably lower body temperature through the activation of CB_1 receptors in the pre-optic area (47). It has been known that analgesic derivatives of aniline have a similar action as cannabinoids, such as mood improvement, psychic relaxation and sedation. Such properties have not been observed so far in the case of paracetamol, although some authors ascribe poor sedative properties to it (29, 48). Furthermore, different concentrations of AM404 have been found to inhibit COX-1 and COX-2 enzymes. This mechanism may be important especially in such areas of the brain in which a high concentration of FAAH enzyme can be observed, e.g., in the mesencephalic trigeminal nucleus, primary sensory neurons. In these areas of the brain an increased production of the active metabolite AM404 can be found, and this in turn may to a certain degree explain the inhibitory action of paracetamol towards cyclooxygenases in the CNS (46).

Inhibition of nitrogen oxide (NO) formation might be also an alternative mechanism of analgesic action of paracetamol. The L-arginine/NO pathway activated by substance P and NMDA receptors leads to NO synthesis, which is an important neurotransmitter in the nociceptive processes of the spinal cord (49, 50).

Summing up, paracetamol acts at all levels of pain stimulus conduction from the tissue receptors through the spinal cord to the thalamus and the cerebral cortex in which pain sensations are evoked. The mechanism of analgesic action of paracetamol is complex. The following possibilities are still taken

Table 1. Advantages and disadvantages of paracetamol therapy.

Advantages (when the drug is administered in the recommended therapeutic doses max. 4 g/24 h)
wide therapeutic application
checked and examined
well tolerated
good bioavailability after oral administration ($t_{1/2}$ 2h)
fast elimination
cheep
a small number of interactions with other drugs
low toxicity at low doses (≤ 2 g / d) to the digestive tract and kidneys
low toxicity in children
rare side effects (main allergic skin reactions)
available in different pharmaceutical forms
Disadvantages
metabolized to a toxic metabolite (N-acetyl-p-benzoquinone imine)
therapeutic index (often not efficient at a low dose)
long-term application may cause: • renal functioning disorder • higher blood pressure • increased prevalence of heart infarction
low therapeutic efficiency • analgesic action at a dose of 1 g administered 2, 3, and 4 times a day • low anti-inflammatory action
hepatotoxicity • increased aminotransferase activity at therapeutic doses • hepatic failure in the case of overuse (two-fold overuse of a therapeutic dose) • enhanced previous liver damage caused by alcohol consumption • combinations with traditional NSAIDs can result in a higher prevalence of digestive tract ulceration

into consideration: affecting both peripheral (inhibition of COX activity) and central (COX, descending serotoninergic pathways, L-arginine/NO pathway, cannabinoid system) antinociceptive processes as well as the redox mechanism (51). The studies on the mechanism of paracetamol action require further verification - they should concern not only the therapeutic action of this drug but also more frequently reported poisoning, especially strong hepatotoxicity resulting from the drug overdose since numerous preparations containing paracetamol are available without a prescription.

Paracetamol on the pharmaceutical market

Paracetamol is available on the market under different trade names in simple (sold over the counter) or more complex preparations combined with an additional active substance obtainable only by prescription (with tramadol) or without it (in combination with codeine phosphate, ascorbic acid or diphenhydramine hydrochloride as well as NSAIDs such as ibuprofen or propyphenazone. Paracetamol occurs in the form of tablets, effervescent tablets, suspension, powder to prepare oral liquid medicine (sachets) and rectal suppositories. When administered orally, clinical effect of paracetamol appears after 30 min. Paracetamol content in oral medicaments differs; most frequently it equals 500 mg, however, there are preparations (most often complex) which contain 325 mg of paracetamol or 750 mg (e.g., Febrisan, Coldrex) or even 1000 mg (e.g., Efferalgan Forte, Codrex MaxGrip, Flucontrol Hot). The fastest action of paracetamol, already after 15 min, occurs in the case of using fast-release tablets, enriched with sodium bicarbonate which enhances stomach emptying. Due to this process, paracetamol quicker passes to the small intestine where it undergoes absorption (e.g., Panadol Rapid®). When administered rectally (suppositories), bioavailability of paracetamol is lower, about two thirds of availability as compared to oral administration. The time necessary to achieve the therapeutic concentration for suppositories is 120-180 min, which means that analgesic action occurs after 2-3 h since the drug intake. Bioavailability and speed of absorption of paracetamol in the form of suppositories depend on numerous factors: the drug dose (in adults usually 650 mg; in children 80-325 mg), the size of the suppository (the smaller and the lower dose the better bioavailability is), the type of vehicle (the higher vehicle lipophilicity, the greater bioavailability and the faster effect but the shorter time of drug action) and the degree of rectal vascularization. Slower absorption of paracetamol applied *via* rectum (suppositories) differs from other analgesic medicaments: e.g., sodium diclophenac in the form of suppositories in the preparations Dicloberl (50 mg of the active substance) or Dicloratio (25, 50 and 100 mg) achieves the maximal blood concentration after 30 min since the application, in the preparations: Diclac and Diclofenac GSK (50 or 100 mg) or Voltaren (25, 50, 100 mg) – after 60 min, and in Olfen (50 and 100 mg) – after 2 h (data according to Pharmindex 2012). These data show that the speed of absorption of an active substance from the drug administered *per rectum* (affecting the occurrence of the therapeutic effect) is influenced by the form and composition of the adjuvant substances contained in suppositories; the same factors affect the suppositories containing paracetamol. Slower absorption of the drug is usually associated with its longer presence in the organism, i.e., with a longer time of action, which in the case pain complaints is of considerable importance.

Paracetamol can be also used intravenously (*i.v.*) and therefore is widely used in the hospital health service, e.g., in the postoperative pain therapy (it has been evidenced that administration of paracetamol especially during the first hour of treatment is more efficient in reducing pain intensity than given orally), in order to quickly decrease high fever or in the case when another route of administration is not possible (52, 53). At the beginning, propacetamol – precursor of paracetamol (Pro-Dafalgan®, Bristol-Myers Squibb; Pro-Efferalgan, UPSA) was used which after the *i.v.* administration underwent hydrolysis to paracetamol and diethylglycine under the influence of plasma esterases. In 2005, an intravenous form of new generation paracetamol was registered as a solution ready to be infused at the concentration of 500 mg/50 mL or 1 g/100 mL (Perfalgan®, Bristol-Myers Squibb) which completely removed proparacetamol from medical practice (53, 54).

Side effects

When appropriate dosage of medicaments containing paracetamol is used, i.e., maximum dose of 4 g/24 h, (as one can read in the leaflet) no serious side effects have been observed, besides possible allergic skin reactions, although after higher doses or prolonged duration of taking the drug, some side effects may occur, especially in the liver (Table 1) (55). Interesting is that at the beginning of 2013, the United States Food and Drug Administration (US FDA) introduced paracetamol on the list of the preparations, which will undergo specific monitoring on the basis of information from the system on

adverse reactions (FEARS, the FDA Adverse Event Reporting System) collected during the period from October to December 2012. The preparations containing paracetamol will be evaluated in terms of inducing adverse skin reactions.

After ingestion of paracetamol, about 90% of the compound undergoes metabolism in the liver in conjugation with glucuronic acid (50-60%), sulfuric acid (25-35%) and cystine (approximately 3%) to form pharmacologically inactive metabolites, which are eliminated with urine. A small amount of the drug (about 5%) is eliminated in an unchanged form by kidneys. Subsequent 5% of paracetamol is subjected to N-hydroxylation in the liver with the involvement of cytochrome P450 enzymes (particularly CYP2E1) to form a toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is very quickly inactivated by glutathione sulfhydryl groups and excreted with urine as mercapturic acid (46).

Severe liver impairment after paracetamol overdose was documented for the first time in Great Britain in 1966 (56). Since then, a steady increase in the number of accidental or intended poisonings has been noted all over the world including Poland. The main cause of this situation is a huge amount of preparations containing paracetamol, which are available on the pharmaceutical market without any prescription (according to the 20th edition of Dugs of Contemporary Therapy, the number of such preparations reaches 92 items, including 39 single and 53 complex products). Depletion of hepatic glutathione stores occurs as a result of the intensive metabolism following intentional and unintentional overdose of paracetamol (ingestion of more than 4 g/24 h, i.e., over 8 tablets, 500 mg each!). In such a situation, paracetamol becomes a dangerous and life-threatening drug because a highly reactive NAPQI metabolite covalently binds to hepatocyte macromolecules leading to impoverishment of enzymatic systems and structural and metabolic damage to the liver (potential lethal hepatic necrosis). In the later stage of poisoning, renal tubular necrosis and hypoglycemic coma may appear (57). It is worth mentioning that the weakened hepatic function (caused by slimming, malnutrition, hepatitis C virus (HCV), human immunodeficiency virus (HIV)), alcohol overuse or application of paracetamol combined with drugs inducing cytochrome P450 (rifampicin, barbiturates, carbamazepine) can lead to hepatic impairment much easier, even when the compound is used in therapeutic doses. Development of acute hepatic failure as a result of paracetamol overuse (i.e., 7.5-15 g /24 h) as well as the methods of its treatment have been precisely discussed in many studies for the last ten years (46, 58-60). The authors of the present study concentrate on other (likely to be potential) adverse reactions of paracetamol, which result from its mechanism of action.

Results of recent reports on paracetamol as a peripheral selective COX-2 inhibitor encourage researchers to analyze this drug more critically. The question arises as to whether paracetamol revealing a similar pharmacological profile to coxibs may induce the same side effects, especially when the drug is used for a long time.[2] A permanent blockade of prostaglandin synthesis through selective COX-2 inhibitors is currently regarded as a cause of adverse cardiovascular reactions in patients after a prolonged use of these drugs (15, 36, 37, 61). Long-lasting COX-2 inhibition decreases the production of vasoprotective prostacyclin (PGI_2) by vascular endothelial cells, which inhibits platelet aggregation and has vasodilational capacity. This impairs the balance between tromboxane and prostacyclin and causes thrombus formation. Contrary to the inflammatory tissue, the endothelial cells possess a low level of peroxides, so they are not likely to inhibit paracetamol activity against COX-2 (14).

It has been shown that oral administration of paracetamol at the dose of 500 mg decreases the amount of excreted with urine 2,3-dinor-6-keto $PGF_{1\alpha}$, the main stable inactive metabolite of prostacyclin, whose synthesis is mediated by endothelial COX-2 (62). Likewise, 50% reduction in this metabolite excretion in the urine of pregnant women was noted after ingestion of 1 g of paracetamol (63). Taking into consideration aforementioned results obtained by Hinz et al. (36), regarding over 80% inhibition of COX-2 in the vascular endothelium caused by paracetamol, it can be speculated that such a mechanism of action would be responsible

[2] Coxibs, NSAIDs selectively inhibiting COX-2 activity, do not affect (in therapeutic doses) COX-1 at the same time. Due to such a mechanism of coxibs, their side effect on the digestive system, which happens in the case of traditional NSAIDs, was eliminated. However, later clinical observations indicated that patients using coxibs for a long time developed adverse cardiovascular reactions. Thus, because of a higher risk of such perturbations in those patients, coxibs (etoricoxib, lumiracoxib, rofecoxib and valdecoxib) have been withdrawn from sale. Rofecoxib known under the trade name of Vioxx (Merck & Co.) was withdrawn as the first one in 2004 after the 5-year existence on the pharmaceutical market; valdecoxib (Bextra, Pfizer) was the next drug withdrawn in 2005. At present, only one drug of this type, celecoxib (Celebrex; Pfizer Europe), is used in Poland.

for adverse cardiovascular reactions in patients who take this drug regularly. It should be emphasized that paracetamol due to its short half-life (approximately 2 h) induces a short-lasting inhibition of COX-2 activity. Thus, in order to eliminate pain it is necessary to administer repeated 1 g doses of paracetamol for maintaining constant (80%) inhibition of COX-2. This fact has to be considered by a doctor prior to making the decision about long-term treatment with paracetamol in order to avoid the drug overdose.

Epidemiological data reveal that long-lasting administration of paracetamol affects blood pressure. *Nurses' Health Studies* present two cohort investigations performed among younger and older women. One of them demonstrated that in patients who regularly took paracetamol (over 500 mg/24 h), a relative risk (RR) for development of hypertension was considerably higher as compared to women who did not use this drug (RR 1.93 for older women; RR 1.99 for younger) (64). Moreover, it worth emphasizing that the risk associated with paracetamol was similar to traditional NSAIDs (RR 1.78 for older women; RR 1.60 for younger). The second cohort investigation carried out in the same study group indicated that in women who frequently used paracetamol (= 22 days a month), the risk of serious cardiovascular events (such as heart infarction or cerebral stroke) was nearly the same as after traditional NSAIDs (RR 1.35 for paracetamol; RR 1.44 for traditional NSAIDs). Similarly, application of paracetamol in the amount of 15 tablets or more per week is associated with the risk of cardiovascular events comparable to traditional NSAIDs (RR 1.68 for paracetamol; 1.86 for traditional NSAIDs) (65). According to the guidelines of the American Heart Association acetaminophen (paracetamol) is nowadays a drug of choice in patients with concomitant cardiovascular disorders (66). The prospective double-blind trial was performed in patients with stable coronary disease who used paracetamol at the dose of 1 g three times a day for two weeks and the drug increased their blood pressure. Its effect was similar to that exerted by diclofenac and ibuprofen.

Paracetamol due to its selective action towards COX-2 and similarly to coxibs but contrary to typical NSAIDs does not possess antiaggregatory properties. The drug does not inhibit blood platelet action when taken at a single oral dose of 1000 mg. However, clinical studies indicate antiaggregatory action of paracetamol in the case of parenteral administration in high doses (67, 68). Paracetamol can be safely used in the digestive tract; on one hand due to its non-acidic chemical structure (unlike acidic NSAIDs gathering in the gastric epithelial cells) and on the other hand, due to a weak impact on COX-1. However, the results of epidemiological studies suggest that paracetamol at daily doses higher than 2-2.6 g increases the risk of serious side effects in the upper segment of the digestive tract such as bleeding or perforations (69). Therefore, it is postulated that a long-term effect of paracetamol on the digestive tract should be examined in randomized studies, especially in patients with osteoarthrisis who require high doses of this drug for a long time. Paracetamol like coxibs does not induce bronchial spasm in patients with aspirin asthma. In the strategy for treatment of pain in asthmatics, it is recommended to ingest this drug at doses lower than 1000 mg in order to avoid potential bronchial spasm (15).

Bearing in mind a preferential action of paracetamol on COX-2, the differences between the drug discussed and coxibs, selective inhibitors of this isoenzyme, should be emphasized. Paracetamol in opposition to selective inhibitors of COX-2, despite a similar mechanism of action, reveals weak anti-inflammatory activity. It is likely to result from the extracellular accumulation of arachidonic acid and peroxides in the inflammatory tissues, which reduce an inhibitory effect of paracetamol on the prostaglandin production (Fig. 3) (14, 35). Indeed, paracetamol did not decrease prostanoid concentrations in the joint fluid of patients suffering from osteoarthrisis (70). On the other hand, paracetamol reduced tissue swelling with similar to ibuprofen efficiency after the oral cavity surgery in humans (71). There have been also some studies which demonstrated anti-inflammatory action of paracetamol, e.g., nociceptive inhibition and carrageenan-induced rat paw edema (72). Therefore, the notion that paracetamol exhibits weak anti-inflammatory properties seems to be more legitimate than the assumption that this drug is devoid of such an action.

As regards safety of paracetamol application in pregnancy, prospective cohort studies in humans have not shown an increase in the prevalence of developmental fetal anomalies in pregnant women who took paracetamol in therapeutic doses, although in some experimental studies on animals paracetamol administered at doses twice as high as the maximum single dose demonstrated embriotoxic action (73). Considering the fact that paracetamol is the drug of choice in pregnant women, it should be emphasized that epidemiological studies report the possibility of the association between application of this drug in pregnancy and development of asthma

in early childhood. The metabolism of paracetamol has been suggested to be responsible for this effect because a large amount of glutathione is used to deactivate the toxic metabolite. Lungs of the developing fetus might deplete glutathione, the main antioxidant of this organ, which can lead to oxidative stress and inflammation of the respiratory airways. In some investigations, the occurrence of wheezing breath in very small children was observed, which however, is a very weak indicator of asthma (74). Epidemiological studies from different research centres provide controversial results on the association between paracetamol ingestion by pregnant women and the development of bronchial asthma later in childhood (74, 75). This happens because a number of other factors such as fever, cold, inflammation of fetal membranes or other infections of pregnant women can induce development of asthma in small children, leading thus to falsification of study results. Thus, a randomized study with placebo as a control could solve this problem. However, such a study would be unethical from the point of view of good clinical practice (GCP) which requires application of a standard drug as a comparator, and as NSAIDs are contraindicated in pregnancy, one group of women with pain and fever would not be treated at all. At present, there is no convincing evidence allowing to unequivocally determine that application of paracetamol in pregnant women may lead to asthma development in small children. Therefore, paracetamol still remains an analgesic and antipyretic drug of choice in pregnant patients. However, it should be stressed that the aforementioned data do not concern complex preparations containing paracetamol or those for *i.v.* infusion (safety for this route of administration has not been determined due to lack of sufficient clinical data).

Precautions and attempts to counteract toxicity of paracetamol

Due to an easy overdose of paracetamol, the US FDA has proposed to implement new solutions, which to a certain degree would limit this growing problem. A decrease in the maximum permissible single dose of paracetamol from 1000 mg to 650 mg seems to be one of the crucial problems. Thus, a question arises what will happen to numerous OTC preparations containing paracetamol in the dose exceeding 650 mg. It has been suggested that higher doses of this drug, i.e., above 325 mg should be available only by prescription (according to the information of the US FDA; www.fda.gov). Another suggested solution postulated by FDA is the withdrawal of packages containing high amounts of paracetamol from the market, e.g., containers comprising even 100 single doses (e.g., Apap - 100 tablets, Codipar - 50 tablets), and the introduction of blisters that should enable the patient to control the amount of ingested drug. Furthermore, the packaging should be labelled with the information about the risk of liver damage caused by the overuse of the drug. It also seems justifiable to use only one international name, either paracetamol or acetaminophen, and not two different names of the same drug because it can be misleading for the patient (if not properly informed the unaware patient can ingest the same active substance under different names). The most drastic proposal suggested by FDA is the withdrawal of all complex drugs, both available over the counter (OTC) and by prescription, because, as the various study results indicate, they are responsible, to a great degree, for acute paracetamol poisoning. The data obtained by the Toxic Exposure Surveillance System (TESS) in 2005 showed that among all acute paracetamol poisonings, 6.3% (i.e., 3,845 of the 61,289 reported) was caused by OTC preparations and 1.5% (41 of the 2,698 reported) involved severe hepatic damage, while 54% of overdoses (i.e., 1,470 of the 2,698 reported) were recorded in the case of using complex drugs available by prescription. As regards the latter drugs, it has not been completely elucidated to which extent a narcotic ingredient present in the preparation contributed to the poisoning (76). At present, in all complex preparations available by prescription in the USA, a single dose of contained paracetamol cannot exceed 325 mg, whereas the way of the drug dosage, despite a decrease in a single dose, remains the same. Although paracetamol is not so toxic for children as for adults (children do not have a well-developed cytochrome P450 system so the toxic metabolite is not formed), FDA also recommends that liquid paracetamol should be available only in a single established dose, e.g., 160 mg/5 mL (according to FDA information; www.fda.gov).

Another solution aimed at prevention of paracetamol hepatotoxicity in Great Britain was the introduction of tablets containing paracetamol and methionine, which after the conversion into cysteine and then glutathione in hepatocytes would inactivate the active metabolite, NAPQI. Moreover, due to such a combination, there is no time wasted from the moment of intentional or unintentional ingestion of a toxic dose of paracetamotol to the application of antidote, e.g., N-acetylcysteine (hepatic damage occurs 24 h after the overdose). Nowadays, the only such preparation registered in Great Britain is

Paradote (Penn Pharmaceuticals) containing 500 mg of paracetamol and 100 mg of methionine. Other preparations of this type, e.g., Pameton (SmithKline Beecham), have been withdrawn. In other European countries and the USA such combinations of paracetamol do not exist on the pharmaceutical market because so far no efficient and safe dosage of methionine has been established for patients; also safety of the long-term application of these preparations has not been investigated yet (some carcinogenic effect of methionine has been suggested). Besides, the price of such a drug is higher than for a preparation containing paracetamol alone (77).

In the light of novel studies, the application of traditional NSAIDs in combination with paracetamol has not been recommended, particularly when active substances occur in higher doses (8, 69). Rahme et al. (69) published the retrospective cohort study performed in 644,183 patients aged over 65 years who had been receiving paracetamol (at daily doses: < 3 g and > 3 g) and/or traditional NSAIDs (with or without a proton pump inhibitor) for 6 years. The risk of hospitalization due to gastrointestinal events (ulceration, perforation, bleeding from the upper or lower segment of the digestive tract) appeared to be two-fold higher in the case of taking paracetamol in combination with traditional NSAIDs as compared to NSAIDs used in monotherapy. The authors of that study (69), as well as other researchers analyzing the problem of interaction between paracetamol and NSAIDs (8), explain the results in relation to the additional COX-1 inhibition caused by paracetamol. This hypothesis seems to be reliable in the light of new data showing that paracetamol synergistically enhances inhibitory effect of diclofenac on platelet activity (68, 78). Thus, safety and usefulness of complex preparations containing paracetamol combined with NSAIDs appearing on the pharmaceutic market are still the matter of discussion. It is worth paying attention to preparations containing paracetamol and NSAIDs (ibuprofen and propyphenazone) available on the Polish market without a prescription (Cefalgin and Saridon - paracetamol + propyphenazone and Metafen and Nurofen ultima - paracetamol + ibuprofen). The aforementioned drugs contain paracetamol at doses of 250-500 mg and NSAIDs: ibuprofen - 200 mg or propyphenazone - 150 mg. According to the manufacturers information, a single dose of these drugs is 1-2 tablets with the possibility of three-fold application per day. Considering the maximum dosage (2 tablets 3 times a day), a total dose of paracetamol would range from 1.5 g to 3 g, which is in compliance with the contemporary knowledge (8, 69) if used sporadically. It should be remembered that a single dose of paracetamol should not exceed 1 g and daily dose 4 g; the US FDA suggests these values should be decreased to 0.65 g and 3.25 g, respectively. In the case of combined application of paracetamol with NSAIDs, paracetamol dosage should be considerably lower than the aforementioned values.

CONCLUSIONS

Summing up, paracetamol monotherapy is efficient, well tolerated by the majority of patients and safe, on condition that the drug is administered at therapeutic doses. Table 1 sums up the advantages and disadvantages of paracetamol. We should, however, bear in mind that the paracetamol overuse or application even at therapeutic doses in some situations like improper slimming, smoking, alcohol abuse or ingestion of other medicines may cause severe hepatic damage or death. Therefore, the question arises as to whether the patient knows that a safe dose of paracetamol (assuming that the abovementioned situations are not present) comprises only eight tablets of 500 mg or four sachets, each one containing 1000 mg, per day and that paracetamol is ''hidden'' in other preparations under different names (here are about 100 simple and complex preparations in Poland). Thus, it is very important to the patient to be warned by doctors or pharmacists about the risk connected with the ingestion and particularly with the overuse of this drug. It appears in the light of new data that despite frequent application of paracetamol as an efficient analgesic and antipyretic drug, the action of this medicament has not been completely understood and this little unknown part may cause irreversible damage to the organism when the drug is overused. A long-term application of high doses of paracetamol carries the risk of adverse reactions typical for COX-2 inhibitors (coxibs) such as hypertension, heart infarction or renal failure. It results from a peripheral selective inhibition of COX-2 by paracetamol. Moreover, it appears that the use of paracetamol combined with NSAIDs is not beneficial because an increase in the occurrence of gastrointestinal events can be observed. On the other hand, *i.v.* administered paracetamol at high doses inhibits platelet aggregation, which is very important in the treatment of patients with disorders of hemostasis.

It should be remembered that despite the fact that paracetamol has a wide clinical application it is not a drug devoid of side effects. Therefore, before taking a decision about the treatment of the patient

with paracetamol, each time a balance of benefits and losses should be made so as to perform the adequate and efficient therapy. The aim of the present study was not to deny the rationality of paracetamol use but only to draw the attention of doctors prescribing this drug and pharmacists selling the drug as well patients taking it to the fact that this drug should be used only in situations which are indispensable. In the light of the contemporary research it is not possible to answer the question included in the title of the present study "Do we know all about paracetamol" but the nearest years will obviously provide the answer whether the decision taken in 1956 to introduce paracetamol as an OCT drug was correct.

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