Short communication

Ibuprofen against Aspirin – you first, please! Thanks go ahead. . .

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Abstract. Aspirin (ASA) and Ibuprofen (IBU), two common NSAIDs, seem to exert a mutual interference on the blood platelet aggregation level. We wish to clarify this controversy on the basis of crystallographic data and molecular modelling.

The ASA–IBU controversy (reported by FitzGerald [1]) is revelatory on several important issues far beyond the fight of some pharmaceutical giants against Bayer as originally presented by the press and in Wolfe paper [2].

The question raised by FitzGerald group is certainly an interesting one. Although several NSAID's are used as effective drugs designed to have similar to ASA activity, acetylsalicylic acid is also a reducing agent of myocardial infarction, stroke or anti-aggregation. The other popular over-the-counter NSAID drug action on vascular level remains unknown.

The popular drugs availability lead inevitably to frequent use of them in conjunction. In this respect already the cross effects of two or more NSAID's taken even in low doses within short laps of time could lead to some conflicting situations. Also, the sequence of use of two drugs should have an important effect on their combined action, especially if one of these compounds does not belong to the same family of NSAID (IBU vs ASA) and displays an additional important activity as in a case of ASA.

One of the facts which should be taken into consideration is also the mechanism of action and especially some minor differences in this mechanism for even closely related chemically drugs. Among commonly prescribed NSAID, those displaying cytoprotective action form five groups:

- propionic acids (e.g., IBU or Naproxen, NAP, for human and rimadyl for animals),
- naphthylalkanone (e.g., nabumetone),
- indoleacetyl acids (e.g., indomethacin),
- phenylacetyl acids (e.g., diclofenac, voltaren),
- salicylic acid (e.g., ASA),

and another large group with, e.g., COXIBS – pyrazole or furanone (rofecoxib, a newer addition to this series).

The last popular drug, acetaminophen used in FitzGerald's study belongs to the group of these drugs but is lacking the anti-inflammatory effect and it should not have an acetylating capacity such as ASA

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because of structural differences (N-acetyl compared to O-acetyl for ASA). However, the acetaminophen does not inhibit platelet aggregation.

One of the problems is that all these drugs, acting well as NSAID are affecting the cyclooxygenase (COX) by accessing the haem channel, except lesser known action of acetaminophen. However, on the molecular level, their mode of action strongly differ one drug from another.

The ASA acts as a potential acetylating agent of Ser 530 according to the simple transesterification. This reaction goes remarkably well because the relatively small ASA is a phenol acetate and the serine has primary alcohol group. In this case, the released salicylic acid's fate is unknown. However, it is worthy of attention that the salicylic acid itself is the first known NSAID which served the further development of the ASA [3]. It is also possible that, in acidic conditions such as stomach acid, the ASA could undergo desacetylation by hydrolysis, hence one of the factors of introduction of enteric-coated tablets.

The salicylic acid in a way does not fit the COX channel mechanism of inhibition of the PG synthesis from arachidonic acid. Numerous NSAID are carboxylic acids (or metal carboxylate salts). Their mode of action is different and, in absence of the acetylating capacity involves different site on access to haem channel.

The interesting hypothesis was confirmed by crystallographic study by analogy to the docking of the arachidonic acid to the COX model. The carboxylic acid should be retained by some basic amino acids of the inner layer of the proteic channel (e.g., Arg), located in different position than Ser 530. The ionic interaction between acidic NSAID and this basic amino acid from the channel wall fully explains the inhibition of PG synthesis, also explains important differences in drug activity according to the chirality of C-2 carbon for propionic acids. In fact, the S-isomer of IBU is an efficient PG-synthesis inhibitor because of the asymmetric C-2 methyl interact with the neighbouring Tyr for S isomer and do not react with R, which favours blocking of access to channel by S and no reaction with R (although some of R isomer propionates are toxic) because of unfavourable position of Arg and the R drug.

Some facts are overlooked in the discussion but can be helpful to further explain the results. Using the model of action of NSAID, as deduced from Garavito works [4], ASA and a group of other NSAID's tested (e.g., IBU but probably also closely chemically related propionic acid, Naproxen) do not have the same docking site in the channel leading to oxidation site of the enzyme. ASA reaches Ser 530, which is at the top of the channel, far from the entry. However, IBU is bound to the channel 19 angstroms away from the haem probably via ionic interaction to the nearby basic Arg 120 [5]. If the IBU is administrated first, it will block the entry to any drug wanting to reach the top of the channel. The channel, as a protein structure is closed, statistically it is difficult to imagine the second molecule of NSAID of any

Fig. 1. Ser 530 acetylation.

Fig. 2. Salt bridge formation at Arg 120.

Fig. 3. 3D representation of COX-1 channel.

sort trying to enter the same channel. It is also worthy of mention that despite its narrow character, the protein channels undergo important changes, thermal modification, enable to considerably change diameter and give way to larger molecules. The inhibition of PG synthesis is based on initial retention of the arachidonic acid via Arg 120, in a banana-like shaped conformation (not as commonly believed U-shape) in order to undergo the oxidation. Finally, the transport of the smaller than arachidonic acid propionates via the channel should take place through some consecutive transfers, from one basic amino acid to another, until reaching the appropriate target (e.g., for ASA, Ser to be acetylated is located far from the entrance). It is necessary to point out that the ionic salt bridge, as for IBU or NAP, and the

chemical transesterification as for ASA, are different chemical reactions. In all cases, however, the free acids are released at once (salicylic acid as a metabolite of ASA deacetylation or corresponding propionic acids) which, in principle, are available for interaction with platelet and thromboxane metabolisms. The size of aryl propionate will probably be discriminatory on this level, comparing to relatively slim and handy ASA metabolites. As far as the access to the active site factor is concerned, both mechanisms of blocking the channel represent the same problem. Once the channel is blocked, it remains unavailable as such for oxidation purpose. In a case of administration of ASA first, the second drug could still bind to the enzyme. The order of administration (ASA followed by IBU or the contrary) should be re-examined in view of these molecular models and our remarks.

Assuming that the ASA is entering first, the salicylic acid is released and Ser acetylated, the IBU becomes available for NSAID activity and can even enter the same blocked channel (as shown on simple molecular modelling model using Garavito's data). This processes is of low statistical probability.

If the IBU enters first, the whole ASA quantity remains entirely available within blood stream and could feasibly compromise blood pH, for example. The potential competition between IBU and ASA for the same channel is well documented by structural models. This explanation stands however because it is assumed that both drugs, ASA and IBU, have a similar binding site to the channel target constants and there is no chemical interaction between these two drugs. However, there is no evidence for "the potential of competitive interaction between both drugs", contrary to FitzGerald's statement [1,6].

The other overlooked problem is that the acetylation of the Ser, although not required for cyclooxygenase activity, is only one of several modes of blocking the access channel. There are probably several other inhibition process mechanisms. For the propionate drugs, the docking via basic amino acid and consequently blocking the access, is a second common mechanism of PG-syntheses inhibition. The PG-synthesis inhibition seems to follow several different pathways.

In this respect, the figure 5 of the FitzGerald paper should be updated accordingly.

The second point is that both drugs are usually used (or rather abused) in quantities many times higher than necessary to generate, e.g., the pain killer action. The remaining "free" ASA or/and IBU, are entering the blood stream and should, for instance, interact competitively with platelets, protecting them against aggregation. The presence of two acids (ASA and IBU) in high concentration will create a disorder in most of pH sensitive systems of solutions controlling blood thickness. It is striking also to point out the low dose of ASA used in this study (81 mg) and the lack of discussion on the effect of different metabolism time for both drugs on the interpretation of results. In order to study a potential competition between ASA and IBU, the equimolar concentration should be used.

The role of NSAID played, e.g., in their gastro intestinal toxicity is very well documented [2]. Despite this warning, it is always practical and somehow handy to have an unlimited access to the over-the-counter pain killer drug. The controversy of ASA-IBU should be seen as one more example of necessity of more rigorous consultation of doctors by patients needy for an efficient pain reliever.

Dr FitzGerald's warning is an excellent contribution to pursue the discussion on the controversy between IBU-ASA which remains entire, and more work is welcomed to clarify this fascinating problem.

References

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