International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685; Impact Factor: RJIF 5.28
Received: 02-01-2020; Accepted: 03-12-2019; Published: 05-03-2020
www.pharmacyjournal.net
Volume 5; Issue 1; January 2020; Page No. 06-09

A brief review on the use of dogs as animal models in *In-vivo* measurement of bio-availability of extended release diclofenac sodium 100mg tablets

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Abstract
Even though it is widely used, Diclofenac Sodium has a short half time it is rapidly and almost completely absorbed and 40% undergoes first-pass metabolism that lead to fluctuations in bio-availability. It also has common side effects that restrict its use such as gastric irritation gastritis, peptic ulcers and bleeding. This is the basis for designing an Extended Release (ER) dosage formulation that would maintain sustained therapeutic levels and minimise the frequency of drug administration and overcome its side effects. Meanwhile, the extended release Diclofenac Sodium tablet formulation is supplied as or contained in medications under a variety of trade names that may not be bio-equivalent. In-vivo testing is applied to measure the active ingredient in the blood supply using High-Performance Liquid Chromatography (HPLC) methods with ultraviolet because it is sensitive, simple, rapid and economic. Animal models such as beagle dogs are preferred because they feasible and less costly.

Keywords: diclofenac sodium, extended release formulation, *In-vivo* testing, dog model

1. Introduction

Diclofenac Sodium belongs to the heterogeneous class of drugs known as Non-Steroidal Anti-Inflammatory (NSAID). It is taken or applied to reduce inflammation, as analgesic to relieve pain and anti-pyretic to reduce rising body temperature (Ganesh *et al*, 2010). Diclofenac Sodium is extensively used as an analgesic to minimise pain in different conditions particularly in acute injury, treating rheumatoid arthritis and gout attacks (Dastidar *et al*, 2000) [8]. The other conditions where it is used to alleviate pain include migraine headache, tooth ache, kidney stones and gall stone pain, and menstrual pain (Brunton *et al*, 2008). It suppresses inflammation and reduces swelling in conditions affecting the joints, muscles and tendons (Aielo *et al*, 2013). It is considered to be very effective managing post-chirurgic, post traumatic pain, moderate post-operative pain and associated pain with bony metastases (Zafar *et al*, 2014).

Diclofenac sodium is a phenylacetic acid derivative. Its structure was specially designed based on information gained about structure activity relationships of other NSAID and its chemical name is 2-[(2, 6-dichlorophenyl) amino] benzene acetic acid mono sodium salt (Altaman, 2015) [1]. The primary mechanism of action responsible for anti-inflammatory, analgesic and anti-pyretic action is that it inhibits prostaglandin synthesis by hindering of the transiently expressed prostaglandin- endoperoxide sythase-2 (PGES-2) alias Cycloxgenase-2 (COX-2) (Patrono, Patrignani and Garcia, 2001: 7:13). Thus, Diclofenac Sodium inhibits the conversion of arachidonic acid to unstable endoperoxide, a reaction catalyzed by cyclooxynase (Feldman, 1998a). It also reduces production of leukotrienes and 5-hydro xyeicosatetraenoic acid, and this contributes to the anti- inflammatory activity. This process prevents production of production of prostaglandin, prostacyclin and thromboxane thereby reducing inflammation, pain and fever (Feldman M 1998_b).

Pharmacodynamics characteristics of Diclofenac Sodium are that in rheumatic diseases, the anti-inflammatory and analgesic properties of Diclofenac sodium elicit a clinical response characterised by marked relief from signs and symptoms such as pain at rest, pain on movement, morning stiffness, and swelling of the joints, as well as by an improvement in function (Sweetman, 2009: 46). In post-traumatic and post-operative inflammatory conditions, Diclofenac Sodium lessens spontaneous pain and pain on movement in such a way that it reduces inflammatory swelling and wound oedema (Dexcel, 2011_a). It has also been found to exert a pronounced analgesic effect in moderate and severe pain of non-rheumatic origin. In primary dysmenorrhoea, Diclofenac sodium is capable of relieving the pain and reducing the extent of bleeding (Dexcel, 2011_b). Even though it is widely used, Diclofenac Sodium has a short half time it is rapidly and almost completely absorbed and 50% undergoes first-pass metabolism that lead to fluctuations in bio-availability (Brunton, 2009_b). It also has common side effects that restrict its use such as gastric irritation gastritis, peptic ulcers and bleeding (Becker, 2016). Therefore, it was the basis for designing an Extended Release (ER) dosage formulation that would maintain sustained therapeutic levels. Minimise the frequency of drug administration and overcome its side effects (Lusiana, Surini & Hayun, 2016).

Since its introduction in 1973, a number of different Diclofenac-containing drug products have been developed with the goal of improving efficacy, tolerability, and patient convenience (Lesney, 2010: 19-20). Moreover, the extended release Diclofenac Sodium tablet formulation is supplied as or contained in medications under a variety of trade names that may not be bio-equivalent (Altaman 2015b) [1]. That is, bioequivalence describes pharmaceutically equivalent products that display comparable bioavailability when studied under similar experimental conditions. This article describes the necessity of conducting in-vivo bio-equivalence of different brands of Extended Release Diclofenac Sodium tablets on animal models with a special reference to dogs.

2. Mechanisms of extended release (ER) oral formulations

Extended-release products are designed to release their medication in a controlled manner, at a predetermined rate, duration, and location to achieve and maintain optimum therapeutic blood levels of drug (Daniel, 2017). The basic rationale of a controlled drug delivery system is to optimize the bio-pharmaceutics, pharmacokinetic and Pharmaco-dynamic properties of a drug in such a way that its utility is maximized through reduction in side effects and cure or control of condition in the shortest possible time by using smallest quantity of drug administered by the most suitable route (Deepus, 2015). Therefore, drugs like Diclofenac Sodium that have a shorter half-life and high dose frequency require a controlled release formulation that offer better patient compliance, maintain uniform drug levels, reduce dose and side effects and increased margin of safety (Chung, *et al*, 2012:57-62) Controlled drug delievery systems are developed as oral extended release matrix systems using hydrophilic polymer as release retardants to optimization of therapeutic effect, by maximizing the bioavailability of conventional drugs (Li Xl, 2006) [13]. These retardant polymers have influence on release characteristics and include cellulose derivatives such as hydro xypropylmethy cellulose (HPMC) or hypromellose that are particularly used in extended release Diclofenac Sodium oral formulations (Dixit *et al*, 2013_a) [18]. Accordingly, a matrix tablet is defined as a oral solid dosage form in which the active drug is homogeneously dispersed throughout the hydrophilic matrices that serves as a release rate retardant (Dixit *et al*, 2013_b) [18]. Drug release from hydrophilic matrices is known to be a complex interaction between dissolution, diffusion and erosion mechanisms: That is, these systems release a drug in a continuous manner by dissolution or diffusion controlled mechanism. Hydrophilic matrix system, in particular, is characterised by swelling, diffusion and polymer dissolution. This combination of mechanisms modulates drug release kinetics. Essentially, drug release from these matrices typically includes penetration of fluid, followed by dissolution of drug particles and diffusion through fluid filled pores. Thus, the diffusion of the drug through the matrix is a rate limiting step (Colombo et at, 1993) [7].

The mechanism of drug release from the matrix tablets is that the drug in the outside layer exposed to the bathing solution is dissolved first and then diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving toward the interior (Omidian, 2008) [20]. It follows that for this system to be diffusion controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix (Kapil *et al*, 201) [12]. The most important factors to be considered when developing a formulation based on hydrophilic matrices are the percentage, solubility and particle size of drug as well as polymer; drug/polymer ratio, type of polymer and its degree of viscosity, compression force among the formulation factors (Maderule, Zarzuelo Lanao, 2011:2-19) [16]. Other micro pharmaceutical factors that affect drug release from a matrix system are drug solubility, polymer diffusivity, thickness of hydrodynamic diffusion layer, surface area and volume of drug delievery device and diluents effect (Wise, 2005) [9].

It is important to note that appropriate type, quantity and quality of hydrophilic polymer retardant excipients can influence the dissolution characteristics of a modified drug product, rate of absorption and ultimately influence its therapeutic quality. This article is mainly focused on explaining the importance of in-vivo quality assessment of extended release Diclofenac Sodium. This is because even though *in vitro* tests present in-vivo predictability of the therapeutic effect in conventional drug formulations, it is not the case with extended release oral formulations. Most controlled release preparations are designed for prolonged release and therapeutic effect, such that variabilities in in-vivo conditions (such as presence and nature of food in the gastrointestinal tract, time of the day the dosage form is administered) can substantially affect the release profile of the drug. Hence, in-vivo testing is the precise method of predicting therapeutic effect of extended release Diclofenac Sodium oral formulation.

3. *In-vivo* testing of extended release solid oral dosage forms

The absorption of a solid dosage form after oral administration depends on three factors: the release of the substance taken, the dissolution of the drug under physiological conditions and the permeability across the gastrointestinal tract, such that *in vitro* dissolution may be relevant to the prediction of an in vivo performance (Siewert *et al*.; 2003). However, like disintegration, dissolution test does not prove that the dosage form will release the drug in-vivo in a specific manner but a step closer to the absorption process. It is important to note that the main purpose of designing a pharmaceutical dosage form so as to give a patient a reliable therapy. In this regard, this requires that a dosage form releases the active ingredient in a consistent and reproducible manner. Therefore, the real test for in-vivo drug performance is therapeutic outcome but testing active ingredient in the blood supply the most appropriate and feasible (Ansels, Loyd and Nicolus, 2011: 67-79). Thus, comparison between the *in vitro* release kinetics of different matrices and the in vivo performance of extended release Diclofenac Sodium is a vital issue. High-Performance Liquid Chromatography (HPLC) methods with ultraviolet detection for measuring Diclofenac sodium in plasma samples have been preferred because of being sensitive, simple, rapid and economic.

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds (Martin and Guiocho, 2005) [17]. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary Phase, the molecules being analyzed, and the solvent(s) used (Lui and Lee, 1991) [14]. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both miscible combinations of water or organic liquids (Lui and Lee, 2006:198-202) [15]. Separation has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase. The choice of

solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column ((Aniszewski, 2015) [2].

In determining concentration of Diclofenac Sodium in blood plasma, a simple high performance liquid chromatography with Ultraviolet (UV) method is developed. Thus, UV spectroscopy is attached to specifically detect Diclofenac Sodium since organic compounds absorb UV light at respective wave length except the amount of light absorbed depends on the amount of a particular compound that is passing through the beam at the time. The method is validated by using linearity, stability, precision, accuracy and sensitivity parameters according to International Conference on Harmonization (ICH) guidelines. Chemicals to be used should be of analytical grade and appropriate HPLC system should be used. Preparation of plasma samples are processed by liquid to liquid extraction while ultraviolet detection is set at a specified appropriate wave length (Yilmaz, Asci and Saziye, 2010). Thus, HPLC is the most advanced technique for high specific and quantitative measurement of low levels of analytical blood samples. Therefore, HPLC is a versatile and reproducible chromatograph technique for estimation of Diclofenac Sodium in blood plasma, especially in quantitative and qualitative estimation of active molecule.

4. *In-vivo* testing of extended release of diclofenac sodium tablets in beagle dogs

In general, dogs as animal models have been frequently used for prediction of human bioavailability because they trust the human most. It is important to note that in pharmacology, bioavailability is the subcategory of absorption and is the amount of an administered dose of unchanged drug that reaches the circulatory system after first pass effects by either the gut wall or the liver (Nguyen *et al*, 2015). Out the dogs use for research purposes the breed that top most is the beagles.

***Source*:** Australian Research Council- 2014

Fig 1

The reason Beagles are used most is because of their size. They are not too small like the toy sized breeds and they aren't very huge, who are really hard to handle, making them just the apt size (Range, 2014_a). They are equally trusting and loyal, and easy to manipulate. Apparently, choosing just one breed as 'the' breed allows people working in this sector to somehow feel that using dogs for research purposes is more ethical. Hence, these dogs can be used for in-vivo testing of Diclofenac Sodium concentration in bloods plasma. These dogs are stolen, taken from shelters or simply picked from the listings about dogs looking for a home (Range, 2014_b).

In terms of scientific validity, there are shortcomings: That is, there are significant species differences in the liver function between dogs and humans such that it is difficult to precisely predict first-pass effect for Diclofenac Sodium that undergoes extensive first-pass metabolism in humans versus dogs. Furthermore, within the same species, there are differences in liver function between male and females (Onslo, 2009). This is because male and female livers express different subsets of genes, which affect the organ's ability to metabolize certain drugs and hormones. Thus, metabolism of drugs in the liver is differentially expressed between male and female with the female animals expressing about twice the amounts metabolic enzymes on the basis of microsomal protein content. Therefore, female animal models are preferred for in-vivo bio-availability since it is more precise prediction of first pass effects.

Another difference is that maximum concentration in dogs is likely to be higher than in human because of smaller blood volume. On the other aspect, higher destructive mechanical forces in dogs and different enzyme configuration would contribute to differences in exact prediction of bioavailability outcome (Kamba *et al*, 2014: 388) [11].

5. Conclusion

Despite shortcoming, highlighted, beagle dogs are appropriate substitute for human subjects in bioequivalence analysis of different brands of Extended Release Diclofenac Sodium tablets. There not only a cheaper option but they make in-vivo studies feasible, particularly in Zambia where it is almost impossible to access human subject.

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