

INTERACTION ANALYSIS OF ASPIRIN WITH SELECTIVE AMINO ACIDS

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Abstract: This study was conducted to assess the compatibility of aspirin with selective amino acids by studying the effect of amino acids on the solubility of aspirin, so that the attention could be paid towards the use of proteinous foods along with aspirin. Two different types of dissolution media, i.e., 0.5% solution of each amino acid and 100 mL of distilled water (100 mL each), were prepared. Then, 1 g of aspirin was added in both media and shaken gently. Ten milliliters of sample was withdrawn at different time intervals, i.e., 10, 20, 30, 40, 50 and 60 min and analyzed spectrophotometrically at 265 nm. It is evident from results that the absorbance of aspirin increased with the addition of amino acids and this increase was significant ($p < 0.05$). Absorbance after adding amino acid like glycine, tyrosine, glutamic acid, tartaric acid and aspartic acid was observed to be 2.98, 2.96, 2.92, 3.23 and 3.28, respectively, as compared to that of aspirin alone. The increase in absorbance of aspirin in the presence of tartaric acid and aspartic acid was non-significantly ($p > 0.05$) greater than that in the presence of other amino acids like glycine, tyrosine and glutamic acid. The absorbance of aspirin in the presence of tartaric acid and aspartic acid was 3.23 and 3.28, respectively, while the absorbance of aspirin in the presence of glycine, tyrosine and glutamic acid was 2.98, 2.96 and 2.92, respectively. This study elaborates that the solubility of aspirin increases with concomitant administration of amino acids, thus the use of amino acids (proteinous foods) with aspirin should be prohibited or low dose of aspirin should be recommended in such situation.

Keywords: aspirin, amino acids, solubility, bioavailability, interaction

Aspirin is the acetate ester of salicylic acid (Fig. 1) (1). Aspirin is an acronym for acetyl (a portion of the chemical), spir from spirea (an ornamental plant) and as in salicin (the compound from which aspirin is derived) (2). The chemical formula of aspirin is $C_9H_8O_4$. Aspirin is colorless, odorless and white crystalline powder (3–5). Molecular mass of aspirin is 180.16 g/mol. Its melting point is 135°C. Its 1 g dissolves in 300 mL of water at 25°C and in 100 mL of water at 37°C (6). It is stable in air but gradually hydrolyses on contact with moisture to acetic and salicylic acids. Aspirin is also unstable in solutions of alkali hydroxides and carbonates (pK_a 3.49 at 25°C) (7). Another aspect is the poor water solubility of aspirin, which eventual-

ly results into local irritation of stomach mucosa after oral administration (8). Aspirin exerts most of its effect by inhibiting cyclooxygenase enzymes (COX) thus suppressing the formation of prostaglandins (PGs).

Aspirin inhibits both COX-1 and COX-2 enzyme but the effect on former is more pronounced. Inhibition of COX enzyme is irreversible. The reduction in the formation of prostaglandins diminishes the sensitivity of the nerve fibers to pain producing substances such as serotonin (5-HT) and bradykinin, thus increasing pain threshold (9).

About 10% of a pre-dissolved 250 mg dose of aspirin is absorbed from an acidic solution in the stomach though the main absorption site is upper

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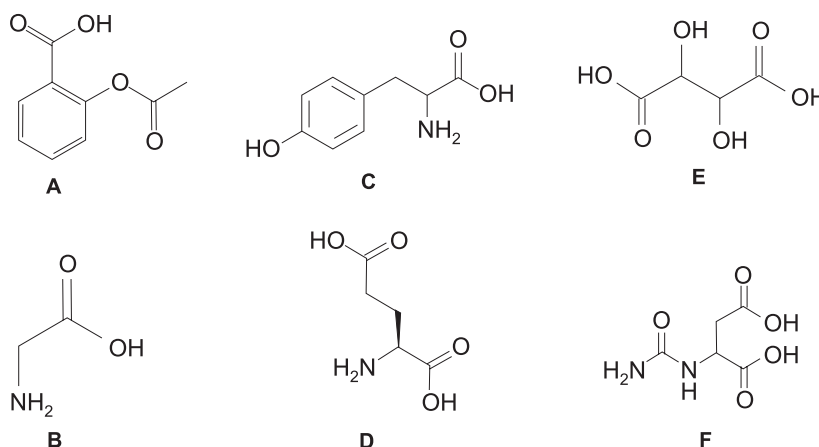


Figure 1. Chemical structures of aspirin (A), glycine (B), tyrosine (C), glutamic acid (D), tartaric acid (E) and aspartic acid (F)

intestine. This is partially because of poor solubility of compound at the strong acidic pH but also the comparably small absorption surface of the stomach mucosa (7).

Like most of other drugs, aspirin is mainly absorbed in upper intestine by passive diffusion of the non-ionized form (5).

Amino acids (AA) are the basic building blocks of proteins. Fundamentally, AA are joined together by peptide bonds to form the basic structure of proteins. AA are organic compounds that contain the basic amine group, NH_2 and acid carboxyl group, COOH .

AA are classified into different ways based on polarity, structure, nutritional requirement, and metabolic fate. Generally used classification is based on polarity. The nature of R-group attached to AA determines their polar nature. The classification of AA on the basis of polarity is as follows: non-polar AA, polar AA with no charge, polar AA with positive charge and polar AA with negative charge. Non-polar AA are neutral (R-group is chargeless), hydrophobic and possess equal number of amino and carboxyl groups. The amino acid in this group includes glycine. Polar AA with no charge possess no charge on the 'R' group and undergo participation in hydrogen bonding of protein structure. The AA in this group include tyrosine. Polar AA with positive charge possess positive charge on the R-group and have greater number of amino groups as compared to carboxyl groups, which makes it basic, for example arginine. Polar AA with negative charge (dicarboxylic mono-AA) possess negative charge on the R-group and have

greater number of carboxyl groups as compared to amino groups which makes them acidic, for example aspartic acid and glutamic acid (4).

This study was conducted to assess the effect of AA on the solubility of aspirin, so that the attention could be paid towards the use of proteinous foods along with aspirin. Literature survey has elaborated that no such study has been conducted previously. This study will help in the future to avoid any biological incompatibility, i.e., incompatibility between drug and food, particularly in the development of total parenteral nutrition containing aspirin.

EXPERIMENTAL

Materials

Aspirin (99.6% purity) was gifted by Mega Pharmaceuticals, Sheikhpura, Pakistan. Glycine, tartaric acid, L-aspartic acid, L-tyrosine, glutamic acid and phenol red were purchased from Pak Chemical Traders, Lahore, Pakistan. Sodium hydroxide (NaOH) was procured from Merck, Darmstadt, Germany. Double distilled water using glass apparatus was prepared in our laboratory.

Methodology

First of all, two different types of dissolution media i.e., 0.5% solution of each amino acid and 100 mL of distilled water (100 mL each), were prepared. Then, 1 g of aspirin was added in both media and shaken for 5 min. The sample (10 mL) was withdrawn at different time intervals, i.e., 10, 20, 30, 40, 50 and 60 min and analyzed spectrophotometri-

cally at 265 nm (10) using double beam spectrophotometer (1601, Shimadzu, Japan). Each sample was compensated with an equal volume of dissolution medium.

Using calibration curve, concentration of drug was determined for each sample. The value of regression coefficient (R^2) for aspirin was 0.9925, which indicated a good correlation between the concentration and absorbance within the concentration range (2–64 $\mu\text{g/mL}$) tested. The Y-equation for aspirin was $y = 0.042x - 0.004$. Each experiment was conducted three times.

Statistics

One way ANOVA was applied to elaborate the significance of difference between various parameters. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Glycine is an organic compound with the formula, $\text{NH}_2\text{CH}_2\text{COOH}$ (Fig. 1). With a hydrogen substituent as its 'side chain', glycine is the smallest of the 20 AA commonly found in proteins. It is unique among the proteinogenic AA in that it is not chiral (11).

Plasma levels of aspirin $> 300 \mu\text{g/mL}$ are considered obviously toxic. Severe toxic consequences are correlated with plasma concentrations $> 400 \mu\text{g/mL}$. A single poisonous dose of aspirin in adults is not recognized with confidence but death may be anticipated at a dose $\geq 30 \text{ g}$. The blood salicylate levels are measured to determine the severity of aspirin intoxication (12, 13). Thus, it is very important to determine the agents which interact with aspirin and ultimately potentiate or diminish its plasma concentration to avoid any undesired situation. In this context, present study was designed to elabo-

rate the possible interaction between AA and aspirin.

It is evident from results that the absorbance of aspirin increased with the addition of amino acids and this increase was significant ($p < 0.05$). This increase in absorbance represents the increase in the ionization and thus enhanced solubility of aspirin. Absorbance after adding amino acid like glycine, tyrosine, glutamic acid, tartaric acid and aspartic acid was observed to be 2.98, 2.96, 2.92, 3.23 and 3.28, respectively, as compared to that of aspirin alone (Tab. 1). The possible reason of increase in aspirin solubility in the presence of these selective AA could be due to the coating of aspirin particles by amino acid particles forming capsule-like structure and thus masking its hydrophobic properties, imparting hydrophilic properties and preventing the aggregation of aspirin particles (14).

This reason could be responsible for the enhanced wettability of aspirin, which ultimately improves its solubility (14). This phenomenon not only increases the solubility of aspirin, but also protects the mucosal membrane from the irritation of aspirin due to the application of coating of AA on the surface of aspirin. It reduces the contact between aspirin and mucosal membrane (15). It has also been proposed that carboxylic acid group interacts with the gastric mucosa, which is responsible for gastric irritation. AA bear the free amino group which reacts with the carboxylic acid group of aspirin instead of reacting with that of gastric mucosa (16). Thus gastric irritation can be minimized by concomitant administration of aspirin with the selected AA.

However, this increase in solubility of aspirin in the presence of AA also exhibits increased solubility of aspirin itself. It would result in the increased bioavailability and thus therapeutic toxic-

Table 1. Influence of various amino acids on the solubility of aspirin (Results are expressed as the mean \pm standard deviation, $n = 3$).

Time (min)	Absorbance of aspirin	Absorbance of aspirin after adding various amino acids				
		Glycine	Tyrosine	Glutamic acid	Tartaric acid	Aspartic acid
10	0.95 \pm 0.02	2.97 \pm 0.25	2.90 \pm 0.23	2.91 \pm 0.11	3.21 \pm 0.41	3.13 \pm 0.54
20	0.96 \pm 0.04	2.97 \pm 0.31	2.91 \pm 0.32	2.92 \pm 0.19	3.22 \pm 0.38	3.22 \pm 0.33
30	0.97 \pm 0.04	2.97 \pm 0.29	2.93 \pm 0.37	2.92 \pm 0.39	3.22 \pm 0.52	3.25 \pm 0.40
40	0.98 \pm 0.03	2.97 \pm 0.30	2.94 \pm 0.18	2.92 \pm 0.32	3.22 \pm 0.35	3.26 \pm 0.49
50	0.99 \pm 0.01	2.97 \pm 0.21	2.95 \pm 0.20	2.92 \pm 0.28	3.22 \pm 0.50	3.28 \pm 0.22
60	0.99 \pm 0.02	2.98 \pm 0.27	2.96 \pm 0.26	2.92 \pm 0.20	3.23 \pm 0.47	3.28 \pm 0.26

Table 2. General properties of selective amino acids.

Name	Solubility (g/100 mL of water at 25°C)
Aspirin	0.0032
Glycine	24.9910
Tyrosine	0.0453
Glutamic acid	0.8641
Tartaric acid	0.1002
Aspartic acid	0.7781

ity can be observed. It is therefore suggested to the prescribers, to instruct the patients to avoid the use of amino acids along with these selective AA (5).

It is also evident from the results that the increase in absorbance of aspirin in the presence of tartaric acid and aspartic acid was non-significantly ($p > 0.05$) greater than that of other AA like glycine, tyrosine and glutamic acid. The absorbance of aspirin in the presence of tartaric acid and aspartic acid was 3.23 and 3.28, respectively, while the absorbance of aspirin in the presence of glycine, tyrosine and glutamic acid was 2.98, 2.96 and 2.92, respectively (Tab. 1). The possible reason for this increased absorbance by aspirin in the presence of tartaric acid and aspartic acid could be due to the presence of high number of hydroxyl groups present in these amino acids (Fig. 1).

This study is interesting not only for pharmacists but also for nutritionists, rather for clinical nutritionists dealing with parenteral feeding. There are several studies in the literature in which interaction of aspirin with high protein products of vegetable or animal origin has been discussed. Odou et al. found significant ($p < 0.05$) effect of concomitantly administered beverages (milk, spirit, and beer) on the pharmacokinetics of orally administered aspirin in healthy human volunteers (17). Yoovathaworn et al. (18) and Thithapandha (19) also determined the influence of caffeine on the pharmacokinetic parameters of aspirin in twelve healthy male human. Both research groups observed that caffeine significantly ($p < 0.05$) increased the rate of aspirin absorption (18, 19).

LIMITATION OF STUDY

In a normal diet for a healthy person or patient (except for parenteral nutrition), amino acids are not generally present as a single molecule, they must

compete with many other components. This study should therefore, be expanded to a complex system of nutrients.

CONCLUSION

This study elaborates that the solubility of aspirin increases with concomitant administration of AA, thus the use of AA (proteinous foods) with aspirin should be prohibited or low dose of aspirin should be recommended in such situation. The results of this study will help in prescribing aspirin with these selective AA to avoid any biological incompatibility, i.e., incompatibility between drug and food.

REFERENCES

- Schorr K.: Acetylsalicylic acid. 4th edn., p. 25, Ameer Publishers, Canada 2006.
- Enger E.D., Ross F.C., Bailey D.B.: Concepts in biology. 12th edn., McGraw-Hill, Boston 2007.
- IARC Working Group on the Evaluation of Cancer Preventive Agents, International Agency for Research on Cancer, Non-steroidal Anti-inflammatory Drugs, Spain.
- Stoker H.S.: General, Organic, and Biological Chemistry. 5th edn., p. 15, Huvian Publishers, Amsterdam 2002.
- Connors K.A., Connors K.A., Amidon G.L.: Chemical Stability of Pharmaceuticals. 2nd edn., p. 222, Saber Publishers, Barcelona 2005.
- Saeb K.: Instant pharmacology. p. 187, Nui Publishers, Auckland 2003.
- Seager S.L., Slabaugh M.R.: Chemistry for Today: general, organic and biochemistry. 3rd edn., p. 203, Saber Publishers, Barcelona 1996.
- Stolerman L.P.: Encyclopedia of Psychopharmacology. 7th edn., p. 1, Nevian Publishers, Sydney 1994.
- Fried B., Sherma J.: Thin layer chromatography. vol. 4, p. 289, SRS Publishers, Kolkata 1999.
- Murtaza G., Khan S.A., Shabbir A., Mahmood A., Asad M.H.H.B., Farzana K., Malik N.S., Hussain I.: Sci. Res. Essays 6, 417 (2011).
- Agarkar P.H., Maheshwari V.L., Kulkarni Y.A.: Biochemistry for Nursing. 3rd edn., p. 3, CBC Publishers, Mumbai 1987.
- The Merck Index, Merck & Co., Inc., Whitehouse Station, NJ 1996.
- Gupta V.: Handbook of Chemistry and Physics. p.58, CRC Press, Cleveland 1977.

14. Agarkar P.H., Maheshwari V.L., Kulkarn Y.A.: Biochemistry for Nursing. 5th edn., p. 3, CBC Publishers, Mumbai 1992.
15. Rasool F., Ahmad M., Khan H.M.S., Khan S.A., Murtaza G.: Asian J. Chem. 23, 3815 (2011).
16. Murtaza G.: Latin Am. J. Pharm., 30, 1389 (2011).
17. Odou P., Barthelemy C., Robert H.: J. Clin. Pharm. Therap. 26, 187 (2001).
18. Yoovathaworn K.C., Sriwatanakul K., Thithapandha A.: Eur. J. Drug Metab. Pharmacokinet. 11, 71 (1986).
19. Thithapandha A.: J. Med. Assoc. Thai. 72, 562 (1989).

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