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**Transdermal Aspirin : Influence of Platelet Aggregation and Serum Lipid Peroxides**

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Aspirin (ASP) inhibits H cyclo-oxygenase (COX) irreversibly and hence thromboxane A<sub>2</sub> (TXA<sub>2</sub>) to prevent platelet aggregation. Orally administered ASP requires high and frequent dosing because it undergoes extensive presystemic hydrolysis in the gut and the liver into salicylic acid (SAL) which is devoid of antiplatelet activity. Continuous exposure of new platelets to ASP is necessary to achieve prolonged inhibition of platelet aggregation. We developed a matrix type transdermal patch of ASP using polyvinyl alcohol (with and without hydrolysis inhibitor and a penetration enhancer). The transdermal flux of ASP, in rat skin was 11.85 and 52.92 µg/cm<sup>2</sup>.h without and with penetration enhancer respectively. Transdermal administration in human volunteers resulted in a significant reduction in serum lipid peroxides (MDA levels: 0.998±0.899 to 0.236±0.076 nM/ml) and inhibition of platelet aggregation.

The most important advantages of transdermal delivery include reduction of side effects due to optimization of the blood concentration-time profile and extended duration of action, which allows greater patient compliance owing to elimination of multiple dosing schedules. Transdermal delivery may also improve the therapeutic value of many drugs by obviating specific problems associated with the drug e.g., gastrointestinal irritation, low absorption, 'first-pass' effect and short half-life necessitating frequent dosing'. The role of platelets in the pathogenesis of atherosclerosis, thrombosis and thromboembolism has been well established. In view of the recognized role played by platelets in the complications associated with coronary artery disease and cerebrovascular disease, there is considerable interest on platelet aggregation inhibitory drug ASP<sup>2,3</sup>.

ASP, but not SAL inhibits COX in platelets permanently by acetylating the serine residue. Since platelets

lack DNA, they cannot resynthesize the new enzyme. Prostacyclin (PGI<sub>2</sub>) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) the predominant COX-mediated metabolites of arachidonic acid in vascular endothelium and platelets, respectively, have potent and opposite effects on vascular tone and platelet function<sup>4,5</sup>. The platelet inhibitory properties of ASP result from its ability to acetylate and irreversibly inhibit platelet COX, thereby reducing the formulation of TXA<sub>2</sub>. ASP at doses above 100 mg. p.o. and SAL reduce the biosynthesis of PGI<sub>2</sub> which is however, recovered to normal levels within 36 h. The platelets released into blood subsequently would have normal enzyme activity because by then ASP is already hydrolyzed<sup>6</sup>. The half-life of ASP is about 15 min while that of SAL is 2.5 h<sup>7</sup>. ASP could be delivered transdermally by patch at a dose that suppresses platelet COX, but low delivery rates due to extensive hydrolysis in skin, have been reported<sup>8</sup>. In the present investigation, we developed a matrix type ASP patch with or without a penetration enhancer. Since long-term, very frequent, oral dosing with ASP tablet (every

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2-3 h) is not practical, we tried in our study to administer ASP transdermally to meet the requirements of controlling or preventing thrombosis. An attempt was also made to reduce the hydrolysis of ASP in the formulation and in the skin. The pharmacodynamic response was measured by recording the platelet aggregation produced by adrenaline challenge and by serum lipid peroxidation end products (as malondialdehyde, MDA) in the samples obtained from subjects before and after application of the patch.

## MATERIALS AND METHODS

ASP was a kind gift by Natco Pharmaceuticals, Hyderabad and polyvinyl alcohol (PVA) was purchased from S.D. Fine Chemicals (P) Ltd., Bombay. Methanol and acetonitrile of HPLC grade were purchased from Ranbaxy Labs, New Delhi. The other chemicals used in the study were of AR grade.

### Preparation of matrix patch:

PVA was used to fabricate matrix device. In formulation 1 (FM1), 500 mg of PVA and ASP (50 - 200 mg) were dissolved in methanol, whereas in formulation 2 (FM2), 100  $\mu$ l dimethyl formamide (DMF) to enhance penetration and 250 mg of sodium fluoride (NAF) to prevent hydrolysis<sup>9</sup> of ASP were also included. 350  $\mu$ l of drug-polymer solution containing 70 mg of ASP were casted as a thin film on a sheet of aluminium foil, which served as backing membrane. After overnight drying at room temperature in a desiccator, the patches were stored in an airtight container. For the *in vivo* studies the composition of the patch was similar to that of FM2 but without DMF and the size of the patch was 12 cm<sup>2</sup>.

### *In vitro* skin diffusion studies:

The skin of albino rats was removed from the backside and stored at - 20°. The epidermal layer (comprising of stratum corneum and viable epidermis) was separated by immersing the skin in hot water (approx. 60°) for 2 min and used for diffusion studies.

A specially designed teflon diffusion cell, which was described in earlier studies<sup>10</sup>, was used in the present investigations. The two compartments of the cell, the receiver and the donor compartments were filled with distilled water. The temperature of diffusion cells was maintained at 37°. The cells were continuously agitated. The contents of receiver compartment were emptied at predetermined intervals of time and replaced with fresh medium, so that 'sink' conditions are maintained

constantly. Samples were stored at 20° and analyzed by HPLC within 12 h.

### HPLC Analysis:

The *in vitro* skin diffusion study samples were analyzed for ASP and SAL by HPLC method reported earlier<sup>11</sup>. The high performance liquid chromatograph of Shimadzu (Japan) equipped with an SPD-M6A. Photodiode array detector, LC-10AS pump and a Rheodyne injector was employed. A 30 cm x 3.9 mm I.D. Bondapack C18 column (Waters-USA) with 5- $\mu$ m spherical particles was used. A mixture of methanol and water (80 : 00, pH was adjusted to 2.5 with sulfuric acid) was used as mobile phase. The analysis was carried out at a flow rate of 1 ml per minute and at 234 nm.

Benzanilide served as internal standard. 250  $\mu$ l samples were acidified by the addition of 150  $\mu$ l of 50% HCl and shaken with 6 ml of dichloromethane for two minutes. The organic layer was separated, after centrifugation at 3000 g for 10 min and dried by application of vacuum. The residue was reconstituted in 50  $\mu$ l methanol and 20  $\mu$ l were injected into the HPLC column. The concentrations of ASP and SAL were calculated from standard graphs.

### Studies in human subjects:

Eight healthy male volunteers aged between 23-27 years and weighing 60-72 kg. participated in the study. Informed consent from volunteers and the approval of the local ethical committee were obtained. On the forearm of each volunteer a transdermal patch EM2 (with 200 mg ASP and without DMF) was placed. Ten-ml blood samples were collected before application of the patch and after six hours, at the time of its removal.

### Platelet aggregation:

Platelet aggregation study was performed in the plasma, by modified method of O'Brien<sup>12</sup> using Systronics photocolormeter. Adrenaline solution at a final concentration of 5  $\mu$ M was used to induce platelet aggregation and the absorbance was measured after 1,2,3,4,5 and 6 min while gently mixing the contents of the cuvette with a fine stirrer.

### Serum MDA (lipid peroxides) levels:

Serum levels of MDA before and after administration of ASP patch were determined by thiobarbituric acid (TBA)-HPLC method<sup>13</sup>. HPLC system with the same column, described under HPLC was employed.

**TABLE 1 : SERUM MDA LEVELS (nM/ml) FOLLOWING THE APPLICATION OF ASP TRANSDERMAL PATCH IN HEALTHY VOLUNTEERS**

Volunteer	Before	After 6 h
I	2.84	0.36
II	1.14	0.29
III	1.67	0.26
IV	0.45	0.21
V	0.34	0.28
VI	0.31	0.21
VII	0.23	0.13
VIII	1.00	0.15
MEAN±SD	0.998±0.899	0.236±0.076

#### Statistical Analysis:

Statistical analysis was performed using Graphpad Prism Software, Inc., San Diego, CA). Paired 't' test at 5% level of significance, was conducted to test the differences in mean(±SD) aggregation half-life and mean (±SD) serum MDA levels before and after the application of the patch.

#### RESULTS

The mean cumulative percentage diffused through rat skin Vs profiles of ASP patch formulations are shown in figure 1. The diffusion of ASP from both formulations was nearly linear. The mean±SD cumulative percentage of dose diffused after 24 h from FM2 was 2.46±1.87 which significantly higher than that diffused from FM1 (0.52±0.16). The concentrations of ASP and SAL (from hydrolysis) in receiver compartment at different sampling time points were determined and the ratio between the two has been plotted against time (Fig. 2).

The platelet aggregation profiles, before and after transdermal administration of ASP (from FM2 without DMF) are shown in figure 3. The aggregation profiles showed a biexponential fall, which is initially rapid and later slow. Terminal aggregation half-lives were determined in each subject and the mean±SD values before and six hours after application of the patch were 13.46±12.93 and 30.87 ±21.27 h respectively. The increase in the aggregation half-life was significant (p<0.05).

The serum level of MDA before and after application of the ASP patch are given in the table. It is evident from

the data that transdermal application of ASP resulted in a significant reduction (p<0.05) in MDA levels from 0.998±0.899 to 0.236±0.076 nM/ml.

#### DISCUSSION

Among numerous substances inhibiting platelet function, ASP is unique in causing an irreversible defect persisting for the life span of the exposed platelets. The mechanism underlying this long-lasting defect involves acetylation by ASP of the active site of platelet COX, which results in irreversible inactivation of the enzyme<sup>14</sup>. Oral ASP doses as low as 150 mg result in a permanent defect in the ASP exposed platelets<sup>12</sup>. Larger doses have little additional effect, a characteristic feature of a saturable process. Human platelets exposed to 40µM ASP for 15 min show marked inhibition of the collagen-induced release reaction *in vitro*<sup>15</sup>, as do rabbit platelets incubated with 30 µM ASP for 10 min<sup>16</sup>.

From the *in vitro* studies it is evident that ASP was hydrolyzed during skin diffusion, both in donor and receiver compartments. The figure showing ratio of ASP/SAL at different time points in the receiver compartment indicates that in FM2 which contained NaF, the hydrolysis of ASP was significantly lower (by 50%) than in FM1. ASP diffusion through rat skin from FM2 was superior to that FM1, which is evidently because of the presence of DMF. The experimental flux from the two formulations FM1 and FM2 was 11.85 and 52.92 µg/cm<sup>2</sup>.h respectively.

The theoretical transdermal flux of ASP calculated by using the following equation<sup>17</sup> is 303.322 µg/cm<sup>2</sup>.h.

$$J = 36 Cw/2.82 + (29.6/P)$$

Where Cw is aqueous solubility of the drug (10mg/ml)

P is the Octanol/Water partition coefficient (0.025)

Such a wide deviation between the theoretical and experimental flux values has been reported in many instances and could be attributed to many reasons<sup>18</sup>.

ASP is extremely sensitive to hydrolysis in the biological fluids and estimation of low concentration of ASP in blood requires special techniques like isotope labeling.

Therefore in the present study an attempt has been made to measure the systemic availability of ASP in terms of its pharmacodynamic activities, inhibition of platelet aggregation and serum MDA levels. Different methods using aggregometer<sup>19</sup> and platelet counter<sup>20</sup> have

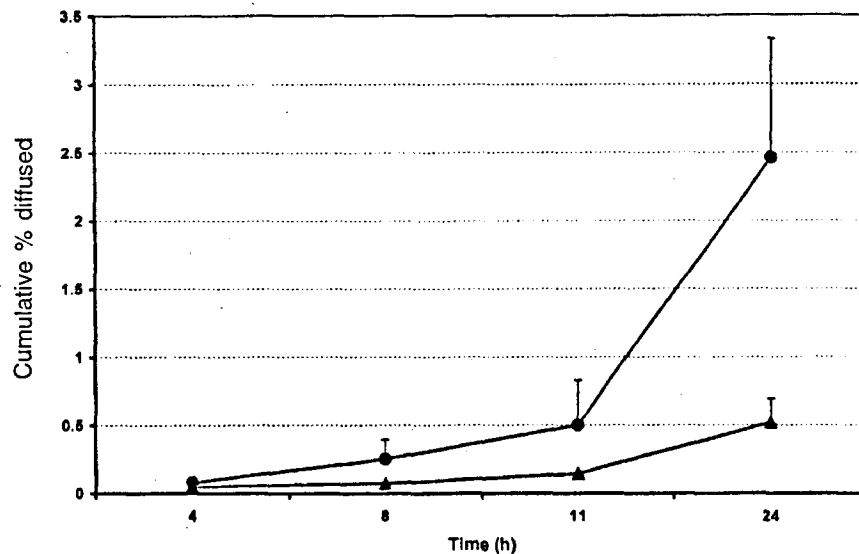


Fig. 1: *In vitro* diffusion of aspirin through rat skin.

Each point is mean plus or minus standard deviation of 4 determinations representing cumulative per cent drug diffused from transdermal patch FM<sub>1</sub> -▲- and transdermal patch FM<sub>2</sub> -■-

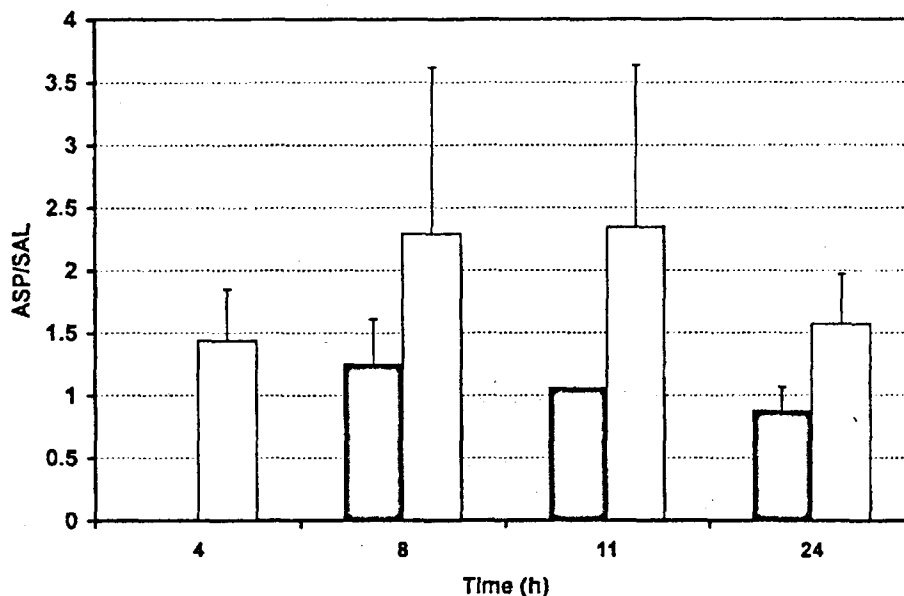


Fig. 2: Hydrolytic stability of aspirin during diffusion experiments.

Hydrolytic stability of aspirin (ASP) is measured in the receiver compartment and expressed as the ratio of ASP and salicylic acid (SAL) from transdermal patch FM<sub>1</sub> -■- and transdermal patch FM<sub>2</sub> -□-

been reported to study platelet aggregation. In the present investigation the method of O'Brien was employed<sup>12</sup>. The platelet aggregation could be induced in plasma by any known pro-aggregating agent like adenosine diphosphate (ADP), thrombin, 5-HT and adrenaline<sup>15</sup>. We observed that aggregation induced by adrenaline was satisfactory. The fall in optical density with time was found to be directly

proportional to the degree of aggregation and presence of ASP prolongs the aggregation induced by adrenaline. Misselwitz and co-workers successfully employed the determination of 'aggregation rate (AR)' induced by ADP in studying the long-term activity of ASP in-patients of atherosclerosis<sup>16</sup>. The aggregation profiles exhibited a non-linear pattern and the characteristics of the aggregation

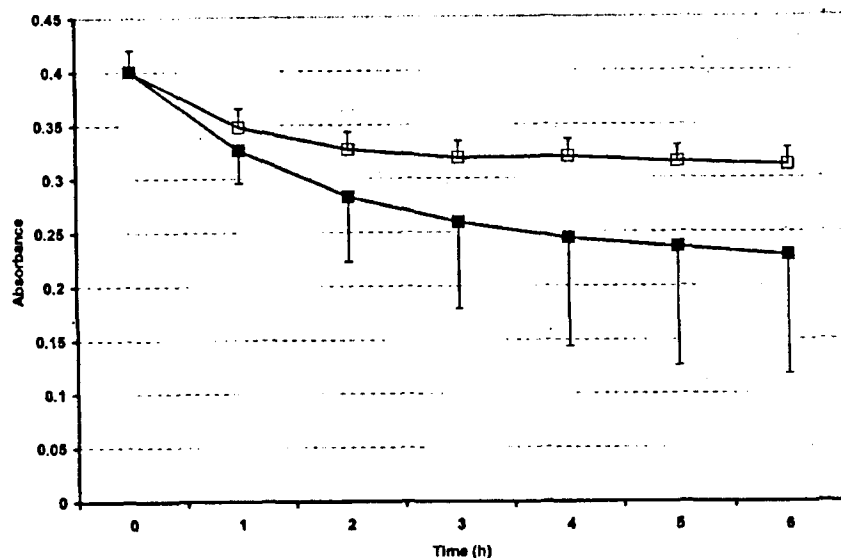


Fig. 3 : Platelet aggregation in healthy human volunteers

Platelet aggregation of blood samples collected from healthy human volunteers (n=8) before —■— and six hours after —□— the application of 200 mg ASP patch

could be analyzed by regression analysis.

Many workers have reported earlier that there is a significant fall in MDA levels after administration of oral aspirin and they unequivocally agree that the serum MDA level is a useful indicator of platelet TXA<sub>2</sub> synthesis and therefore, aspirin availability/activity at the platelet sites<sup>21-24</sup>. The results of the present study clearly indicate that transdermal aspirin (200 mg) is effective in significantly inhibiting the platelet aggregation and formation of serum MDA ( $p < 0.05$ ) 6 h after application. It is therefore concluded that ASP could be delivered by transdermal route to alter the platelet function effectively. It is, however, important to conduct experiments to prove the efficacy of such formulation on long-term administration.

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