Background and Purpose—The widespread use of aspirin requires clarification of the aspirin resistance phenomenon. Most studies on this field are focused on patients which may affect the action of aspirin.

Methods—We evaluated the biological efficacy of aspirin in healthy subjects.

Results—Agonist–induced platelet aggregation was fully abrogated by 100 mg of aspirin in all individuals. By contrast, with the platelet function analyzer-100 device, 33.3% of the subjects displayed no response. This failure was overcome by 500 mg or by in vitro treatment of blood with 30 μmol/L acetylsalicylic acid. Intake of 100 mg of aspirin efficiently reduced by 75% the level of 11-dehydro thromboxane B2 (11-dTxB2) in all cases. However, variability on the pre-aspirin level (range 72.4 to 625.9 ng/mmol creatinine) led to substantial differences in the residual amount of the metabolite between subjects treated with aspirin (range 12.9 to 118.0 ng/mmol creatinine). Finally, there was no influence of platelet glycoprotein IIb/IIIa (Pro33Leu), platelet glycoprotein Ia/IIa, (C807T), and FXIII (Val34Leu) polymorphisms on the efficacy of aspirin. However, the cyclooxygenase (Cox)-1 507 allele associated with higher level of 11-dTxB2, both before and after aspirin. Moreover, the Cox-2 765C variant displayed a slightly higher reduction in 11-dTxB2 level on treatment with aspirin.

Conclusions—Our findings suggest that full resistance of healthy subjects to aspirin is rather unlikely. However, differences in aspirin absorption, or pharmacokinetic, or other unrecognized factors may lead to lack of effect of low dose of aspirin in some subjects when using tests like platelet function analyzer-100. Whether Cox polymorphisms are thrombotic risk factor for patients under aspirin will require further research. (Stroke. 2005;36:276-280.)

Key Words: aspirin ■ platelet function tests ■ polymorphisms

A high percentage of patients still experience a vascular event despite aspirin treatment giving rise to the concept of aspirin resistance.1,2 The definition of the aspirin resistance phenomenon is controversial, and thus the reported range varies broadly, from 5% to 40%, depending on the assay used for identification and the population studied.2–4

From a biochemical point of view, aspirin resistance would refer to patients who are taking aspirin but do not display an adequate degree of platelet inhibition.3 Several laboratory tests are being used to assess the response to aspirin. Early reports have provided contradictory results based on measurements of platelet aggregation.5,6 More recent studies assessed the response to aspirin by measuring the urinary level of 11-dehydro thromboxane B2 (11-dTxB2) as an index of systemic generation of thromboxane A2. Again, inconclusive results have been reported.7,8 Currently, the most appealing test in clinical practice for assessment of platelet inhibition by aspirin is the platelet function analyzer (PFA)-100.9 By using this test, a high prevalence of aspirin resistance (20% to 40%) has been reported in various recent reports.9–11

Few studies have assessed the efficiency of the aspirin treatment in the same population using those different tests. Although some authors have reported correlation between the results of optical aggregometry and PFA-100,6 discrepancies have been also reported.4,10 Indeed, the lack of a suitable tool for identifying biological aspirin resistance is nowadays an aim of debate.12

Discrepancies in results among previous studies may likely rely on the fact that the individual response to aspirin is a continuous variable and may depend on interaction of acquired and genetics factors.13 Most previous studies involved patients whose particular atherothrombotic and inflammatory environment would largely influence the response to aspirin. Our current study investigating healthy subjects should reduce the degree of influence of acquired factors and would...
help to identify genetic factors involved in aspirin resistance. Our search has been focused on common genetic variations previously suggested to be associated with this phenomenon: cyclooxygenase (Cox)-1 (C50T),\textsuperscript{14} Cox-2 (G-765C),\textsuperscript{15} the fibrinogen receptor platelet glycoprotein (GP) IIb/IIIa (Pro33Leu), the platelet collagen receptor, GP Ia/IIa, (C807T), and the FXIII (Val34Leu).\textsuperscript{16,17}

**Materials and Methods**

The study involved 24 white healthy subjects from our staff (13 men and 11 women; 35.6±5.9 years). Among them, 4 were selected because of their GP IIa 33Leu genotype, because this polymorphism had been often related to aspirin resistance. All of them had hematological and biochemical parameters within the normal range. Participants abstained from taking any drug for at least 2 weeks before enrolment. The study was conducted in agreement with the Declaration of Helsinki and was approved by a local ethical committee.

**Study Design**

Participants took 100 mg of aspirin (Bayer) after lunch on days 1 and 2 of the study. This guideline has been shown efficient to block production of TxA\textsubscript{2} for the whole platelet life.\textsuperscript{18} Citrate anticoagulated venous blood and first morning urine specimen were collected on fasting conditions at day 1 (pre-aspirin sample) and thereafter at day 3.

In 7 individuals selected because of an apparent failure of response to 100 mg of aspirin, the study was repeated 2 weeks later with a dose of 500 mg. Also in these subjects, in vitro studies were performed by incubating untreated citrated whole blood with 30 \(\mu\)mol/L acetylsalicylic acid (Sigma-Aldrich) for 30 minutes at room temperature. Such concentration approaches the plasma concentration found in patients treated orally with 100 mg per day.\textsuperscript{19}

When needed, platelet rich plasma and platelet poor plasma were obtained from citrated blood samples (0.129 mol/L citrate-containing tubes; Vacutainer, Becton Dickinson, Meylon, France). Functional studies were performed in platelet-rich plasma or whole blood within 2 hours of extraction. Urine and platelet-poor plasma samples were stored at \(-70^\circ\text{C}\) until analysis. For cell counts, biochemical determinations and genotyping, additional blood samples were obtained in EDTA-containing tube (Vacutainer, Becton Dickinson).

**Platelet Aggregation**

Platelet aggregation was evaluated in citrated platelet-rich plasma (3\(\times\)10\textsuperscript{5} platelets/\(\mu\)L). Aggregation was induced with either 1 mmol/L arachidonic acid or collagen 10 \(\mu\)g/mL (Menarini Diagnostics). Changes in light transmission were recorded for a total time of 5 minutes using an Aggrecorder II aggregometer (Menarini Diagnostics).

**11-Dehydro Thromboxane B\textsubscript{2} Levels**

The pre- and post-aspirin levels of 11-dTxB\textsubscript{2} were determined in urine samples, by means of a commercially available ELISA (11-dehydro Thromboxane B\textsubscript{2}, EIA Kit Cayman Chemical) following the manufacturer’s instructions. All urine samples were assayed in duplicate, and the mean intra-assay coefficient of variation (CV, \%) was 3.72±3.5. The interassay CV from 3 samples assayed in duplicate in 2 different days was 10.1±2.7.

**Platelet Function Analyzer-100**

Citrated whole blood was stabilized at room temperature for 30 minutes and tested in the PFA-100 (Dade Behring) using collagen-epinephrine cartridges. The closure time was recorded up to the upper device limit of 300 s.

**Genotyping**

Genotyping of GP IIa, GP Ia, and FXIII polymorphisms was performed by polymerase chain reaction as previously described.\textsuperscript{20–22} Determination of the Cox-1 C50T and Cox-2 G-765C genotypes were performed by polymerase chain reaction–allelic restriction assay with Fau-I (Fermentas, Pascual and Furío) and single-stranded conformational polymorphism, respectively, using specific primers (Cox-1F: 5'-GGTCCCGTGGGGAATTTTC3'; Cox-1B: 5'-GGGGGAAAGGOGTTG 3'; Cox-2F: 5'-CCGCCTCCCTTTGTCACATC3'; Cox-2B: 5'-GGCTGTATATCTGC-TCTATG3').

**Statistical Analysis**

Results are expressed as mean value±SD for continuous variables and as percentages for categorical variables. Differences between pre- and post-aspirin values were assessed by 2-tailed paired \(t\) test. Regression analysis was performed to assess significant correlation between pre- and post-aspirin values and between results from different tests. The influence of the investigated polymorphisms in the value of the distinct parameters, was evaluated by analysis of variance. The potential risk for failure of aspirin associated to these polymorphisms was assessed by the \(\chi^2\) test. Differences among groups for each individual test were considered significant when uncorrected \(P<0.05\).

**Results**

**Platelet Aggregation**

All individuals displayed maximal platelet aggregation, both in response to arachidonic acid 1 mmol/L and collagen 10 \(\mu\)g/mL in pre-aspirin samples (mean percentage of aggregation, 88.1±6.6 and 79.31±11.6, respectively). This response was inhibited by more than 90% with aspirin 100 mg (mean percentage of aggregation, 3.7±1.2 and 4.2±1.3, respectively). Thus according to this test, all individuals can be classified as normal responders to aspirin 100 mg.

**11-Dehydro Thromboxane B\textsubscript{2} Levels**

The 11-dTxB\textsubscript{2} pre-aspirin levels were highly variable among subjects (mean, 179.5±142.0; range, 72.4 to 625.9 ng/mmol creatinine; CV=80%). After 100 mg of aspirin, we still observed a significant heterogeneity in the 11-dTxB\textsubscript{2} levels (mean, 39.8±22.9; range, 12.9 to 118.0 ng/mmol creatinine; CV=58%), because all subjects displayed a high and rather uniform reduction in their 11-dTxB\textsubscript{2} levels (mean percentage of reduction, 75.1±9.1; range, 55.1 to 87.5%; CV=12%). Regression analysis demonstrated that the post-aspirin level of 11-dTxB\textsubscript{2} significantly correlated to the pre-aspirin level \((R^2=0.729; P=0.0001)\).

**Platelet Function Analyzer-100**

We observed a moderate variability on the pre-aspirin values of the closure time (128.7±26.3 s; range, 84 to 186 s; n=24; CV=20.5%). In 16 of 24 subjects (66.7%) the post-aspirin (100 mg) closure time exceeded the 300 s measurable by the device. All these subjects were therefore considered as normal responders to this dose of aspirin. However, in 8 of 24 subjects (33.3%), the closure time after aspirin remains below 300 s, and these subjects were considered as nonresponders to this aspirin dose (Table 1).

Of mention, the pre-aspirin closure time in these 7 nonresponders subjects (117.5±22.7 s) was not significantly different from that in the normal responders (134.4±26.8 s; \(P=0.14\)). Moreover, there were no significant differences among normal and nonresponders to 100 mg of aspirin in either hematocrit (41.8±3.8 versus 44.5±4.3%, \(P=0.13\)),
leukocyte counts (6.2 ± 1.2 versus 6.5 ± 1.4; P = 0.63) (×10⁹/L), and platelet counts (244 ± 47 versus 256 ± 58; P = 0.58) (×10⁹/L). The von Willebrand factor antigen plasma levels (vWF:Ag, %) were also similar in these normal and abnormal responders to aspirin treatment (93.9 ± 23.5 versus 111.7 ± 21.7, before aspirin, P = 0.21; 85.6 ± 15.3 versus 93.7 ± 16.1, after 2 days with aspirin, P = 0.41).

Two weeks later, the individuals with apparent resistance to 100 mg of aspirin (subject K was excluded as he developed a mild allergic response to aspirin) were further investigated. We also selected 2 subjects with complete response to 100 mg of aspirin as controls (I and J). The pre-aspirin closure times were similar to those found previously (Table 1). As shown, both the intake of 500 mg of aspirin or the in vitro incubation caused prolongation of the closure times >300 s in all cases. According to these data, no subject can be classified as nonresponder to this high dose of aspirin or to the in vitro incubation of blood with a low acetylsalicylic acid dose.

Correlation Between 11-dTxB₂ Level and PFA-100 Closure Time

The regression analysis revealed no statistical association between 11-dTxB₂ levels and PFA-100 closure times, neither pre-aspirin (P = 0.519) nor post-aspirin (P = 0.445).

Genotype and Efficacy of Aspirin

The prevalence of C807T and FXIII genotypes was similar to that described in Mediterranean populations (Table 2). Moreover, the Cox-1 50T and Cox-2 G-765C polymorphisms displayed similar frequencies than that previously described in other populations (Table 2).

In all subjects, the platelet aggregation was maximal in absence of aspirin and negligible after intake of 100 mg of aspirin irrespective of their genotypes. Neither did we find association between these polymorphisms and PFA-100 values either pre- or post-aspirin (Table 2). Interestingly, the levels of 11-dTxB₂ were significantly higher in carriers of the Cox-1 50T allele than in 50C carriers, both pre-aspirin (375.9 ± 275 and 140.3 ± 54.2 ng/mmol creatinine, respectively; P = 0.001) and post-aspirin (62.1 ± 43.7 and 35.4 ± 14.7 ng/mmol creatinine, respectively; P = 0.031). However, we observed no significant influence of this polymorphism on the reduction in 11-dTxB₂ level after aspirin intake (P = 0.104; Table 2). In contrast, we found a slight association between the impairment of 11-dTxB₂ caused by 100 mg of aspirin and the Cox-2 genotype. Thus, the −765C carriers seemed to be more sensitive to this dose of aspirin than homozygous −765G subjects (P = 0.041; Table 2).

Discussion

Many studies have addressed the aspirin resistance phenomenon, providing controversial results and reflecting the biological complexity of this concept. A current limitation of most previous studies is that they involved patients with an atherothrombotic and inflammatory background and already on aspirin treatment. These designs may underscore the aspirin response, because the pre-aspirin values for the different parameters investigated are not recorded. Our study largely differs from others by investigating the response to aspirin in healthy subjects, primarily lacking risk factors that may influence the effect of the drug. Moreover, the study of this population could help to clarify the role of common genetic changes on the heterogeneous response to aspirin.

Our results testing platelet aggregation before and after intake of low dose of aspirin (100 mg) further indicated that this test would unlikely be useful to screen for aspirin resistance in healthy people as it has been previously reported. By contrast, with PFA-100 testing we identified a high percentage of healthy subjects, similar to that found in

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**TABLE 1. Effect of In Vivo Treatment With High Dose of Aspirin (500 mg) or In Vitro Exposition of Platelet to Acetylsalicylic Acid on the PFA-100 Closure Times (s) of Healthy Subjects Displaying No Response to Low Dose (100 mg) of Aspirin**

<table>
<thead>
<tr>
<th>Subject</th>
<th>100 mg Aspirin Pre</th>
<th>500 mg Aspirin Pre</th>
<th>In Vitro 30 μmol/L Acetylsalicylic Acid Pre</th>
<th>100 mg Aspirin Post</th>
<th>500 mg Aspirin Post</th>
<th>In Vitro 30 μmol/L Acetylsalicylic Acid Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>126</td>
<td>144</td>
<td>135</td>
<td>&gt;300</td>
<td>136</td>
<td>&gt;300</td>
</tr>
<tr>
<td>B</td>
<td>156</td>
<td>197</td>
<td>152</td>
<td>&gt;300</td>
<td>150</td>
<td>&gt;300</td>
</tr>
<tr>
<td>C</td>
<td>88</td>
<td>103</td>
<td>88</td>
<td>&gt;300</td>
<td>100</td>
<td>&gt;300</td>
</tr>
<tr>
<td>D</td>
<td>124</td>
<td>163</td>
<td>94</td>
<td>&gt;300</td>
<td>120</td>
<td>&gt;300</td>
</tr>
<tr>
<td>E</td>
<td>121</td>
<td>266</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<td>139</td>
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<td>84</td>
<td>178</td>
<td>91</td>
<td>&gt;300</td>
<td>90</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

Normal responders (n=16) 134.4±26.8 >300

**Values for normal responders (mean±SD) are given for reference.**

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**TABLE 2. Genotype Distribution of the Studied Subjects and Influence of Different Polymorphisms on PFA-100 Values and on Urine 11-dTxB₂ Levels Before and After 100 mg of Aspirin**

<table>
<thead>
<tr>
<th>FXII Val4Leu</th>
<th>GP IIa Pro33Leu</th>
<th>GP IIa C807T</th>
<th>Cox-1 50T</th>
<th>Cox-2 G-765C</th>
</tr>
</thead>
<tbody>
<tr>
<td>W (n=15)</td>
<td>P</td>
<td>CC (n=12)</td>
<td>CT + T (n=12)</td>
<td>P</td>
</tr>
<tr>
<td>135.6±22.8</td>
<td>117.3±29.2</td>
<td>125.0±19.4</td>
<td>0.696</td>
<td>128.6±26.6</td>
</tr>
<tr>
<td>P200</td>
<td>128.9±22.4</td>
<td>129.5±26.9</td>
<td>0.951</td>
<td>132.0±28.9</td>
</tr>
<tr>
<td>128.7±24.5</td>
<td>120.8±26.7</td>
<td>0.144</td>
<td>128.6±26.6</td>
<td>125.1±20.5</td>
</tr>
<tr>
<td>P</td>
<td>0.677</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PFA-100 closure time pre-aspirin, s**

**PFA-100 nonresponders** 6 2 0.657 7 1 0.621 2 6 0.193 7 1 1.0 4 4 0.167

**11-dTxB₂, pre-aspirin (ng/mmol creat)**

| 178.7±126.2 | 180.9±174.9 | 0.972 | 168.0±120.7 | 213.5±205.4 | 0.513 | 132.0±52.4 | 227.1±186.7 | 0.104 | 140.3±54.2 | 375.9±275.0 | 0.001 | 161.1±128.9 | 224.4±174.3 | 0.334 |

**11-dTxB₂, post-aspirin (ng/mmol creat)**

| 38.9±16.3   | 41.3±35.2     | 0.817 | 37.9±17.8   | 48.3±35.3   | 0.308 | 31.7±14.3  | 47.9±27.6   | 0.083 | 35.4±14.7  | 62.1±43.7   | 0.031 | 39.5±23.5  | 40.5±23.7   | 0.927 |

**11-dTxB₂, inhibition, %**

| 75.3         | 74.9          | 0.934 | 75.5        | 74.3        | 0.781 | 74.9        | 75.3        | 0.919 | 73.8        | 81.8        | 0.104 | 72.6        | 80.9        | 0.041 |

*Subjects whose closure time was <300 s. Creat indicates creatine. Continuous variables are expressed as mean±SD. The influence of the genotype was evaluated by analysis of variance. The potential risk for failure of aspirin associated to these genotypes was assessed by Fisher exact 2-tailed P value.*
patients as nonresponders to low dose of aspirin. However, these individuals are by no means fully resistant to aspirin, because in all, a 5-fold increase in aspirin dose caused prolongation of the closure time above 300 s. Moreover, these subjects displayed closure times >300 s when untreated blood samples were incubated in vitro with 30 μmol/L aspirin. These findings suggest that the lack of response to 100 mg of aspirin may be related with reduced bioavailability of the drug. Indeed, individuals treated orally with low dose of aspirin display great interindividual differences in the plasma concentration–time profiles for acetylsalicylic acid. Our data are also in agreement with those reported by Alberts et al., who showed that the prevalence of aspirin-resistant patients in PFA-100 testing declines by ~50% by raising the aspirin treatment from 81 to 325 mg per day. Yet, there is still no conclusive evidence that increasing aspirin to tolerable levels could be clinically useful in patients behaving as resistant to low dose of aspirin. Although in this study the PFA-100 results of nonresponders to low dose of aspirin seem unrelated to significantly different hematocrit, platelet counts, or reduced vWF:Ag levels, the PFA-100 assay is not a specific test to measure the full resistance to aspirin is unlikely. Thus, the apparent failure of response to low dose of aspirin observed in some individuals could be a predictor of worse outcome or if it could identify patients who may benefit from alternative therapies that more effectively reduces in vivo thromboxane production, should be further investigated.

In this study, we found no relationship among the studied polymorphisms and a defective response to aspirin as evaluated with aggregometry or PFA-100. However, we observed that the Cox-1 50T variant associated with significantly higher levels of 11-dTxB2 (both in absence and presence of aspirin). In addition, the Cox-2 −765C allele associated with a mild but significantly increased sensitivity to aspirin. This result supports the recent report of Cipollone et al., suggesting this polymorphism as an inherited protective factor against myocardial infarction and stroke. In summary, this study in healthy subjects indicates that a full resistance to aspirin is unlikely. Thus, the apparent failure of response to low dose of aspirin observed in some individuals by PFA-100, which is not a specific test to measure response to this drug, is corrected by higher doses. Moreover, although the post-aspirin levels of 11-dTxB2 are largely dependent on the pre-aspirin levels, low dose of aspirin efficiently reduced by ~75% the 11-dTxB2 production in all individuals. In addition, the level of 11-dTxB2 might be influenced by genetic characteristics. Keeping in mind the small numbers of individuals studied and the multiplicity of the analysis that might affect the statistic power, our current findings should be interpreted with precaution and will await further demonstration on larger studies.

Acknowledgments
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References


Biological Assessment of Aspirin Efficacy on Healthy Individuals: Heterogeneous Response or Aspirin Failure?

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