The Effect of Recombinant Tissue Plasminogen Activator (r-tPA) on Quantitation of Neutralising Anti-Streptokinase Antibodies

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ABSTRACT

The measurement of anti-streptokinase antibodies can distinguish the patients who may benefit from streptokinase and those who should be treated with some other thrombolytic regimen. Neutralising titration test is a commonly used classical assay for measuring anti-streptokinase antibodies and in this assay the ability of anti-streptokinase antibodies in patients' sera in preventing the lytic effect of streptokinase is assessd. As we showed previously the presence of r-tPA in the serum may interefere with the neutralising titration test, we investigated this interference in vitro. The level of neutralising anti-streptokinase antibodies in a serum sample with known levels of the antibodies were measured in absence and presence of increasing amount of r-tPA including therapeutic values. Increasing amount of r-tPA in vitro induced a sudden reduction in the measurable titre of anti-streptokinase antibody levels in a serum with elevated levels of anti-streptokinase antibodies. This effect of r-tPA, which is through activation of plasminogen has not been reported previously.

We suggest this assay is unsuitable for clinical diagnosis of anti-streptokinase antibodies.

Keywords: Antistreptokinase, Antibodies, Tissue Plasminogen Activator

INTRODUCTION

Streptokinase released from streptococci is a commonly administered thrombolytic agent [1-7]. The presence of anti-streptokinase antibodies in patients' sera as a result of previous streptococcal infection or/and previous fibrinolytic therapy with streptokinase may provoke hypersensitivity reaction[8-10] and reduce the efficacy of the treatment.[11-17] The measurement of anti-streptokinase antibodies in patients' sera can differentiate between those patients who may benefit from streptokinase and those who should be treated with some other regimen.[18]

There are a variety of methods for measuring these antibodies.

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Counter current immuno-electrophoresis, [19,11] radial immunodiffusion,[19] radio-immunoassay [20,12,21] and enzyme-linked immunoassay (ELISA) [22,15,10] are immunological methods. Neutralising titration test is the classical assay for measuring anti-streptokinase antibodies, which was first introduced by Kaplan[23] and is a functional assay. In this commonly used assay the ability of anti-streptokinase antibodies in patient's sera in preventing the lytic effect of streptokinase is assessed.

We have previously studied the effects of pre-existing anti-streptokinase antibodies on streptokinase administration.[17,10] In those studies, the levels of isotype specific (measured by ELISA) and neutralising anti-streptokinase antibodies (measured by neutralising titration assay) were monitored in patients with acute myocardial infarction (AMI) pre-treatment and post-treatment with streptokinase and r-tPA. In the patients treated with streptokinase an

immediate and significant fall in the levels of anti-streptokinase antibodies was observed with the either method mentioned above.[17] This decrease was predictable and has been reported before.[24,25] In the patients treated with r-tPA the levels of isotype specific anti-streptokinase antibodies (measured by ELISA) did not change after administration of r-tPA. However, to our surprise the levels of neutralising anti-streptokinase antibodies (as measured by neutralising titration test) showed significant reduction as compared with the pre-treatment levels,[17] therefore, in the present In vitro study the intereference of r-pPA on the neutralising titration test results was studied.

MATERIALS AND METHDS

Quantitation of Neutralising Anti-Streptokinase Antibodies

Anti-streptokinase neutralising antibodies were quantified in patient's serum samples by the classical neutralising titration assay following manufacturer's instructions (ASK, anti-streptokinase kit, BioMerieux Ltd, Gaften House, Garftway, Basing stokes, Hampshire).

At time intervals from pre-treatment every 15 minutes up to hour 2 after streptokinase administration and at days 1 to 7 samples were taken to monitor the levels of anti-streptokinase antibodies as described previously.[17,10]

The samples were diluted 1/10 in phosphate buffered saline (PBS, pH 7.4) and 25 micro liter was applied to round-bottomed micro plate (Greimer Labs Ltd, Slotion Road, Cambridge) and double diluted across the micro plate up to 1/640. 25 micro liter streptokinase was then added to each well. The micro plate was covered and incubated at 37˚C for an hour. 50 micro liter of a mixture of fibrinogen and plasminogen was added to each well. Rabbit erythrocytes were washed in PBS and resuspended in PBS to give a 5% suspension and then was added to dried bovine thrombin.

20 micro liters of the erythrocytes' suspension was then applied to the wells and the micro plate was covered and incubated at 37˚C for 2 hours. The last dilution that prevented the lytic effect of streptokinase on formed clots and prevented sedimentation of the erythrocytes was considered as the end point titre of the sample and expressed in reciprocal titre of neutralising anti-streptokinase antibodies per milliliter.

Determination of Intra and Inter-Assay Variations of the Titration Assay

Intra and inter-assay variations of the classical method for quantitatin of anti-streptokinase antibodies were determined to assess the accuracy and reproducibility of this method. Known positive sample, which contained 80 units/ml, neutralising anti-streptokinase antibodies were measured 12 times by the method described above to determine the intra-assay variation. Coefficient variation (C.V.) of the measurements was determined by the following formula: C.V.=standard deviation/mean $\times$ 100.

The levels of neutralising anti-streptokinase antibodies in the known sample (80 units/ml) were measured on 7 different days to determine the inter-assay variation and the C.V. were calculated as described above.

In Vitro Effect of r-tPA on Quantitation of Neutralising Anti-Streptokinase Antibodies

The levels of neutralising anti-streptokinase antibodies in serum sample with known levels of these antibodies (80 units/ml) were measured in absence and in presence of r-tPA (Actilyse, Boehringer Ingelheim Ltd, Brackmel Berkshire) as below; this sample was diluted 1/10 in PBS (total volume 500 micrlolitre) or was diluted in PBS containing increasing amount of r-tPA from 0.3 microgram per millitre to 5000 microgram per millitre (total volume 500 microlitre).

Therapeutic dose of r-tPA is about 1000 microgram per millitre. 25 micro litre from each tube was then applied to micro plate and the titre of the neutralising anti-streptokinase antibodies was measured as described above.

RESULTS

Intra and Inter-Assay Variations of the Anti-Streptokinase AntibodiesTitration Assay

The C.V. of intra-assay and inter-assay of the titration test were 21% and 20 % respectively (Table 1).

The Levels Anti-Streptokinase Antibodies

Table 1. Intra and inter-assay variations of neutralizing titration assay (the values are expressed in units/ml).

Assay	Number of measurements	Mean	Median	S.D.	C.V.
Intra-assay	12	73.3	80	15.6	%21
Inter-assay	7	74.3	80	15	%20

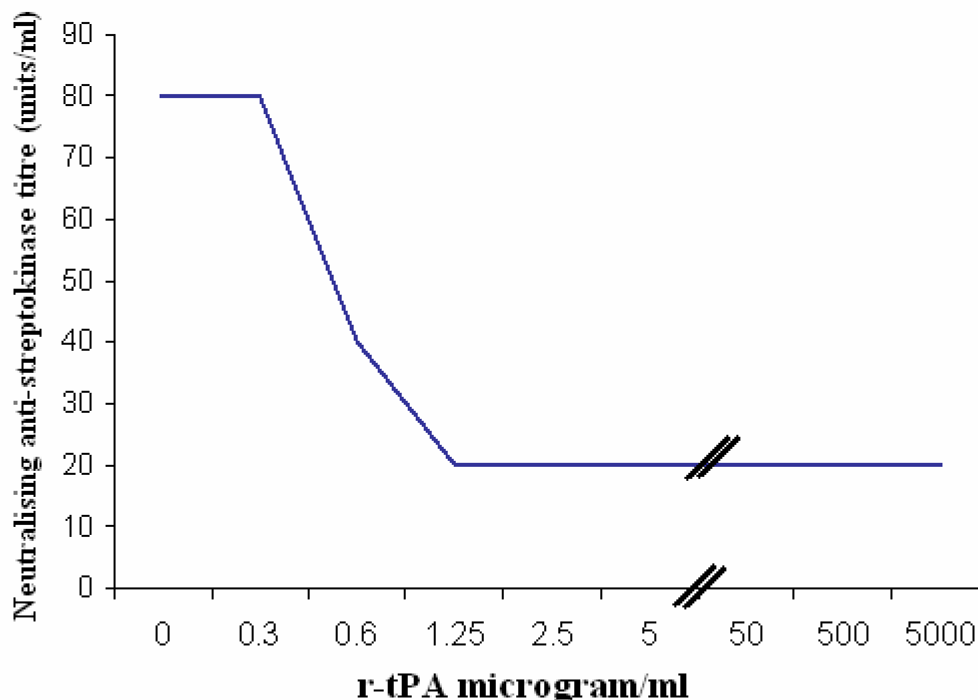

Figure 1. In vitro effect of r-tPA on the titre of neutralising anti-streptokinase antibodies.

We previously showed that administration of streptokinase in patients with AMI, as measured by immunoassay, resulted in an immidiate fall in the levels of anti-streptokinase antibodies.[10]

Administration of streptokinase also resulted in a significant fall in the levels of neutralising anti-streptokinase antibodies as measured by functional neutralising titration assay.[10]

The levels of isotype specific anti-streptokinase antibodies in patients treated with r-tPA did not show statistically significant changes as measured by ELISA (Table 2).

However, quantitation of neutralising anti-streptokinase antibodies showed a significant reduction in post-treatment samples compared with pre-treatment values at 15 minutes after administra-tion of r-tPA ($p<0.01$, Table 2).

Table 2. Pre-treatment and post-treatment (15 minutes) mean values of IgG, IgA, IgM and neutralizing anti-streptokinase antibodies in patients treated with r-tPA (The values are expressed in units/ml).

Isotype	Pre-treatment (n=10)	Post-treatment (n=10)
IgG (ELISA)	5.1	5.6
IgA (ELISA)	0.4	0.5
IgM (ELISA)	8.3	9.4
Neutralising abs. (titration)	28	2.2

In Vitro Effect of r-tPA on Quantitation of Neutralising Anti-Streptokinase Antibodies

When serial amounts of r-tPA were added to a serum with known elevated levels of neutralising anti-streptokinase antibodies (80 units/ml) a reduction in

the measurable titre of these antibodies was observed (Figure 1). The presence of 0.5 microgram/ml r-tPA dropped the titre of anti-streptokinase antibodies in the serum from 80 units/ml to 40 units/ml. In the presence of 1.25 microgram/ml of r-tPA, the levels of anti-streptokinase antibodies decreased to 20 units/ml. From this point by increasing r-tPA the levels of anti-streptokinase antibodies did not decrease (Figure 1).

DISCUSSION

Streptokinase is now commonly administered as thrombolytic agent in patients with AMI. Streptokinase promotes clot lysis and reduces the mortality rate.[26,6,7] With the same efficacy of r-tPA (4, 26), although it is about 10 to 12 times cheaper than r-tpa.[3,27] One of the main disadvantages of streptokinase administration is the streptokinase resistance due to pre-existing anti-streptokinase antibodies as a result of previous streptococcal infection or previous treatment with this agent. These antibodies may provoke hypersensitivity reactions or may reduce the lytic effect of streptokinase. [11,13,25,16,17,18] Many types of methodologies have been employed to measure anti-streptokinase antibodies responses; radio-immunoaassay, counter-current immuno-electrophoresis, radial immuno-diffusion and ElISA have been infrequently employed for detecting anti-streptokinase antibodies; the latter being sensitive and a reproducible technique.[24,21,25,28,29]

Neutralising anti-streptokinase antibodies titration assay is a functional assay that measures these antibodies by using the effect of these antibodies in inhibiting the lysis of clots by streptokinase. In this study the levels of isotype specific anti-streptokinase antibodies (measured by ELISA) in patients treated with r-tPA did not change after the administration (Table 2). However, neutralising titration test in these patients showed a significant reduction in the levels of detectable antibodies at 15 minutes ($p<0.01$, Table 2).

In vitro study of the effect of r-tPA on the quantitation of anti-streptokinase antibodies revealed that the presence of administered r-tPA in the patients' sera was responsible for these observation (Figure 1). This effect of r-tPA, is through activation of plasminogen since t-PA (usually released from injured tissu) converts plasminogen to plasmin and disolves the clot.[27] The presence of r-tPA in sera therefore results in a false low titre of anti-streptokinase antibodies and has not been reported before. We did not look at the levels of anti-streptokinase antibodies in these patients as long as patients were treated with streptokinase but we could conclude that due to clearance of r-tPA from the circulation, the false low titre of anti-streptokinase antibodies in these patients would be improved.

From this effect of r-tPA on the neutralising titration funcional assay we assume that this assay is unsuitable for clinical diagnosis of anti-streptokinase antibody levels and alterations in coagulation system due to other thrombolytic and anti-coagulants treatment (i.e. heparin) may also interfere with assay which gives false negative results. Moreover, this method has a poor accuracy and reproducibility as we showed by intra-assay and inter-assay variations (Table 1).

REFERENCES

1. Walker MG, Dhall DP, Kasenally, Mavor GE. The administration and stability of streptokinase. Postgrad Med J 1973; 49:33-4.
2. Thayer C. Results of post-marketing surveillance program on streptokinase. Curr Ther Res 1981; 30:129-40.
3. Sharma GVRK, Cella G, Parisi A, Sashara AA. Thrombolytic therapy. New Eng J Med 1982; 306(21):1268-76.
4. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI). GISSI-2. A factorial randomized trial of anistreplase versus streptokinase and heparin versus no heparin among 12490 patients with acute myocardial infarction. Lancet 1990; 336(8701):65-71.
5. Hogg KJ, Gemmill JD, Burns JMA, Lifson WK, Rae AP, Dunn FGG, et al. Angiographic patency study of anistreplase versus streptokinase in acute myocardial infarction. Lancet 1990; 335(8684):254-8.
6. Anderson JL, Sorensen SG, Monreno FL, Hackworthy RA, Brown KF, Dale HH, et al. Multicentre patency trial of intravenous anistreplase compared with streptokinase in acute myocardial infarction. Circulation 1991; 83(1):126-40.
7. Chouhan LK, Hajar HA, George T, Pomposiello JC. Clinical and angiographic features of patients from the Indian subcontinent with streptokinase treatment for acute myocardial infarction: experience in Qatar. Angiology 1992; 43(8):641-6.
8. Ong ACM, Handler CE, Walker JM. Hypersensitivity vasculitis complicating intravenous streptokinase therapy in acute myocardial infarction. Internat J Cardiol 1988; 21(1):71-3.
9. Lee HS, Yule S, McKenzie A, Cross SG, Reid T, Davidson R, et al. Hypersensitivity reactions to streptokinase in patients with high pre-treatment anti-

streptokinase antibody and neutralization titres. Eur Heart J 1993; 14(12):1640-3.
10. Afshari A, Holme ER, Hillis WS. Antibody response to Streptokinase. J Qazvin Uni Med Sci (Persian) 2000; 15(3):8-13.
11. Lew AS, Neer T, Rodriguez L, Geft IL, Shan PK, Ganz W. Clinical failure of streptokinase due to an unsuspected high titre of anti-streptokinase antibody. JACC 1984; 4:183-5.
12. Hoffmann JJML, Fears R, Bonier JJRM, Strandring R, Ferres H, DeSwart JBRM. Significance of antibodies to streptokinase in coronary thrombolytic therapy with streptokinase or APSAC. Fibrinolysis 1998; 2:203-10.
13. Massel D, Turiep AGG, Carins AJ, Ofsu FA Buchanan MR. Previous streptokinase therapy inhibits subsequent streptokinase thrombolysis. Circulation 1991; 84:476.
14. Buchalter MB, Suntharalingam G, Jennings I, Hart C, Luddington RJ, Chakraverty R, et al. Streptokinase resistance: when might streptokinase administration be ineffective. Br Heart J 1992; 68(5):449-53.
15. Lynch M, Pentecost BL, Littler WA, Stockley RA. The significance of anti-streptokinase antibodies. Clin Exp Immunol 1994; (96)3:427-31.
16. Gemmill JD, Hogg KJ, Dunn FG, Rae AP, Hillis WS. Pre-dosing antibody levels and efficacy of thrombolytic drugs containing streptokinase. Br Heart J 1994; 72(3): 222-5.
17. Afshari A. The role of humoral mediators in immunological reactions to Streptikinase. PhD thesis (Department of immunology, Glasgow University)1995; 69-118.
18. Juhlin P, Bostrom PA, Torp A, Bredberg A. Streptokinase antibodies inhibit reperfusion during thrombolytic therapy with streptokinase in acute myocardial infarction. J Intern Med 1999; 245(5):483-8.
19. Spottl F, Kaiser R. Rapid detection and quantitation of precipitating streptokinase antibodies. Thrombos Diathes Haemorrh 1974; 32(2-3):608-16.
20. Moran DM, Sandring R, Lavender ER, Haris GS. Assessment of anti-streptokinase antibody levels in human sera using a micro-radio-immunoassay procedure. Thromb Haemostas 1984; 52(3):281-7.
21. Fears R, Heam J, Standring R, Anderson JL, Marder VJ. Lack of influence of pretreatment anti-streptokinase antibody on efficacy in multicentre patency comparison of intravenous streptokinase in acute myocardial infarction. Am Heart J 1992; 124(2):305-14.
22. McGrath KG, Patterson R. Anaphylactic reactivity to streptokinase. JAMA 1984; 252(10):1314-7.
23. Kaplan MH. Nature and role of lytic factor in hemolytic streptococcal fibrinolysis. Proc Soc Exp Biol Med 1994; 57:40-3.
24. Lynch L, Littler WA, Pentecost BL, Stockley RA. Immunoglobulin response to streptokinase in acute myocardial infarction. Br Heart J 1991; 66(2):139-42.
25. Fears R, Ferres H, Glasgow E, Strandring R, Gemmill JD, Burns JMA, et al. Monitoring of streptokinase resistance titre in acute myocardial infarction patients up to 30 months after giving streptokinase or anistreplase and related studies to measure specific anti-streptokinase IgG. Br Heart J 1992; 68(2):167-70.
26. White HD, Williams BF, Norris RM. Safety and efficacy of repeat thrombolytic treatment after acute myocardial infarction. Br Heart J 1990; 64(3):177-81.
27. Massel D. Potential cost effectiveness of tissue plasminogen activator among patients previously treated with streptokinase. Can J Cardiol 1999; 15(2):173-9.
28. Bom VJ, Brugemann J, van der Schaaf W, van Wijik RT, van der Meer J. Rapid enzyme immunoassay of anti-streptokinase antibodies in human plasma. Clin Chem Acta 1993; 218(2):121-9.
29. Elliot JM, Cross DB, Stewart A, White HD. Neutralizing antibodies to streptokinase four years after intravenous thrombolytic therapy. Am J Cardiol 1993; 71(8):640-5.