DOES NIPRISAN® RETARD THE EVOLUTION OF SICKLE CELL RETINOPATHY?

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SUMMARY

Objective:

To investigate the efficacy of Niprisan®, an antisickling agent, in the management of sickle cell retinopathy.

Methods: The study was designed as a phase IIb double-blind, placebo-controlled crossover trial. Eighty-eight patients aged between 5 and 36 years (mean 15.3 years) were randomized into 2 treatment groups. One group received Niprisan® at a dose of 12mg/kg per os per day and the other group a placebo in a similarly encapsulated form, for an initial period of six months. After a crossover without interval washout, the treatment was continued for a further six months. Ocular signs, including jaundice and corkscrew/comma sign in the anterior. segment, and signs of non-proliferative, pre-proliferative and proliferative retinopathy in the posterior segment, were assessed with a view to identifying deteriorations within these parameters.

Results: A within-person analysis provided no evidence that Niprisan® reduced the risk of anterior segment deterioration (odds ratio = 0.91; 95% c.i. 0.35, 2.36; p=1.00). Thirteen individuals contributed to the posterior segment analysis, 3 of whom experienced deterioration whilst receiving Niprisan® (odds ratio = 0.30, 95% c.i. 0.05-1.17; p=0.09; Mcnemar chi^2 = 3.17, p=0.05).

Conclusion: This study provides evidence that Niprisan® may reduce substantially the risk of posterior segment deterioration.

Key words: Niprisan®, sickle cell retinopathy, antisickling agents

INTRODUCTION

Haemoglobinopathies are a recognized cause of retinopathy.[1] In Jamaica, proliferative sickle cell retinopathy (PSR) has been reported in 32% of HbSC and 6% of HbSS subjects.[2] In Nigeria, a PSR prevalence of 6% has been reported in a cohort of predominantly HbSS patients.[3] Current standard treatment of PSR is with photocoagulation of vasculo-proliferative lesions.[4] Peripheral scatter photocoagulation is probably beneficial if extensive neovascularization or vitreous haemorrhage is present. This treatment, however, is not without risk, and neovascularization from the choroid, retinal tears and occlusion of the posterior ciliary arteries can and do occur.[5, 6, 7] Furthermore, there are many areas in the developing world where laser equipment is not available or not accessible. The role of antisickling agents that inhibit the polymerization of haemoglobin S in the management of sickle cell retinopathy has not been properly addressed. These antisickling agents include phenylalanine and hydroxybenzoic acid, used singly or in combination,[8] hydroxyurea,[9] Piracetam,[10] and Cromolyn sodium.[11] Hydroxyurea is probably the most commonly used of these remedies. It is, however, relatively cytotoxic and may cause life-threatening cytopenia.[9]

Niprisan® is a phytomedicine developed by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, from indigenous medicinal plants for the management of sickle cell disease (SCD).[12] It is formulated into standardized capsule form from freeze dried extracts of *Piper guineensis* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *Sorghum bicolor* leaves. It has been shown to inhibit polymerization of HbS *in vitro*, in addition to displaying analgesic and anti-inflammatory properties.[13] It has also been found to be non-toxic in laboratory animals[14] and healthy volunteers.[15] Pilot clinical (phase IIa) studies indicated its safety and

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efficacy in the management of sickle cell disease (SCD).[15] In the pilot study, 73% of the 30 patients who volunteered for the study did not experience any crisis during the 12 months of the trial as against an average of more than three crises per year prior to treatment with Niprisan®. The remaining 27% of the study population experienced less frequent and less severe crises. In addition, studies of acute toxicity, assessed by liver enzyme activity, creatinine and blood urea nitrogen, suggested that Niprisan® is safe. Recently, phase IIb clinical trials were carried out to investigate in more detail the safety and efficacy of this preparation for the treatment of SCD. This communication reports the findings with respect to ocular manifestations of SCD.

PATIENTS AND METHODS

The study was designed as a double-blind, placebo-controlled, crossover trial. The trial was publicized via the Sickle Cell Club in Abuja and through the mass media, to residents of the Federal Capital Territory, Nigeria. Subjects were volunteers who asked to be included in the trial. They were told about the new phyto-medicinal preparation for the treatment of severe symptoms of sickle cell disease and offered the opportunity to participate in a controlled trial. Information concerning the safety of the preparation in laboratory animals and healthy volunteers was provided. Individuals were eligible for inclusion if they had experienced moderate to severe recurrent episodes of bone pain and at least three painful or vaso-occlusive crises in the preceding year. These crises must have required treatment at a health care facility or at home with a parenteral or equianalgesic dose of oral narcotics or non-steroidal anti-inflammatory drugs, confirmed from medical records. Episodes of acute chest syndrome, splenic/hepatic sequestration or priapism were all considered. Patients were excluded from the trial if they were known to be HIV positive, ill with hepatitis or tuberculosis, pregnant or breast-feeding, or unwilling or unable to follow instructions regarding treatment. Ethical clearance for the trial was obtained from the National Agency for Food and Drug Administration and Control (NAFDAC) of Nigeria and the Ethical Committee of NIPRD. Sample size calculations were carried out based on detecting an impact on general physical parameters such as frequency of painful, haemolytic and other crises.

Judging from the results of the pilot study, a reduction in incidence of 70%[15] was targeted for detection by the selected sample size. One group received Niprisan® at a dose of 12mg/kg p/o once per day and the other group a placebo in a similarly encapsulated form, for an initial period of six months (Animal studies on spontaneous motor activity and nociceptive stimuli had suggested that the drug effect peaks at 90 minutes and lasts for 24 hours[13]). After a crossover without any washout period, trial subjects received the other treatment for another six months. Before receiving any Niprisan® or placebo, all patients underwent a baseline ocular evaluation that included visual acuity (corrected and uncorrected), anterior segment assessment for corkscrew vascular formation or comma sign using a pen-torch, and dilated binocular indirect ophthalmoscopy. They were assessed for signs of background, non-proliferative sickle cell retinopathy (NPSR: schisis cavities/iridescent bodies, black sunburst sign, salmon patch haemorrhage), as well as pre-proliferative and proliferative retinopathy, (peripheral vascular occlusions, arterio-venous anastomosis, sea fan neovascularization, pre-retinal and vitreous haemorrhage).

All posterior segment ocular findings were drawn on a chart. Other incidental eye disease was also noted. The same ocular examination was repeated at six months post enrolment, just before crossover, and at twelve months, at the end of the second treatment period. The same observer (OEB), who was unaware of the treatment group allocations, performed all ocular examinations. The findings at six months were compared with those at baseline, and those at twelve months were compared with findings at six months by OEB, who remained unaware of each individual's treatment group. Individuals were classified into two groups: those showing deterioration and those with no deterioration. Deterioration was considered to have occurred only if a new lesion was seen, or there was a definite worsening of an existing, previously identified lesion on the retinal chart. Initially, each eye was considered separately and then the results from both eyes were combined to categorize each individual as either 'showing deterioration' (in either eye) or 'not showing deterioration'. Data were recorded in a pre-coded format and entered using Borland Dbase 5 software. Analysis was carried out using EPI-info version 5 and Stata version 6.0 (http://www.stata.com).

RESULTS

Ninety individuals were identified as eligible for inclusion in the trial and gave written informed consent. Before randomization, two of these individuals declined to participate, largely due to the logistic difficulties of travelling from their homes to the study centre. Of the remaining 88 individuals, 86 were HbSS and 2 were HbSC. Using the concealed envelope method, 42 individuals were randomized to treatment group 1 (receiving Niprisan® for 6 months followed by the placebo), and 46 to treatment group 2 (first receiving the placebo, then Niprisan®). The two treatment groups had similar age distributions (table 1). The proportion of males was higher in treatment group 1 (55% versus

43%). The pre-trial frequency and severity of sickle cell disease crises were broadly similar in the two treatment groups.

At baseline, the prevalence of SCD-related anterior segment signs was 20% for clinically apparent jaundice and 38% for significant corkscrew vessel formation or comma sign. Overall, anterior segment signs were present in 48% of the subjects. These signs were more common in treatment group 2 than in treatment group 1 (57% versus 38%, see table 2). SCD-related posterior segment signs were observed in 23% of the subjects, with a higher prevalence in treatment group 1 than treatment group 2 (29% versus 17%). Non-proliferative lesions were more prevalent (23%) than proliferative lesions (6%). Posterior segment signs (NPSR and PSR) were more common in males than females (33% versus 17%) even though females were, on average, older (mean age 16.3 years for females versus 14.2 years for males). Other findings included maculopathies (6 individuals), white without pressure lesions (3), snail track lesions (1), paving stone degeneration (1), old toxoplasmosis scar (1), and Bergmeister's papilla (1). Details of these findings have been published elsewhere.[3]

At the end of the first dosing period, posterior segment data were complete for 66 (75%) individuals and anterior segment data were complete for 65 (74%) individuals (figure 1). Twelve individuals (30%) were lost to follow-up from treatment group 1 (Niprisan® then placebo) during the first treatment period, while 10 individuals (22%) were lost from treatment group 2 (placebo then Niprisan®). A further 2 individuals (6%) were lost from treatment group 1 during the second treatment period while 4 individuals (11%) were lost from treatment group 2. In total, 16 individuals were lost to follow-up whilst receiving Niprisan® while 12 individuals were lost while receiving the placebo (p=0.20). The most common reason for loss to follow-up was individuals moving away from the study area, either beyond a convenient distance to the study centre, or to an unknown address.

Individuals lost to follow-up were compared with those remaining in the trial with respect to baseline characteristics. Mean ages were similar in the two groups (15.2 years in those lost to follow-up versus 15.4 years, p=0.87). A higher proportion of males (43%) than females (20%) were lost to follow-up by the end of the trial (p=0.02). The prevalence of anterior segment pathology at baseline was 32% (9/28) among those lost to follow-up compared with 55% (33 of 60) among those with follow-up (p=0.05). For posterior segment pathology, the prevalence for those who remained in the trial was 18% (11 of 60) and 32% (9 of 28) for those lost to follow up (p=0.15).

During the first follow-up assessment at the end of six months, 17 individuals showed deterioration in the anterior segment. Eleven of these individuals had received the placebo while six had received Niprisan® (table 3). Eighteen individuals showed posterior segment deterioration; 12 of whom had received the placebo (table 3). Details of the posterior segment deteriorations are presented in table 4.

After the second treatment period, anterior segment data were available for 57 individuals. During this period, there were 9 deteriorations in individuals receiving Niprisan® and 5 in individuals receiving placebo. Complete posterior segment data were available for 60 persons, of whom 5 were judged to have deteriorated. Of these 5 individuals, 4 were receiving the placebo during this period. Risk of posterior segment deterioration appears to have been higher in the first period (18/66) than in the second period (5/60). This difference is statistically significant (p=0.01).

Within person analysis

A within-person analysis was performed to control between-person variation in age, sex, genotype and severity of pre-existing disease. Only individuals with follow-up during both treatment periods and who experienced a deterioration in one period but not in the other contributed to this analysis. Exact 95% confidence intervals for the odds ratio were obtained and the exact tests of null hypothesis of no treatment effect were also performed.

Twenty-one individuals contributed to the anterior segment analysis. Ten of these had experienced deterioration whilst receiving Niprisan® (odds ratio for effect of treatment = 0.91; 95% c.i. 0.35, 2.36; p=1.00) providing no evidence that Niprisan® reduces the risk of anterior segment deterioration. However, the 95% confidence interval is wide and does not exclude the possibility of either a substantial beneficial or a substantial negative effect. Only 13 individuals contributed to the posterior segment analyses, 3 of whom experienced deterioration whilst receiving Niprisan® (odds ratio =0.30; 95% c.i. 0.05, 1.17; p=0.09; Mcnemar's Chi^2 = 3.17 p=0.05). Thus there is some evidence that Niprisan® reduces the risk of posterior segment deterioration.

DISCUSSION

Results published elsewhere indicate that individuals on Niprisan® reported severe pain, 65% less often than individuals on a placebo (p<0.05).[16] It is possible that Niprisan® might also have an impact on SCD related retinopathy by inhibiting the polymerization of HbS, through its effect on rheological variables and by improving peripheral retinal perfusion.

Table 1. Distribution of selected parameters at baseline by treatment group

Parameter	Treatment group 1 (Niprisan® followed by placebo) N=42	Treatment group 2 (Placebo followed by Niprisan®) N=46
Median age (inter-quartile range)	14.5 (11-18)	14.5 (12-17)
Number (%) of males	22 (55%)	20 (43%)
Number (%) of individuals with anterior segment lesions	16 (38%)	26 (57%)
Number (%) of individuals with posterior segment lesions	12 (29%)	8 (17%)
Mean frequency of SCD related mild to moderate pain during 4 month pre-trial period	18.4 (0-89)	15.1 (0-96)
As above, but severe pain (requiring narcotic treatment or hospital admission)	12.7 (0-79)	11.5 (0-61)

Table 2. Sickle cell disease-related eye findings at baseline

Finding		Treatment group 1 (Niprisan® followed by placebo) N=42	Treatment group 2 (Placebo followed by Niprisan®) N=46
Posterior segment:			
Non proliferative retinopathy (NPSR)	Iridescent spots/schisis cavities	8 (19%)	5 (11%)
	Sunburst lesions	4 (10%)	2 (4%)
	Vitreo-retinal fibrosis	2 (5%)	1 (2%)
	Salmon patch	2 (5%)	0
	Pre-retinal haemorrhage	0	1 (2%)
Proliferative retinopathy (PSR)	Vascular occlusion	0	2 (4%)
	Arterio-venous anastomosis	1 (2%)	1 (2%)
	Sea fan neo-vascularization	0	1 (2%)
Any SCD related posterior pathology		12 (29%)	8 (17%)
Anterior segment:			
	Jaundice only	3 (7%)	4 (9%)
	Corkscrew vessels/ comma sign only	9 (21%)	15 (33%)
	Both of the above	4 (10%)	7 (15%)
Any SCD related anterior pathology		16 (38%)	26 (57%)

NB: Figures in this table are not additive due to overlaps in pathology.

Table 3. Occurrence of anterior and posterior segment deteriorations by treatment group by treatment period.

	Treatment group 1	Treatment group 2
Treatment allocation: 1st 6 month period	**Niprisan®**	**Placebo**
Number of individuals with deterioration of anterior segment/number examined	6/30	11/35
Number of individuals with deterioration of posterior segment/number examined	6/30	12/36
Treatment allocation: 2nd 6 month period	**Placebo**	**Niprisan®**
Number of individuals with deterioration of anterior segment/number examined	5/27	9/30
Number of individuals with deterioration of posterior segment/number examined	4/28	1/32

Table 4. Definite posterior segment deteriorations

Identity number	Treatment during period when deterioration occurred	Right eye	Left eye
1st six month period			
5	Placebo	Iridescent spots	Nil
9	Placebo	Black sunburst lesion. Retinal breaks	Pre-retinal haemorrhage, iridescent deposit, and new breaks within pre-existing Snail track lesions.
10	Placebo	Iridescent spots	Nil
11	Placebo	Nil	Black sunburst
14	Niprisan®	Iridescent spots	Nil
15	Niprisan®	White without pressure lesions	
21	Placebo	Retinal breaks within pre-existing Snail track lesions, raised fibro-vascular mantle	(Previous sea fan neovascularization), Vitreo-retinal fibrosis associated with pre-existing Snail track lesions
25	Niprisan®	Iridescent deposits, black sunburst lesions	Arterio -venous anastomosis
29	Niprisan®	Iridescent spots, raised fibrous mantle	Raised fibro-vascular mantle, vascular occlusion
32	Placebo	Schisis cavity, arteriolar occlusion	Nil
33	Niprisan®	Black sunburst	Nil
38	Placebo	Iridescent spots	Iridescent spots
42	Placebo	Nil	More extensive sunburst ++
52	Niprisan®	Iridescent spots	Iridescent spots/schisis cavity
59	Placebo	Nil	Schisis cavity, black sunburst
67	Placebo	Iridescent spots	Nil
76	Placebo	Nil	Vitreo-retinal fibrosis
78	Placebo	Venous tortuosity	Venous tortuosity
2nd six month period			
89	Placebo	Nil	Iridescent spots
86	Niprisan®	Salmon patch (Previous iridescent spots maintained)	Pre-retinal haemorrhage (Previous AV malformation; black sunburst)
76	Placebo	Iridescent spots	New black sunburst
47	Placebo	Nil	Black sunburst

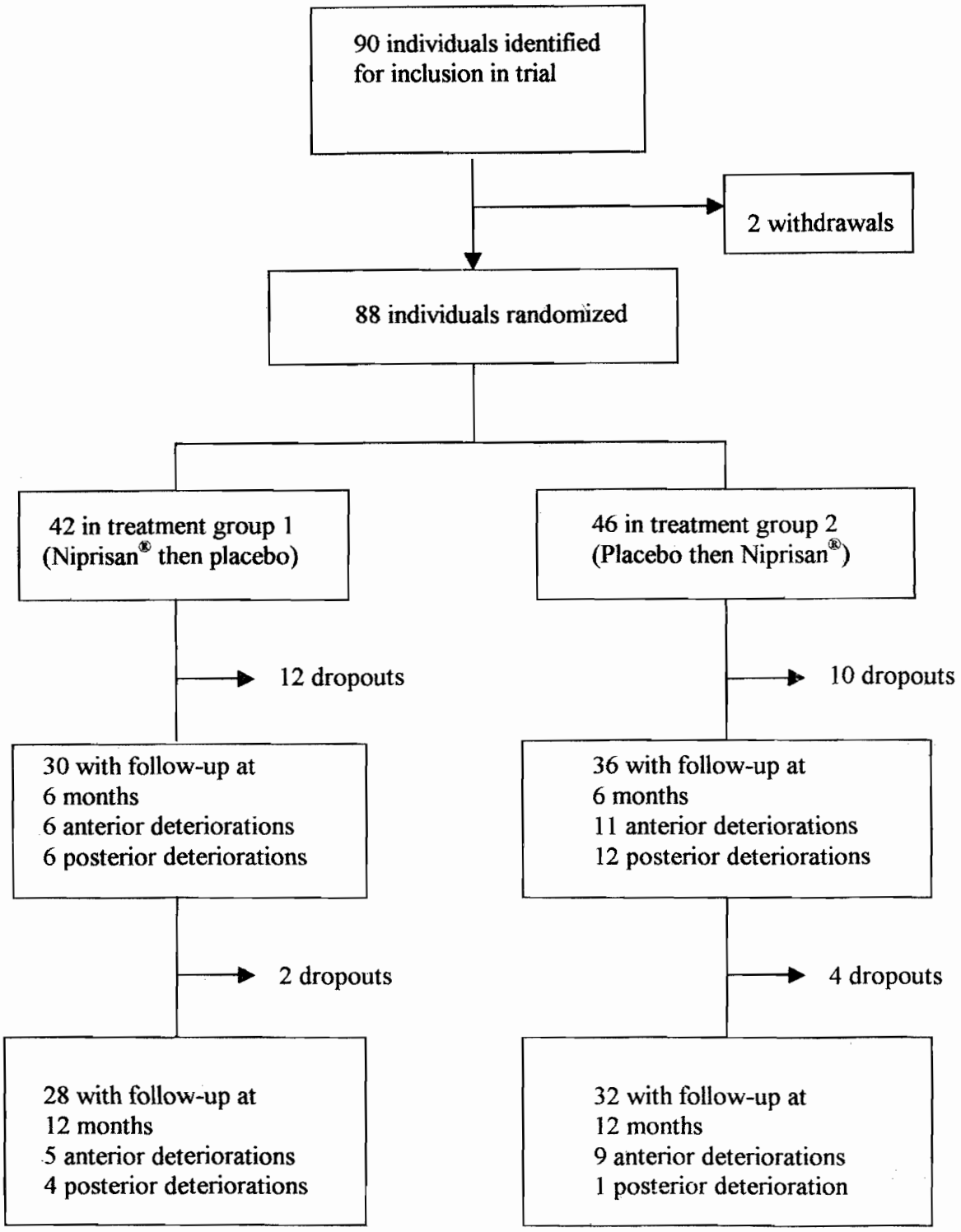

Figure 1. Consort diagram

The results of our small trial, designed to detect the impact of Niprisan® on bone pain and other crises rather than on ocular manifestations of SCD, suggest that Niprisan® may indeed reduce substantially the progression of SCD retinopathy.

Our trial had a number of limitations which basically reflected the conditions under which we practice in West Africa. First, the sensitivity and specificity of our ocular findings would probably have been enhanced with the use of fluorescent angiography and retinal photography, with which we might have been in a better position to make statements on vascular re-modelling. However, the double-blind, placebo-controlled crossover design of the study means that any lack of sensitivity and specificity in our measurement of outcome would be expected to mask rather than exaggerate any impact of Niprisan®. Second, most of the patients in this study had HbSS, which is less strongly associated with sickle cell retinopathy than HbSC. The decision to recruit mainly HbSS individuals, however, reflects the primary outcome investigated in this trial, bone pain and other crises, which are more strongly associated with the HbSS genotype.

Within these limits, our findings suggest that there may be a place for Niprisan® therapy in the prevention of sickle cell retinopathy in susceptible individuals. The exact mechanism of action of Niprisan® in this instance is not clear, but may be connected with its effect on rheological variables, which may in turn affect peripheral perfusion. *In vitro* polymerization studies carried out at NIPRD [13] indicate that Niprisan® markedly inhibited polymerization and gelation of HbS which increases the deformability of sickled red cells.[17] A substance that inhibits gelation might have a beneficial effect on peripheral retinal perfusion and thus inhibit the progression of sickle cell retinopathy, since most of the lesions are due to vascular occlusion with resultant ischaemia, infarction or haemorrhage in various retinal layers. Niprisan® did not appear to have the same effect on the anterior segment signs of jaundice and comma sign/vascular tortuosity. The reason(s) for this is not clear. Jaundice is a reflection of the level of unconjugated bilirubin, which in turn is a reflection of the degree of red cell haemolysis. Abnormalities of the bulbar conjunctival blood vessels provide evidence of the vaso-occlusive process and are believed to result from flow obstruction or impedance by sickled cells. Levels of bilirubin were not measured in the study. However, from the point of view of the potential threat to sight, posterior segment lesions are more important.

These ocular findings mirror to some extent the systemic results from the study. This may reflect the common pathogenesis of pain and peripheral retinal disease in SCD - microvascular closure due to thrombosis or intravascular sickling. Niprisan® significantly reduced the frequency of SCD crises associated with severe pain. Liver and renal enzyme analysis following use of the drug indicated that it had no significant liver or renal toxicity, suggesting that it is a safe phytomedicine.[13] The potential benefits of medical therapy in the management of sickle cell eye disease have not been paid much attention in the literature. A lot of research effort has, however, focused on photocoagulant therapy. Our findings suggest that the use of Niprisan® may substantially reduce the incidence of deterioration in the posterior pole. It is noteworthy that the overall number of cases of deterioration events lessened in the second half of the trial, (18 in the first half of the trial versus only 5 in the second half). A similar effect was noticed with respect to the general physical parameters - severe pain being reported an average of 30 times per person during the first period of treatment against an average of 11 times during the second half of the trial. The reason for this is not clear at present because the drug is taken daily and on the basis of its half-life, we would not expect its benefit to extend many days after cessation of treatment.

The possible use of Niprisan® in combination with other antisickling remedies also needs to be studied. Other workers have commented upon the importance of synergism in antisickling medication.[3] Tucaresol, for instance, increases the oxygen affinity of haemoglobin[13] and its use in combination with other antisickling agents which inhibit HbS polymerization, such as Niprisan®, merits investigation. Tucaresol has the added advantage that it has already been administered to healthy volunteers, and its tolerance and dosage is the subject of ongoing research.

In conclusion therefore, our results from a relatively small study involving less than 100 individuals, provides evidence that Niprisan® may reduce the risk of SCD-related posterior segment deterioration. They also highlight the possibility that antisickling remedies may have a role to play in slowing the progression of sickle cell retinopathy, which may in turn reduce the need for (or complement) photocoagulant therapy. A larger trial to provide a more precise estimate of the effect of Niprisan® on ocular disease is needed, and plans to undertake a multicentre study in the six geopolitical zones of Nigeria, with at least six hundred participants, are being developed.

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